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Why have clinical trials in sepsis failed?

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The systemic inflammatory response is biologically complex, redundant, and activated by both infectious and noninfectious triggers. Its manipulation can cause both benefit and harm. More than 100 randomized clinical trials have tested the hypothesis that modulating the septic response to infection can improve survival. With one short-lived exception, none of these has resulted in new treatments. The current challenge for sepsis research lies in a failure of concept and reluctance to abandon a demonstrably ineffectual research model. Future success will necessitate large studies of clinical and biochemical epidemiology to understand the course of illness, better integration of basic and clinical science, and the creation of stratification systems to target treatment towards those who are most likely to benefit.

The landscape of sepsis clinical trials

The first clinical trial of the sepsis hypothesis, that the endogenous inflammatory response of the host determines the outcome of life-threatening infection, was published in 1976 [1]. In a study of 172 patients with septic shock, William Schumer reported that administration of large doses of methylprednisolone could reduce mortality from 39% to 11%. Forty years later, although the use of high dose steroids has been largely abandoned, controversy continues regarding the efficacy of lower doses, and the underlying promise of the mediator-targeted therapy remains unachieved. The reasons have been the subject of numerous editorials and opinion pieces [2,3]; we have failed to address these systematically and to alter research designs accordingly, and success is as elusive now as it was a generation ago.

There have been more than 100 Phase II and Phase III clinical trials of strategies to modify the systemic inflammatory response by selectively or nonselectively targeting its endogenous mediator molecules (Figure 1). The majority of these have been commercially funded studies conducted with the goal of marketing a proprietary compound. Strategies have included nonselective suppression of inflammation using corticosteroids or ibuprofen; the selective neutralization of microbial products such as endotoxin or host inflammatory mediators such as tumor necrosis factor (TNF), interleukin-1 (IL-1), platelet-activating factor, and nitric oxide; nonselective targeting of inflammatory mediators using polyvalent immunoglobulin; the administration of proteins that stimulate some aspects of immune function, including granulocyte colony-stimulating factor (G-CSF) and interferon γ; and the administration of anticoagulant molecules such as activated protein C (APC), tissue factor pathway inhibitor (TFPI), anti-tissue factor antibody, antithrombin, thrombomodulin, or heparin (Table 1).

The basic approach to these trials has been similar [4,5]. Each has targeted patients who have, or are suspected to have, infection as the cause of the acute clinical crisis, and has sought to show that the specific candidate intervention could reduce rates of mortality in treated patients. Each has used simple physiologic parameters to identify an at-risk study population, and has assumed that the resultant mortality signal will be large. Finally, each has proceeded with an expectation of success, and thus has not incorporated an analytic plan to probe the reasons for failure. Moreover, it is apparent that although no specific strategy has led to a dramatic improvement in survival for patients with sepsis, in aggregate the studies have shown a small but consistent signal for clinical benefit (Figure 1).

There is not a single simple explanation for the disconnect between promise and reality in sepsis research, but rather a complex matrix of factors that range from our understanding of biology to our assumptions and expectations in clinical research and to the social, economic, and regulatory constraints of the research environment.

Sepsis and the biology of innate immunity

The work of Pasteur and others in the nineteenth century established the critical role of exogenous microorganisms in the pathogenesis of infection, and created the basic architecture of the germ theory of disease. The consequences have been transformative. Annual mortality from infectious disease in the USA declined from 800 cases per 100 000 in 1900 to approximately 70 cases per 100 000 a century later [6]. Strikingly, however, the most impressive declines reflected public health measures such as vaccination, the development of public health departments, the chlorination of water, and the pasteurization of milk, rather than the development of effective antimicrobial interventions in the form of antibiotics, or of effective patient support measures with the emergence of intensive care units.

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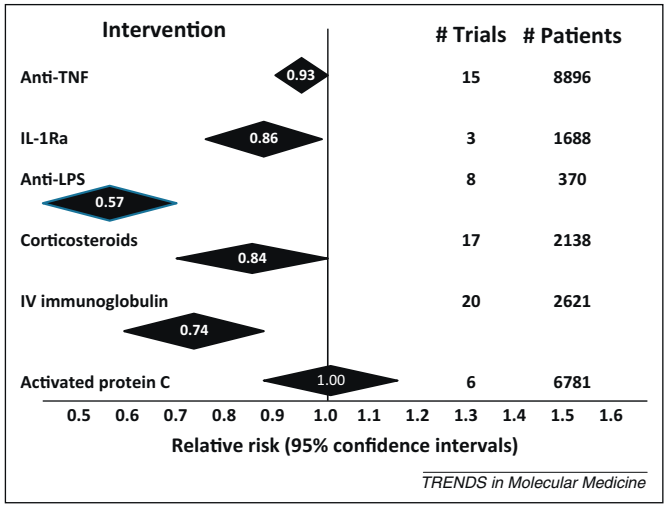

Figure 1. Therapeutic efficacy of mediator-targeted therapy for sepsis. Pooled data from randomized controlled clinical trials of commonly studied interventions to modulate the host response in patients with sepsis. Data are presented as relative risk (center of diamond) and 95% confidence intervals (extremes of diamonds). Relative risk is the ratio of the probability of death in treated patients compared with untreated controls; a value of 1 indicates no effect, whereas a value of less than 1 indicates that the experimental intervention is superior. Anti-LPS treatments are restricted to studies of extracorporeal hemoperfusion using a polymyxin B column. Abbreviations: LPS, lipopolysaccharide; TNF, tumor necrosis factor; IV, intravenous; IL-1Ra, interleukin-1 receptor antagonist.

