SUCH STUFF AS DREAMS ARE MADE ON: MEDIATOR-DIRECTED THERAPY IN SEPSIS

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Sepsis, a life-threatening disorder that arises through the body's response to infection, is the leading cause of death and disability for patients in an intensive care unit. Advances in the understanding of the complex biological processes responsible for the clinical syndrome have led to the identification of many promising new therapeutic targets, including bacterial toxins, host-derived mediators, and downstream processes such as coagulation and the endocrine response. Diverse therapies directed against these targets have shown dramatic effects in animal models; however, in humans, their impact has been frustratingly modest, and only one agent — recombinant activated protein C — has achieved regulatory approval. This review summarizes the approaches that have been evaluated in clinical trials, explores the reasons for the discordance between biological promise and clinical reality, and points to approaches that may lead to greater success in the future.

MENINGOCOCCAEMIA
An uncommon, but fulminant, infection with substantial mortality and morbidity in the form of tissue necrosis leading to the loss of digits and even limbs.

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Prospero, the ageing Shakespeare's alter ego, musing on the magic of art and the transience of brave ideas declared:

> …These our actors,
> As I foretold you, were all spirits and
> Are melted into air, into thin air:
> And, like the baseless fabric of this vision,
> The cloud-capp'd towers, the gorgeous palaces,
> The solemn temples, the great globe itself,
> Ye all which it inherit, shall dissolve
> And, like this insubstantial pageant faded,
> Leave not a rack behind. We are such stuff
> As dreams are made on…

The history of clinical research into novel therapies that target the host inflammatory response in sepsis might equally be dismissed as an illusory endeavour, the persistent exercise of naive hope over pragmatism[1,2]. Several billions of dollars have been expended in pursuit of the hypothesis that the survival of critically ill patients can be improved by targeting the endogenous host response to infection. Research has been fuelled by the potential for enormous financial returns in the treatment of a disease process that generates estimated costs of more than US $16 billion annually in the United States, and by the promise of averting some of the more than 200,000 deaths it causes per year[3]. Yet the translation of this possibility into clinical reality has been, at best, modest. During the past two decades, only one novel drug has achieved regulatory approval, and a second has found a new therapeutic indication. But such an interpretation ignores the complexity of the challenge, the small but persistent therapeutic signal that is driving attempts to understand that complexity, and the enormity of the human problem that might be overcome once the challenge has been successfully addressed.

Sepsis — generically defined as the response of the host to microbial infection — encompasses a broad spectrum of processes, ranging from the response to rhinoviral infection, which is mild, self-limited and beneficial, to that of overwhelming MENINGOCOCCAEMIA which is fulminant, progressive and lethal. In clinical parlance, the word 'sepsis' is used to indicate what has also been called 'severe sepsis' (sepsis resulting in physiological organ dysfunction) or 'septic shock' (sepsis

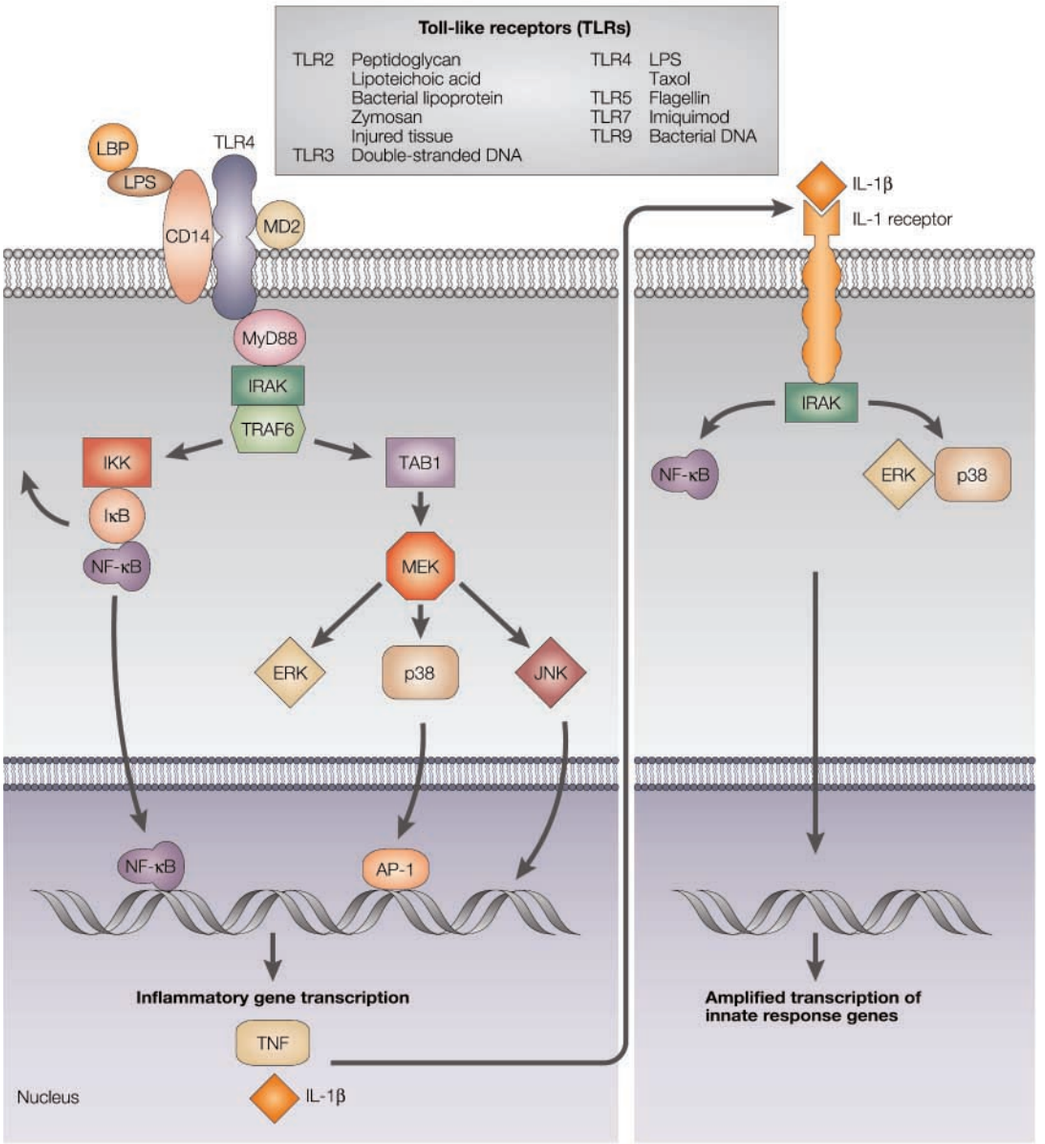

Figure 1 | **Activation of an innate immune response to invading microorganisms.** Engagement of a Toll-like receptor (TLR) by one of its ligands triggers an intracellular signalling cascade that includes activation of the latent cytoplasmic transcription factor NF-κB with its translocation to the nucleus, and activation of the mitogen-activated protein (MAP) kinase pathway. The coordinated action of these intracellular proteins results in the transcription of pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF). IL-1, in turn, engages its receptor, which shows substantial homology to receptors of the TLR family, and leads to further transcription of genes that serve to amplify, modulate and antagonize previously expressed inflammatory genes. AP-1, activated protein 1; CD14, endotoxin receptor; ERK, extracellular signal-regulated kinase; IKK, IκB kinase; IRAK, IL-1 receptor-associated kinase; IκB, inhibitor of κB; JNK, Jun N-terminal kinase; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; MEK, MAP kinase kinase; MyD88, myeloid differentiation protein 88; TAB1, transforming growth factor-β activated kinase 1 binding protein; TRAF6, TNF receptor-associated factor 6.

from bacterial flagellae, whereas TLR9 recognizes and binds to conserved motifs in bacterial DNA. The ligands for TLRs are incompletely identified, and are not limited to microorganisms, but rather to stimuli that might be perceived as danger signals by the host, including heat-shock proteins and injured tissue, the latter being recognized through TLR2. So, the initiation of a septic response does not depend on the presence of viable microorganisms, but rather on the presence of specific patterns that identify a complex molecule as a potential threat to the host. It follows that strategies that can modulate this response will be useful not only in sepsis (arbitrarily delimited by demonstration of infection), but also in acute diseases in which an innate immune response has been triggered by endotoxin, other bacterial products or tissue trauma.