Table 1. Clinical trials of biologic response modifiers in sepsis

Target	Strategies
Endotoxin (LPS)	Monoclonal antibodies
	LPS: HA-1A, E5
	Enterobacterial common antigen
	Toll-like receptor 4 (TLR4) antagonists
	Eritoran
	TAK-242
	Anti-CD14
	Bactericidal permeability increasing protein
	Taurolidine
	Alkaline phosphatase
	Polymyxin B
	Conjugate
	Extracorporeal column
	Lipid emulsion
Tumor necrosis factor (TNF)	Monoclonal or polyclonal antibodies
	Soluble receptor constructs
Interleukin-1 (IL-1)	Recombinant IL-1 receptor antagonist
Platelet activating factor (PAF)	Small molecule inhibitors
	PAF acetylhydrolase
Eicosanoids	Ibuprofen
	Soluble phospholipase A2 (sPLA2) inhibitor
Nitric oxide	L-NMMA
	Methylene blue
Hypercoagulability/disseminated intravascular coagulation (DIC)	APC, Protein C concentrate
	TFPI
	Antithrombin
	Anti-tissue factor antibody
	Heparin
	Thrombomodulin
Immune suppression	Intravenous immunoglobulin
	G-CSF, GM-CSF
	Interferon γ
Endocrinopathy	Corticosteroids
	Vasopressin
Others	Selenium
	Lactoferrin
	Bradykinin antagonists
	Statins
	Extracorporeal hemoperfusion

Experimental work in the 1950s first suggested that the morbidity of invasive infection involved more than the intrinsic cytopathic activities of the infecting microorganism, but rather arose through the response that the organism evoked in the infected host [7]. Fever, for example, was attributed to the expression of an endogenous pyrogen, later identified as the cytokine IL-1. A report by Michalek and colleagues in 1980, using adoptive transfer of bone marrow cells between endotoxin-sensitive and endotoxin-resistant mice, found that the lethality of endotoxin was transferred with the bone marrow cells: animals died not because they were exposed to endotoxin, but rather because they responded to that exposure [8].

Over the ensuing three decades we have learned an enormous amount about the biology of the septic response that is responsible for organ injury and death. We have identified key host-derived mediators of that response, including IL-1 [9] and TNF [10], and have learned that the coagulation cascade and the innate immune system are intimately inter-related [11]. We have identified the cellular mechanisms through which infection and other forms of danger are recognized by the host [12], and in the process, have learned that the response is a generic response to danger, rather than a specific response to infection [13]. We have identified endogenous modifiers of the potentially harmful host response and, at the same time, have learned that microorganisms and their products may also mediate the adaptive aspects of the host response to injury [14]. Finally, we have begun to unravel some of the cellular processes that alter basal transcriptional patterns in innate immune cells, and thus both enable the host to respond effectively to a threat and mediate the inadvertent host injury that results from that response.

These processes are enormously complex. Using a reductionist animal model of acute inflammation, the administration of a bolus infusion of endotoxin to a mouse, it has been shown that more than 100 discrete biochemical species are necessary for disease evolution because their ablation or administration alters mortality risk [15] (Table 2). In a corresponding human model, low dose endotoxin challenge alters the expression of 3714 genes in circulating leukocytes [16]. A conclusion from this body of work is that inflammation is a complex interdependent process whose deleterious effects can be ascribed to multiple host-derived biochemical mediators. A corollary to this conclusion is that it is improbable that modulating the activity of any one of these will have more than a modest effect on the clinical course of illness.

Table 2. Biochemical species implicated in the lethality of murine endotoxemia[a]

Target	Neutralization improves survival	Administration improves survival
Cytokines and related proteins	IL-1, IL-12, IL-18, IL-33, TNF	IL-1Ra, IL-1α, IL-4, IL-10, IL-13
	IL-25, IFN-α, Gelsolin, Ghrelin	HGF, LIF, CRP
	MIF, LIF, IFN, HMGB1, G-CSF	MCP-1, BPI
	MIP-1α, MFP-14, LBP, PTH-RP	CAP-18, TSG-14, VLDL, VIP
	VEGF, VIP, Leptin, Activin A	C3, C4, melatonin, APO-A1
	TREM-1, IRF-2	Protein C, Apolipoprotein E
	Annexin-1, Annexin-A5	Hemoglobin, Urocortin
	Adrenomedullin	Cortistatin
	S100A8, Fetuin A, HSF-1	Lipocalin-2, Follistatin, SFlt-1
	Pro-insulin C peptide, CGRP	
Receptors and membrane proteins	IL-1R, TNFR1, PAF-R, LECAM-1	SR-B1, PAR2, P2X(1), BKβ1,
	TREM-1, LDLr, CD11a, CD14	Caveolin-2, EPCR, Tie-2
	IFN-1R, Caveolin-1, MMP8	Adenosine A3R, VIPR, α7-nicotinic Ach receptor
Intracellular proteins	IFIT2, ZBTB20 zinc finger protein	Stat-2, Stat-4, Stat-6, IκB, HSP70
	mTORC1-S6K, Caspase-3	Hemoxygenase, A20 zinc finger protein,
	Caspase-7, Caspase-11, PTEN	SENP6, ApoA1, Miz1
	Platelet glycoprotein 1b-1X	Gilz, MCPIP1, Tak1, BKβ1
	Rab27a, Farnesyltransferase	Bcr, Abr, Platelet factor 4
	Uncoupling protein 2, DAP12	
	Transglutaminase II, eNOS, Hck	
	P38, JNK, iNOS	B-Arrestin
Transcription factors	NF-κB, BET proteins	ATF3, KLF2
Coagulation factors	Tissue factor, PAI1	TFPI, APC, Platelet factor 4
Others	PAF, PLA2	Vitamin B12, vitamin D3
	Nicotinamide, bilirubin	

[a]Each of these molecular species has been implicated in the pathogenesis of murine endotoxemia based on the ability of blockade (by genetic deletion or inhibitor) or administration (by direct administration or overexpression) to alter the lethality of systemic endotoxin challenge.

Although the biologic complexity of the innate immune response has been increasingly clarified over the past few decades, this knowledge has not led to changes in the way that clinical research is conducted. It is instructive to assess the assumptions that guide clinical research in sepsis and to explore the ways in which each of these is wanting. This paper will address specific assumptions regarding the population studied, the interventions used, and the outcomes expected, and then propose an alternate conceptual model.

Deconstructing the research model

The study population

The entry criteria for most clinical trials targeting host- or microbial-derived mediators of a systemic inflammatory response are those of sepsis syndrome [4], or a closely related constellation of clinical manifestations known as the systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, or septic shock [17]. Elizabeth Ziegler and colleagues reported in 1982 that administration of a polyclonal serum neutralizing endotoxin could reduce mortality rates in patients with Gram-negative bacterial infections [18]. Her study relied on the expert clinical judgment of experienced clinicians to delineate the population of patients who might be recruited; remarkably, this expertise proved efficacious. However, the vagaries of clinical expertise were clearly inadequate to establish an objective set of criteria to guide clinical management, and it was felt to be important that treatment be started as soon as feasible, and before culture data became available. Thus, when Bone and colleagues met to develop a clinical trial to evaluate the efficacy of high dose corticosteroids in sepsis, they recognized the need to identify a clinical syndrome that would facilitate the recruitment of patients prior to the availability of culture data. Their proposal, the product of an intense day in a hotel room in Las Vegas utterly unencumbered by data, was an entity they called 'sepsis syndrome' (Table 3) [4]. Sepsis syndrome was not a disease but a hypothesis, that the specific criteria delineated a population of patients who might benefit from a specific intervention, in this case, corticosteroids. When the hypothesis was not supported, it was unclear whether the explanation was a failure of the intervention or a failure of the diagnostic criteria.

The construct of sepsis syndrome had much to recommend it. It had face validity, it looked very much like the complex group of patients intensivists cared for, and it hinted at a common pathophysiologic basis, and thus at the prospect of one or more 'magic bullets' to treat it. This promise was all the more plausible because the biology of inflammation was little understood. TNF, IL-1, and platelet-activating factor had been identified as examples of an apparently limited number of potent proinflammatory

Table 3. Sepsis syndrome and SIRS[a]

Sepsis syndrome	SIRS
Suspected or proven infection	
Heart rate > 90 bpm	Heart rate > 90 bpm
Respiratory rate > 20/min or receiving mechanical ventilation	Respiratory rate > 20/min or $paCO_2$ < 32 mm Hg
Temperature < 35.5°C or > 38.5°C	Temperature < 36.0°C or > 38.0°C
Evidence of organ dysfunction	WBCs > 12 000 or <4000, or >10% band forms

[a]Abbreviations: SIRS, systemic inflammatory response syndrome; bpm, beats per minute; WBCs, white blood cells.

molecules, and thus were an attractive target for intervention; indeed, these were the first molecules to be targeted, and they remain among the most intensively studied. The complexity and interdependence of the network of host-derived molecules that mediated systemic inflammation became apparent only later with an accumulating body of work that showed that the targeting of any of dozens of host molecules could protect mice from lethal endotoxemia [15], and with the advent of techniques such as microarrays that revealed the extent of the transcriptomic effects of endotoxin exposure [16]. Using the criteria of sepsis syndrome to define an at-risk population generated estimates of more than 750 000 cases per year in the USA [19], making it a very attractive disorder to the pharmaceutical industry, promising substantial profits from treating a common lethal illness that currently lacks effective treatments.