The engagement of TLRs on macrophages, neutrophils and endothelial cells activates multiple signal-transduction cascades that lead to new gene expression, in particular gene expression mediated through nuclear factor κB (NF-κB), a transcription factor that is centrally involved in pro-inflammatory cytokine gene expression (FIG. 1). As complicated as this process might seem when illustrated schematically, it oversimplifies the enormously complex interactions that occur *in vivo*. A single stimulus, such as endotoxin binding to a single cellular receptor, activates multiple intracellular signalling cascades, and leads immediately to the *de novo* expression of hundreds of new genes. But each of these, in turn, can act on the cell in an autocrine fashion, on neighbouring cells in a paracrine manner, or on distant tissues as an endocrine mediator, evoking a new signalling cascade and repressing or activating new patterns of gene expression.

Most of what is known about the innate immune response derives from studies carried out within the first few hours of cell stimulation, when the predominant cellular products include such classic pro-inflammatory cytokines as tumour necrosis factor (TNF) and interleukin-1 (IL-1). But other inflammatory proteins whose importance could well equal that of TNF and IL-1, including, for example, high-mobility group box-1 (HMGB-1)[9], are not expressed for many hours. Gene expression is profoundly altered in multiple tissues, and the interactions of individual mediators are better understood through complexity theory than through a conventional endocrine model in which a single protein evokes a reproducible and constrained series of effects. The ultimate mechanisms responsible for lethality in the host are unknown, but might stem from the ability of this process to induce microvascular thrombosis and anoxic cell death, alterations in regional blood flow and cellular nutrient uptake leading to impaired and altered cellular function[10], and activation of apoptosis, leading to the programmed cell death of vital tissues[11,12].

Therapeutic promise for targeting any of these hundreds of mediators arises from work in animal models of infection or endotoxaemia in which neutralization of the mediator of interest successfully prevents the lethality of the experimental challenge. Early work that established the pivotal role of TNF as a mediator of sepsis, for example, showed that the overwhelming lethality of endotoxaemia in the mouse[13], or that caused by the infusion of live *Escherichia coli*[14] or *S. aureus*[15] in the baboon, could be completely prevented by pre-treatment with a neutralizing antibody directed against TNF. However, TNF is only one of several dozen endogenous mediators whose neutralization can prevent the lethality of experimental endotoxaemia (TABLE 1), and the benefit of most is only apparent when treatment is given prior to experimental challenge.

This divergence between experimental model and clinical reality underlines two aspects of sepsis whose importance has been under-appreciated. First, the innate host response is complex and redundant, and might respond only incompletely to therapy directed against individual mediators. Second, the timing of intervention is crucial to the efficacy of therapy. Moreover, as investigators seek to translate insights from biological mechanisms into new clinical therapies, it has proven difficult to determine who might benefit from therapy, how the therapy should be instituted and titrated, and how the success of therapy is best measured. We will return to a further consideration of these issues after first reviewing the scope of therapeutic strategies that have been studied.

If sepsis is defined conceptually as the clinical syndrome that results from the host response to invasive infection, then potential sepsis therapies can be categorized as those that target the inciting infection, those that are directed against the response of the host and those that treat the clinical sequelae of this process.

Targeting microorganisms or their products

Systemic antibiotics directed against the infecting organism, and surgical intervention to achieve source control, represent the mainstay of anti-infective measures in the treatment of sepsis, although neither has been evaluated by a randomized placebo-controlled trial, and the attributable effect of each on outcome is unknown. Agents that target microbial toxins are a relatively recent innovation.

Endotoxin (lipopolysaccharide). The interaction of endotoxin with the mammalian host is an intricate one, and is more typical of a hormone than of a toxin (FIG. 1). Endotoxin is carried in the blood by a specific carrier protein, lipopolysaccharide-binding protein (LBP), and interacts with cells of the innate immune system through a distinct receptor, CD14. The endotoxin–CD14 complex then engages a specific receptor, TLR4, to initiate intracellular signalling and the transcription of hundreds of genes[16,17]. The symptoms and biochemical manifestations of a systemic inflammatory response can be evoked in healthy human volunteers by the injection of nanogram quantities of endotoxin[18], whereas larger quantities can produce shock and life-threatening organ dysfunction[19]. Endotoxin exposure can occur during infection with Gram-negative bacteria. In addition, the indigenous flora of the gastrointestinal tract is an endogenous store of endotoxin, estimated to be as much as several grams, and endotoxaemia of gut origin can occur when gut perfusion or barrier function is altered, for example, during the repair of an abdominal aortic aneurysm[20] or during cardiopulmonary bypass[21].

Natural antibodies to endotoxin are known to be present in the blood, and to confer a measure of protection during periods of exposure to endotoxin[21]. Ziegler and colleagues demonstrated that a polyclonal antiserum raised by immunizing healthy human volunteers with a mutant form of endotoxin that was lacking the *O* side chain could reduce mortality when given to patients with Gram-negative bacteraemia; the treatment effect was most striking in patients with septic shock[22]. The prophylactic value of this same antiserum was evaluated in a population of 252 surgical patients at high risk of

Box 1 | Clinical trials of therapies targeting mediators of sepsis

Included are randomized, controlled and blinded clinical trials of novel strategies directed against the host inflammatory response in populations of critically ill patients with sepsis. Studies are considered to show evidence of harm or benefit if, in a single study, there is a statistically significant difference in rates of a pre-defined, clinically important endpoint (mortality unless otherwise specified) in the overall study population, or a pre-specified subgroup; therapies whose efficacy has resulted in the availability of the agent for clinical use are noted. For most approaches, limitations of study design — primarily lack of power and excessive heterogeneity of the study population — render the study results inconclusive, rather than definitively negative.

Harm

- Soluble tumour necrosis factor (TNF) receptor p75 (REF. 47)
- Inhibition of nitric oxide with L-*N*-monomethyl arginine (L-NMMA)[161]

Benefit

- Neutralization of endotoxin with polyvalent anti-serum or monoclonal antibody HA-1A[22,24]
- Neutralization of endotoxin with recombinant bactericidal permeability increasing protein (reduced morbidity)[29]
- Neutralization of TNF by monoclonal antibody[56]
- Acceleration of removal of platelet-activating factor (PAF) with recombinant PAF acetylhydrolase
- Administration of recombinant human activated protein C[4] (clinical use)
- Administration of pharmacological doses of corticosteroids (clinical use)

Indeterminate or no effect

- Neutralization of endotoxin by monoclonal antibody, antibody to enterobacterial common antigen, or polymyxin B
- Inhibition of TNF by monoclonal antibody or soluble receptor p55
- Inhibition of interleukin-1 (IL-1) with recombinant IL-1 receptor antagonist
- Inhibition of PAF with a receptor antagonist
- Inhibition of nitric oxide with L-NMMA or methylene blue
- Inhibition of bradykinin
- Inhibition of prostaglandins with ibuprofen
- Nonspecific suppression of inflammation with high dose corticosteroids or pentoxifylline
- Administration of granulocyte colony-stimulating factor (G-CSF) or interferon-γ
- Inhibition of coagulation with anti-thrombin III or tissue factor pathway inhibitor

resulting in refractory haemodynamic instability). Severe sepsis and septic shock are common in the industrialized world. There are estimated to be ~750,000 new cases each year in the United States; with a crude mortality at 28 days of 30%, annual mortality from sepsis rivals that of myocardial infarction, and exceeds that of almost all common cancers[3]. However, sepsis commonly arises as a complication of other serious disease processes or their treatment, and so its presence as a modifiable disease process is under-appreciated, and its attributable mortality is unknown.