However, continuing evidence of at best a modest effect across a heterogeneous group of interventions (Figure 1) strongly suggests that shortcomings of the diagnostic criteria, rather than of the specific intervention, are responsible for the apparent lack of efficacy. This is not surprising, for it is intuitively evident that the patient population that might benefit from neutralization of TNF, an early proinflammatory mediator, would differ from that which might benefit from administration of granulocyte macrophage colony-stimulating factor (GM-CSF), a growth factor for myeloid cells. It is similarly improbable that a particular intervention, for example, neutralization of endotoxin, would be equally effective across a heterogeneous group of infections from community-acquired pneumonia to catheter-related bloodstream infections, and caused by microorganisms ranging from methicillin-resistant *Staphylococcus aureus* (MRSA) to the influenza virus.

As importantly, the physiologic criteria of sepsis syndrome do not delineate a biochemically homogeneous population of patients [20]. A clinical trial of 2634 patients, stratified by a baseline level of IL-6 above or below 1000 pg/ml, found that circulating levels of IL-6 ranged from 8 to 1 550 000 pg/ml, and that levels of TNF ranged from 7 to 57 000 pg/ml [21]. The assumption that all patients in the trial would respond equally to TNF neutralization is implausible.

Finally, patients with severe sepsis manifest striking heterogeneity not only with respect to the site or microbiology of the inciting infection but also with respect to the genetic background within which the infection arises, and the comorbid conditions present in the patient at the time of onset. Because infection has been a prominent threat during human evolution, innate response genes are highly polymorphic, and genetic factors play a much more important role in the risk of premature death from infection than they do in the risk of death from cancer or heart disease [22]. Moreover, clinical criteria for sepsis syndrome may be met by a teenager with meningococcemia, but more commonly by an octogenarian with a urinary tract infection in the setting of dementia and congestive heart failure; advanced comorbidities impact not only the attributable mortality of sepsis but also the ethical framework within which care decisions are made.

The intervention

Inflammation is an adaptive process that evolved to protect the host, thus it follows that its manipulation might be harmful in certain infections. A systematic review of 480 preclinical studies of the neutralization of TNF found that although anti-TNF therapies increase survival when the experimental challenge is endotoxin, viable Gram-negative bacteria, or Gram-positive organisms such as *S. aureus*, they are ineffective in a complex model of polymicrobial infection and tissue ischemia induced by cecal ligation and puncture, and associated with increased mortality when the challenge organism is *Streptococcus pneumoniae*, *Listeria*, *Candida*, or *Mycobacterium tuberculosis* [23]. Similarly, subgroup analyses of trials of the neutralization of endotoxin have shown increased mortality when endotoxin is neutralized in patients with Gram-positive infections [24,25]. Conversely, initial studies of the efficacy of APC in sepsis syndrome suggested that the greatest efficacy was in those patients who had infection caused by *S. pneumoniae* [26], although this subgroup effect was not evident in the subsequent PROWESS Shock study that failed to demonstrate overall efficacy for APC. Both the biologic recognition systems and the evoked cellular responses vary by the nature of the infecting microorganism, and just as no single antibiotic would be expected to be efficacious across all infections that cause sepsis, it is thus implausible that targeting any single host-derived mediator molecule would be uniformly effective in all patients. How they might vary is largely speculative, for we have not incorporated analyses to test these interactions into the design of large efficacy trials, nor have we delineated plausible subgroups that might be differentially affected by a given intervention.

Optimal therapeutic efficacy further rests on the assumptions that the agent is biologically active *in vivo*, and that both the dose and duration of therapy have been optimized. These assumptions have also been found wanting. Although early studies suggested that neutralization of endotoxin with a monoclonal antibody directed against the core glycolipid of endotoxin could improve survival during Gram-negative infections [27], subsequent work failed to support this conclusion [24]; experimental studies suggested that the antibody was not able to neutralize endotoxin [28]. A monoclonal antibody against TNF that showed no clinical benefit in patients with sepsis syndrome proved incapable of neutralizing circulating TNF bioactivity in these same patients [29]. It is possible that changes in the activity of recombinant IL-receptor antagonist [30] or of APC [31] contributed to the lack of efficacy apparent in follow-up studies; however, evidence of *in vivo* activity was not explicitly sought in these trials.