Sepsis is a clinical syndrome whose pathophysiology reflects the activation of an innate host response to infection. The apparent simplicity of this definition belies a complex process whose rational therapy is as frustratingly elusive as it is biologically compelling. More than 70 well-designed randomized clinical trials have been performed to test the hypothesis that manipulation of one of the more than 200 putative mediators of systemic inflammation can improve the survival of critically ill patients with sepsis. One new therapy has made it to market[4], and a second agent, hydrocortisone, has found a new clinical indication[5]. But dozens of other strategies have yielded results that can variously be interpreted as evidence of harm, lack of efficacy or limited benefit, or, more frequently, inconclusive evidence of utility (BOX 1).

The challenges of modulating the host inflammatory response in sepsis are as numerous and diverse as the potential targets for intervention. It is timely, therefore, to review the successes and failures of the enterprise to date, and the implications these have for its future directions.

What is the biological rationale?

Microorganisms can produce disease in one of three ways. The uncontrolled proliferation of organisms can, by competing for energy substrate or using host tissues as that substrate, exhaust host reserves and jeopardize normal physiological function. Such a mechanism of disease is not only rare, but also impractical from an evolutionary perspective, for destruction of the host deprives the parasite of its source of sustenance. Microorganisms can also kill through the expression of toxins that interfere with fundamental cellular functions (for example, the toxins of diphtheria or *Clostridium botulinum*), or that promote the spread of microorganisms through host tissues and the neutralization of intrinsic host defences (for example, the exotoxins of *Streptococci* or *Clostridia*, or the production of coagulase by *Staphylococcus aureus*). With advances in surgery and the availability of antibiotics, this second mechanism of bacterial pathogenesis, which is characteristic of infection with exogenous organisms, has been supplanted in importance by a third mechanism — the induction of illness through the systemic activation of innate immune defences, or sepsis, by bacterial products. Endotoxin or lipopolysaccharide from the cell wall of Gram-negative bacteria is the best studied of these bacterial triggers.

Building on early work by Michalek and colleagues[6], the discovery in 1998 that the host response to endotoxin is mediated by a receptor known as Toll-like receptor 4 (TLR4)[7] opened the door to a new understanding of innate immunity, and, in turn, to the potential for a new sophistication in the development of therapies to target sepsis (FIG. 1). TLRs represent the interface between the microbial and mammalian worlds, where a molecule is recognized as foreign to the host, and a protective immune response is activated. The family of ten TLRs serves as the innate immune system's equivalent of the antibody or T-cell receptor, binding to stereotypical and highly conserved biochemical structures that identify a molecule as foreign and potentially threatening, and activating the complex biochemical cascades of inflammation[8]. As mentioned, TLR4 binds endotoxin from the cell wall of Gram-negative bacteria, whereas TLR2 recognizes, among other molecules, peptidoglycan from Gram-positive bacteria and bacterial lipoprotein from Gram-negative bacteria. TLR5 binds flagellin

Table 1 | **Endogenous molecules whose manipulation alters survival in murine endotoxaemia**

	Activity increases lethality	Activity decreases lethality
Cytokines and other extracellular proteins	IL-1, IL-12, IL-18, TNF, IFN-γ, TGF-β, MIF, MIP-1α, HMGB-1, MFP-14, LBP, PTHrP, tissue factor	IL-1ra, IL-4, IL-10, IL-13, IVIG, IFN-α, HGF, LIF, CRP, KBP, MCP-1, BPI, LIF, CAP-18, LL-37, TSG-14, ApoE, VLDL, complement components C3 and C4, melatonin, VIP, PACAP
Cell-surface receptors	IL-1 receptor, TNF receptor, PAF receptor, LDL receptor, CD11a, CD18, LECAM-1, TREM-1	Macrophage FcγR, VIP receptor, adenosine A3 receptor
Signal-transduction molecules and other intracellular proteins	Hck, COX2, inducible NOS, caspase-3	Stat-4, Stat-6, I6B, haemoxygenase, HSP70
Miscellaneous	PAF	Vitamin B_{12}, vitamin D_3, oxidized phospholipids