Evaluation of the pharmacokinetics and pharmacodynamics of a new intervention has been minimal. Initial dosing is typically inferred on the basis of results in animal models, and the final dose selected by an optimistic interpretation of mortality data from a Phase II trial. In the absence of specific markers to guide the duration of therapy, an arbitrary duration is selected. A Phase III clinical trial of N^G-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of inducible nitric oxide synthase, hinted at the limitations of this approach. Dosing was titrated to

mean arterial pressure, and the overall result showed significantly increased mortality in treated patients, but enhanced survival in the subgroup of patients who received the lowest doses of the agent [32].

The outcome

Sepsis is a lethal condition. Even in the face of improved global management of patients with sepsis, mortality rates of 20% to 25% are commonly seen in sepsis trials, and even higher rates are reported in unselected case series. It follows, therefore, that the primary measure of efficacy for a novel therapy must be evidence that the agent can increase survival in treated patients. A mortality endpoint, however, presents three significant challenges in trial design: (i) its interpretation depends on the time horizon over which mortality is measured; (ii) it is an insensitive measure of biologic activity, and thus a poor tool to use in early phase clinical research to optimize selection of the study population or the dose or duration of therapy; and (iii) it provides no insight regarding clinical efficacy in attenuating nonmortal morbid events that may be of substantial importance to the patient who survives an episode of sepsis.

Regulatory agencies have considered 28 day all-cause mortality to be the optimal measure of clinical effectiveness in sepsis trials. This somewhat arbitrary time frame arose following early studies with an antiendotoxin antibody that showed greater efficacy at 28 days than at 14 days [33]. More recently, there is evidence that a 28 day time horizon may not be long enough to detect a differential treatment effect in a complex patient population for whom death is usually the result of a deliberate decision by the family and the attending team to discontinue supportive measures. A study of the effects of targeting normoglycemia in more than 6000 critically ill patients, for example, showed no evidence of a differential treatment effect at 28 days, but a significant difference at 90 days that favored less rigorous control of plasma glucose levels [34]. Although a longer time horizon may favor a more stable mortality estimate, it does increase the complexity of patient follow-up and may increase random noise as patients succumb to unrelated comorbidities.

For reasons articulated above, there is a compelling need for early phase sepsis trials to focus on identifying an optimal study population and an optimal dose and duration of therapy, the latter, ideally, guided by a readily measured biomarker. Although measures of biochemical or physiologic activity are poor surrogates for clinical efficacy, the corollary is also true; in the absence of studies of many tens of thousands of patients with clearly articulated *a priori* subgroup analyses, mortality is an insensitive tool for optimizing trial design. For example, a trial of anti-TNF therapy in severe sepsis tested the hypothesis that patients with hyperinflammatory sepsis (reflected in an elevated IL-6 level) would benefit from anti-TNF therapy. A rapid assay to detect IL-6 levels greater than 1000 pg/ml was developed based on an analysis of just over 100 patients in a Phase II trial [35]. The study showed an equivocal effect on mortality when the cut-off value was 1000 pg/l, but a much larger and sustained effect when levels were more than 7000 pg/l [21,36]. Subgroup analyses commonly suggest differential efficacy in different study populations; although they are frowned on in the context of Phase III trials, they are potentially important elements of adequately powered Phase II studies undertaken to refine study entry criteria, particularly when conducted based on *a priori* hypotheses deriving from preclinical or observational studies.

Finally, as the mortality of sepsis continues to decline, the focus is increasingly shifted to long-term morbidity in sepsis survivors. Natural history studies show that an increased mortality risk is sustained for years after an index episode of sepsis [37], and that survivors experience a substantially diminished long-term quality of life [38]. A study of recombinant bactericidal permeability increasing protein (rBPI) in pediatric meningococcemia failed to show a significant mortality reduction from a baseline mortality rate of less than 10%, but did find that survivors experienced fewer major morbid events such as amputations [39]; the absence of a mortality benefit precluded approval of rBPI as a therapy for pediatric meningococcemia. As mortality declines, the need to attenuate morbidity in those patients who survive the episode becomes more compelling.

Multiple other factors are recognized to contribute to unmeasured heterogeneity in the populations of patients recruited into sepsis trials. Mortality risk varies with such factors as the adequacy of initial resuscitation [40], the location of the patient at the time of initial diagnosis [41], and the time between onset of illness and presentation to the hospital. As peak levels of many of the most widely studied inflammatory mediators occur early in the course of illness [42], delayed presentation may preclude the possibility of clinical benefit.

Defining the challenge

The challenge of reorienting our approach to the study of modulating the host response in critical illness is a daunting one. It requires a fundamental reassessment of the assumptions of the concept of sepsis, building on contemporary understanding of biology and the complexity of innate immunity and adaptive immunity. It also requires a critical reconsideration of the prevailing research paradigm, from the role of preclinical models to the sources and impact of heterogeneity in clinical populations and, based on this, a reengineered approach to the evaluation of preclinical models, disease epidemiology, and clinical trial design and conduct. Even beyond this, it requires a commitment to new models of collaborative research that can accommodate the stark reality that it will take time to generate stable insight and that the payoff for doing so will be, at least at first, modest.

Limitations of the concept of sepsis

The word 'sepsis' is attributed to Hippocrates, who held that there are two fundamental processes of tissue breakdown: (i) pepsis; a life-sustaining process exemplified by the digestion of food or the fermentation of grapes to produce wine; and (ii) sepsis; a process associated with putrefaction and exemplified by foul-smelling vapors and the process of rot [43]. These concepts preceded the identification of microorganisms by more than two millennia;

however, the word 'sepsis' came to be synonymous with the process of infection in the twentieth century, and sepsis or septicemia indicated a severe disease resulting from disseminated infection and is associated with circulating microorganisms in the bloodstream. Work over the past three decades has revealed that the clinical syndrome is a product of the host response rather than a direct effect of bacterial invasion, and that the response is both variable across differing infectious agents and evoked by noninfectious as well as infectious threats.

Much time, effort, and debate has gone into establishing a contemporary operational definition of sepsis [17,44]. The current iteration, a clinical syndrome reflecting the maladaptive aspects of the host response to infection, is useful in that it emphasizes the importance of seeking undiagnosed infection as a cause of otherwise unexplained clinical deterioration. However, it has proven itself inadequate in defining discrete populations of patients who might benefit from an intervention that targets a particular element of that response. Conversely, an innate immune response can be triggered by noninfectious as well as infectious stimuli [45,46], and many patients who might benefit from a particular therapy are systematically excluded from trials because they are not infected.

We have made assumptions about who might best benefit from interventions such as the neutralization of TNF based on studies from three decades ago that showed benefit in animal models of Gram-negative infection [10]. Yet, clinical trials have shown that TNF neutralization is most effective in noninfectious disorders such as rheumatoid arthritis [47] and inflammatory bowel disease, and that infection can be a side effect of such therapy [48]. The construct of sepsis as currently defined is both overly nonspecific to identify patients for recruitment to clinical trials and too restrictive to encompass all acutely ill patients who might benefit from an intervention that targeted the innate host response. Moreover, the patient with active infection faces the greatest potential for the adverse effects of an intervention that modulates innate immunity. The solution is not to yet again refine a global definition but to recognize that just as there is no single disease called cancer, the concept of sepsis embraces a host of disorders. Our challenge in developing effective adjuvant therapies is to better characterize this heterogeneity, and thus to determine discrete patient populations who might benefit from an equally heterogeneous group of interventions.

Interpretation of preclinical studies

Clinical trials of novel biologic agents typically proceed on the basis of a limited portfolio of preclinical data showing efficacy in simple rodent models such as endotoxemia or cecal ligation and puncture. These bear little relation to human disease. Whereas human sepsis is characteristically a disorder of elderly patients with significant comorbidities, the models employ healthy young animals. Human illness is aggressively treated and supported by intensive care unit technologies, whereas animal sepsis receives little adjuvant support. In human sepsis, treatment is started at a variable time after the onset of illness; in animal models, it is typically started coincident with or even before the infectious challenge. Finally, the transcriptomic changes of murine sepsis bear little resemblance to those of human illness [49].

Preclinical research has an important role to play in the development of novel therapeutics. This role, however, is less the prediction of clinical efficacy than the delineation of a plausible biologic target and a demonstration of the consequences of manipulating that target. In developing a research program, it is understandably attractive to focus on the models that show evidence of efficacy. In practice, however, once the possibility of efficacy has been established, a much more important role for preclinical studies is to delineate those situations where intervention may produce no effect or even harm. A systematic review of preclinical studies of TNF neutralization in experimental sepsis, for example, suggests that anti-TNF therapies are most efficacious in models of systemic endotoxemia or Gram-negative infection, ineffective in models of complex polymicrobial infections such as cecal ligation and puncture, and harmful when the challenge organism is *S. pneumoniae*, or an opportunistic pathogen such as *Candida*, *Listeria*, or *M. tuberculosis* (Figure 2).

Understanding heterogeneity

Heterogeneity in sepsis arises at multiple levels. The inciting infection is heterogeneous with respect to its microbiology, site, and timeliness of diagnosis. Patients are heterogeneous with respect to their underlying genetic makeup, their comorbidities, and their preferences for care. The treating system is heterogeneous with response to the knowledge, treatment practices, and available resources of the treating team and their capacity to provide differing modalities of advanced care. Each of these factors can independently impact the response to a particular therapeutic intervention.