ApoE, apolipoprotein E; BPI, bactericidal permeability-increasing protein; CAP, cationic antimicrobial peptide; CD14, endotoxin receptor; COX, cyclooxygenase; CRP, C-reactive protein; HGF, hepatocyte growth factor; HMGB-1, high-mobility group box-1; HSP, heat shock protein; IFN, interferon; IL, interleukin; IL-1ra, IL-1 receptor antagonist; IVIG, intravenous immunoglobulin; KBP, Kallikrein-binding protein; LBP, lipopolysaccharide-binding protein; LDL, low-density lipoprotein; LECAM-1, leukocyte endothelial cell adhesion molecule 1; LIF, leukaemia inhibitory factor; LL-37, cathelicidin; MCP, monocyte chemoattractant protein; MFP, multi-functional protein; MIF, macrophage inhibitory factor; MIP, macrophage inflammatory protein; NOS, nitric oxide synthase; PACAP, pituitary adenylate cyclase activation polypeptide; PAF, platelet-activating factor; PTHrP, parathyroid hormone related protein; TGF-β, transforming growth factor β; TNF, tumour necrosis factor; TREM, triggering receptor expressed on myeloid cells; TSG, tumour necrosis factor stimulated gene; VIP, vasoactive intestinal peptide; VLDL, very-low-density lipoprotein.

Gram-negative infection; there was a trend towards a reduction in mortality (odds ratio (OR) = 4.4, 95% confidence interval (CI) 0.9–20.8) and subsequent shock (OR = 2.5, 95% CI 0.9–6.6) in treated patients[23]. Two different monoclonal antibodies against endotoxin — HA-1A, a human antibody, and E5, a murine antibody — were subsequently evaluated in multicentre clinical trials. Pooled data from six published studies revealed a 28-day mortality of 35.5% in the placebo group and 33.6% in patients with Gram-negative infections who received the antibody (OR = 0.92, 95% CI 0.79–1.07, $p = 0.28$) (FIG. 2). The mortality reduction in one of these trials achieved statistical significance[24]; in the largest of these studies[25], however, there was a trend towards *increased* mortality for patients without Gram-negative bacteraemia who received anti-endotoxin antibody (40.5 versus 36.8%, $p = 0.073$). A recently reported study of T88, an antibody to the enterobacterial common antigen, a surface glycophospholipid shared by members of the family Enterobacteriaceae, showed no evidence of benefit, but rather an increased rate of adverse respiratory events in treated patients[26].

Other strategies to neutralize endotoxin have been evaluated. Bactericidal permeability-increasing protein (BPI) is a 55-kDa neutrophil product that binds endotoxin and prevents it from activating host cells[27]. BPI levels are increased in patients with sepsis resulting from Gram-negative infection[28]. A 21-kDa peptide based on the lipopolysaccharide-binding domain of BPI, rBPI21, has been evaluated in trauma, in patients undergoing major liver resection and in children with meningococcaemia. In a Phase III study of 393 children with meningococcaemia, rBPI21 treatment was associated with a modest reduction in mortality from 9.9 to 7.4%, and an improved functional outcome 60 days following treatment[29]. Another neutrophil granule protein, cationic antimicrobial protein 18 kDa (CAP 18), binds endotoxin and has shown promising biological activity in preclinical studies[30]. The amino-acid derivative taurolidine, which is known to exert anti-endotoxin activity *in vitro*, has been evaluated in a single study, but showed no evidence of clinical benefit and no convincing evidence of biological activity *in vivo*[31].

Other strategies to neutralize endotoxin include the administration of high-density lipoprotein[32] or a lipid compound to bind circulating endotoxin, blockade of its interaction with TLR4 using soluble CD14 or a synthetic lipid molecule that serves as a competitive antagonist[33], or acceleration of its degradation by alkaline phosphatase[34]. The antibiotic polymyxin B binds and chelates endotoxin. A polymyxin-B–dextran conjugate failed to show evidence of efficacy in an unpublished Phase II trial; studies of the extracorporeal removal of endotoxin using polymyxin B are ongoing[35].