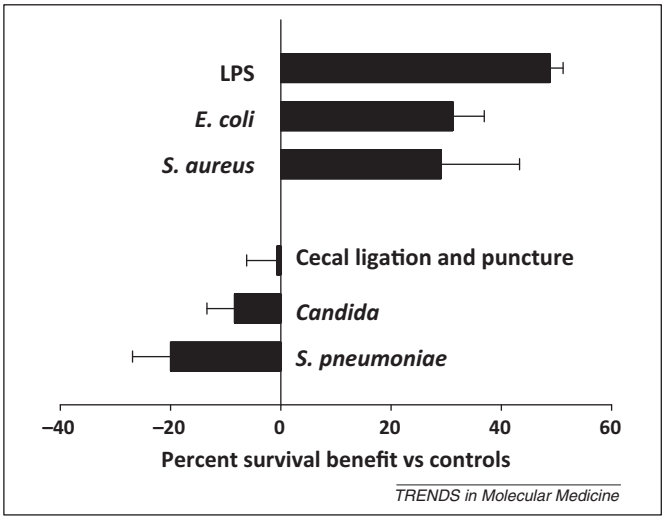

Figure 2. The consequences of neutralization of TNF in preclinical models of sepsis. Data are from a systematic review of 480 studies of the neutralization of TNF in a variety of *in vivo* models involving eight different animal species. Data are presented as increase in percent survival when compared with untreated controls. TNF neutralization was beneficial if the challenge was LPS, *Escherichia coli*, or *Staphylococcus aureus*, inefficacious in a complex model of intra-abdominal infection (cecal ligation and puncture), and harmful when the challenge organism was *Candida* or *Streptococcus pneumoniae*. Adapted from [23]. Abbreviations: TNF, tumor necrosis factor; LPS, lipopolysaccharide.

Heterogeneity has two consequences. First, because the potential to benefit from therapy differs between patients, inclusion of patients who receive little or no benefit from the intervention dilutes the therapeutic signal, and thus reduces the differential benefit. A much larger sample size is needed to show efficacy. More importantly, however, whereas some patients benefit from the intervention, others are actively harmed. Both preclinical [23] and clinical [25,32] studies suggest that this is an important concern in sepsis studies.

Rethinking the research model

The shortcomings of the prevalent approach to the identification, development, and testing of novel therapies of sepsis are many. How to address them is less clear; elements of a possible approach are articulated here.

Defining the epidemiology of sepsis

Successful therapy of coronary artery disease has been guided by a detailed understanding of natural history and risk factors for adverse outcome. Much of this information has come from the Framingham study that, since 1948, has followed an inception cohort of more than 5000 inhabitants of the city of Framingham Massachusetts [50]. A comparable body of epidemiologic data on the clinical course of acute illness does not exist. Although there are many studies that have provided descriptive data regarding the clinical characteristics and prognosis of patients who meet arbitrary criteria for sepsis or SIRS, what is needed is an unbiased natural history study of a much more inclusive population of acutely ill patients that seeks to identify distinct clinical patterns of response and that incorporates a comprehensive study of genetic polymorphisms and plausible biochemical markers of inflammation evaluated over time. By delineating the clinical course of patients with persistent elevation of TNF versus that of patients with impaired expression of human leukocyte antigen (HLA)-DR, for example, such a study could define clinical phenotypes and prognosis that can better inform patient stratification. A further focus on patients with an extreme phenotype, survival in a patient predicted to die, or death in a patient predicted to survive, can aid in defining important biologic determinants of outcome.

Such a study will of necessity be large, complex, and very expensive. Nonetheless, it could provide the data necessary to create a biochemical or pathologic definition of sepsis, rather than a definition grounded in physiology and imperfect consensus.

Identifying plausible targets for intervention

In the past, potential therapeutic targets have been identified on the basis of current biologic knowledge and the availability of interventions that can alter outcome in preclinical models. We now understand that the list of possible targets is enormous and, as a corollary, that the chances that targeting any one of these will result in a dramatic improvement in survival is small. As a consequence, there is a need to refocus efforts on the most plausible treatment strategies, using both the available body of clinical and preclinical evidence and a systematic approach to integrating expert opinion.

Secondary analyses of completed sepsis trials are potentially a valuable source of insight into differential clinical efficacy on the one hand, and problems of dosing or clinical activity on the other. Although most of the data is currently held by pharmaceutical companies who are often reluctant to release the data because of intellectual property concerns, the lack of a viable commercial product and the potential for important insights to guide future research may well go a long way to alleviating these concerns. The development of an independent professional collaboration to define analyses and to oversee the process would facilitate such an effort.

Systematic reviews of preclinical work can also provide useful insight into differential efficacy (Figure 2). Whether these translate into differential effectiveness in human populations is unknown, but this is a hypothesis that could be tested using available clinical trial data.

Beyond a critical analysis of existing data, there is a need to focus future activities on targets that have the greatest prospect of yielding clinical benefit. Criteria to be considered here include the target itself and the ability to measure its presence and activity, the preclinical and clinical studies of efficacy and potential harm, the availability and potential cost of interventions, and the aspect of a systemic inflammatory response that is targeted. It would seem intuitively attractive, for example, to target a cellular process such as activation of a transcription that regulates the expression of multiple genes rather than a single gene. Similarly, the volume of information on the effects of neutralizing TNF both in sepsis and in chronic inflammatory diseases makes this an attractive agent for future work.