Polyvalent immunoglobulin. Antibodies to endotoxin target a single antigen. An alternate strategy is to administer concentrated immunoglobulins pooled from multiple donors, with the expectation that the preparation will provide broad cross-reactivity against a number of potential antigens, both known and unknown, and so will facilitate the opsonization of infecting microorganisms to promote phagocytosis and killing, and the neutralization of microbial toxins. The utility of intravenous immunoglobulin (IVIG) has been evaluated in several small studies. Pooled data from these show a significant mortality benefit for treated patients (OR = 0.64, 95% CI 0.51–0.80), although the total population is small (492 patients)[36]. Preparations enriched for IgG appear to be less effective, perhaps because IgM is able to inactivate complement[37]. Large multicentre trials are needed to clarify the therapeutic role of IVIG.

Targeting early host inflammatory cytokines

The activation of TLRs by ligands such as endotoxin results in the transcription of genes for several early pro-inflammatory cytokines, prominent amongst which are TNF and IL-1.

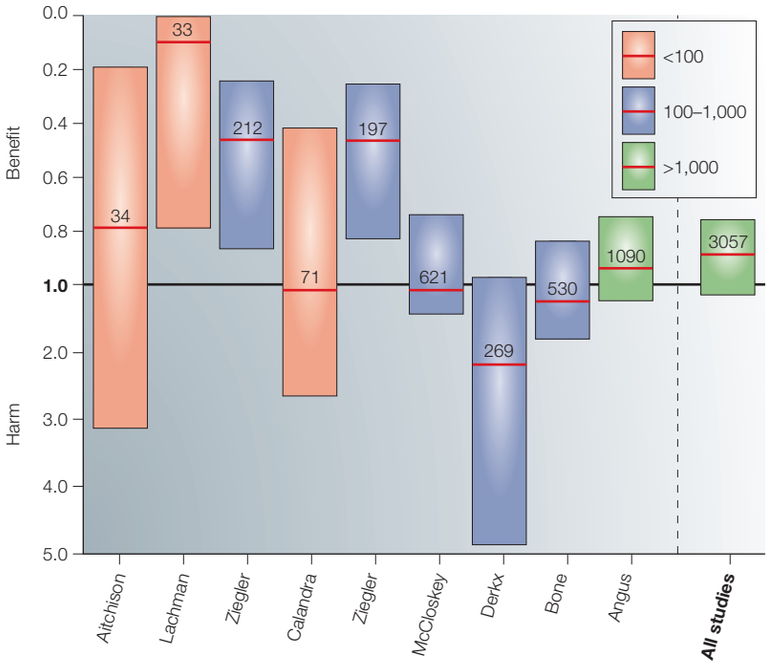

Figure 2 | **Randomized clinical trials evaluating the neutralization of endotoxin by an antibody.** Nine randomized trials have evaluated the effects of neutralization of endotoxin with a polyclonal antiserum or a monoclonal antibody. A value of 1.0 on the *y* axis denotes no effect; numbers less than 1.0 indicate benefit for the study intervention, while values of greater than 1.0 denote harm. For each study, the odds ratio (OR; red line) and 95% confidence intervals (CI) are shown; numbers within each box represent the total number of patients enrolled in the study, and boxes are colour coded by the number of patients involved. In some, data are for patients with Gram-negative infection, whereas others reflect the results for all-comers. Although there is a suggestion of benefit when all studies are pooled (OR = 0.93, 95% CI 0.73–1.18), because the confidence intervals include 1.0, the effect is not statistically significant.

Tumour necrosis factor. TNF, which acts through one of two receptors designated TNFR1 (p55) and TNFR2 (p75)[38], is a crucial early mediator of endotoxin-induced shock[13,14], which makes it an attractive therapeutic target. The administration of endotoxin to healthy human volunteers results in detectable levels of circulating TNF[39], and, conversely, the administration of TNF reproduces the clinical features of endotoxaemia[40,41]. Circulating TNF levels are elevated in sepsis, although the magnitude of the increase is variable, and its correlation with outcome is inconsistent[39,42–44]. Two strategies targeting circulating TNF have been evaluated in multicentre human studies — soluble TNF receptor constructs, and specific neutralizing anti-TNF antibodies.