Improving patient stratification and staging

Adjuvant therapy in oncology only became a realistic option with the development of clinical and pathologic stratification systems that facilitate targeting therapy to those patients most likely to benefit. For example, combination chemotherapy with doxorubicin, cyclophosphamide, and paclitaxel improves disease-free survival for women with breast cancer that has metastasized to regional lymph nodes [51]. These potent agents have significant side effects. They are not given to all patients with a diagnosis of cancer, nor even to all patients with a diagnosis of breast cancer, but rather to a subset of these patients who have breast cancer with histologically documented metastases to regional lymph nodes. In the absence of nodal spread, the side effects outweigh the potential benefits, whereas in the presence of distant metastases, the total burden of illness makes a meaningful response unlikely. The TNM (Tumor, Nodes, Metastases) staging system facilitates the study and use of adjuvant therapy in cancer. Cancer staging has evolved over more than 60 years, and now includes the use of specific tumor markers to further customize therapy. Women with breast cancer that expresses the Her2/Neu receptor additionally benefit from the use of a specific receptor antagonist, herceptin. Intriguingly, Her2 expression also predicts an increased likelihood of benefit from paclitaxel [51].

We currently do not have comparable stratification systems for acute illness that could facilitate the evaluation of adjuvant therapies. The PIRO (Predisposition,

Insult, Response, Organ Dysfunction) model has been proposed as a candidate approach [52]; however, it exists as a concept only. The development of staging systems in oncology has taken more than 60 years and has relied on massive international collaborative efforts, informed by the type of epidemiologic data discussed above. A similar effort will be needed to develop useful models for acute illness.

Rethinking early phase clinical studies

Phase III registration studies for novel sepsis therapies have typically been informed by small Phase II trials whose primary role was to show the possibility of clinical efficacy. These trials have used conventional criteria to identify study participants and have inferred the possibility of efficacy on a statistically nonsignificant trend towards mortality benefit. Having seen this trend, sponsors have been reticent to alter the study design, and thus the definitive trial has been conducted using a similar design. This approach has been uniformly ineffective.

An alternate approach is to plan multiple, potentially overlapping early phase studies to inform the optimal design of a Phase III trial. An early phase research program for an anti-TNF therapy, for example, might include a cohort study to determine the prevalence and risk factors for increased levels of TNF or IL-6 as well as the time course over which levels remained elevated, followed by a dose-finding study to determine an optimal dose and duration of therapy, based on the response of a biomarker. Having accomplished this, a pilot study might be undertaken to determine whether administration of the therapy differentially impacted biomarker levels or measures of organ dysfunction when patients are stratified by variables that plausibly impact response to treatment such as the presence and nature of infection or the presence of specific genetic polymorphisms. Salvage studies in which an agent is administered only to patients who are refractory to conventional therapy, or adaptive designs in which patients are differentially recruited to the better performing study arm are other models that might be profitably employed in early phase sepsis research.

Creating new research models

The common theme that underlies a new approach to sepsis research is that the task of developing new treatments is enormous, and that success will be slow and costly, even if the ultimate promise is substantial. This is not a state of affairs that is attractive to the pharmaceutical industry, whose priorities are driven by financial considerations such as the size of the ultimate need and the duration of patent protection. By contrast, it is an ideal opportunity for large, investigator-led research consortia, which can mobilize international collaborations, given the financial support. Large-scale collaboration has given us the successes of the Large Hadron Collider and the Human Genome Project. Learning how to effectively modulate the host response in critical illness will almost certainly require the same type of dedication.

Academic research groups have played a pivotally important role in advancing knowledge about the treatment of cancer and heart disease. Investigator-led research groups in critical care have arisen in every continent, and are not only generating information about the optimal management of critically ill patients but also demonstrating the feasibility of large-scale international academic collaboration [53]. Working in collaboration with industry and public funders, they could be a powerful tool for advancing sepsis research.

Concluding remarks

Defined as a clinical syndrome resulting from the adverse consequences of the host response to infection, sepsis is arguably the leading cause of preventable death in the world today; four of the World Health Organization's top ten causes of global mortality result from sepsis. It can be conservatively estimated that upwards of $10 billion has been spent with the objective of developing effective adjuvant treatments to meet this unmet need. Yet, with the failure of a recent confirmatory trial of APC [31], no such treatments are currently available, and the prospect that this will change seems remote.

There is a compelling need to revisit our approach to sepsis research, an exercise which will entail a fundamental reconsideration of assumptions, biology, trial design, and research collaboration. As industry abandons the field because of the vanishingly small prospect for success, it is incumbent upon academic investigators and their research consortia to assume the leadership role in launching such an initiative.

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