Both the p55 and p75 receptors for TNF are cleaved from the cell membrane and released into the circulation where they compete for the binding of circulating TNF, and so serve as soluble inhibitors of TNF bioactivity. Levels of soluble receptors for TNF are elevated in sepsis, particularly in those patients who develop organ failure[44]. Fusion proteins consisting of the extracellular domain of the receptor fused to the Fc portion of immunoglobulin have been created for both TNF receptors, and because their binding affinity for TNF is high, they would be anticipated to serve as effective scavengers of circulating TNF. The p55 receptor construct has been evaluated in two studies. Although there was an encouraging trend towards improved mortality in a Phase II trial that enrolled 498 patients, with a 15% relative-risk reduction in the highest dosage group that neared significance in the subset of patients with severe sepsis and early septic shock[45], the therapeutic signal in a larger Phase III trial was weak and statistically insignificant[46]. The administration of a recombinant p75 fusion protein to patients with sepsis resulted in an unanticipated increase in mortality for treated patients[47], although this receptor construct has proven effective in the treatment of patients with rheumatoid arthritis[48].

Monoclonal antibodies to TNF have enjoyed a somewhat more consistent, albeit modest, effect in patients with sepsis (FIG. 3). Ten multicentre randomized trials of various anti-TNF antibodies have been completed[49–57]; in aggregate, they show a statistically significant 3.5% mortality reduction when administered to patients with sepsis, although there is some variability between studies. The largest of these trials, which recruited 2,634 patients stratified by baseline levels of IL-6 greater or less than 1,000 pg/ml, reported a 3.5% overall reduction in 28-day mortality and an adjusted mortality (adjusted for baseline imbalances in illness severity) of 5.8% in the 998 patients with elevated IL-6 levels; both effects were just within the 0.05 alpha level for statistical significance[56].

Interleukin-1. IL-1, a 26-kDa protein, is synthesized by monocytes, macrophages and neutrophils in response to TLR engagement[58]. Gene transcription of IL-1 is accompanied, with a slight temporal delay, by transcription of the gene for the IL-1 receptor antagonist (IL-1ra), a protein that shares 22% sequence similarity with IL-1 but does not transduce an activating signal and so functions as an endogenous competitive inhibitor of IL-1 (REF. 59). The release of IL-1ra is substantially greater than that of IL-1, underlining the important regulatory role exerted by this protein in modulating IL-1 activity[60].

Recombinant IL-1ra has been evaluated in three multicentre trials. The first, an open-label Phase II study, showed a striking dose-dependent mortality reduction from 44 to 16% for treated patients[61]. Two subsequent Phase III trials, however, yielded much more modest and statistically insignificant results[62,63]. Pooled data from these three studies show a significant 4.9% mortality benefit associated with administration of IL-1ra (35.5% placebo, 30.6% IL-1ra; OR = 1.25, 95% CI 1.01–1.53); however, the clinical development of this protein as a therapy for sepsis has been curtailed.

Targeting bioactive lipid mediators

The generation of bioactive lipids from cell membrane components occurs concomitantly with the transcription of pro-inflammatory cytokines. Enzymes of the phospholipase A_2 (PLA_2) family catalyse the conversion of cell membrane phospholipids to arachidonic acid and lysophospholipids such as platelet-activating factor (PAF). Arachidonic acid, in turn, is a precursor for the synthesis of prostaglandins and thromboxane through the cyclooxygenase pathway, and of leukotrienes through the lipoxygenase pathway[64] (FIG. 4).

APACHE II SCORES
A composite measure of acute severity of illness, including variables reflecting acute (A) physiology (P), age (A) and chronic health evaluation (CHE).

ISOFORMS OF NOS
Constitutively expressed nitric oxide synthase is present in endothelial cells, neurons, and hepatocytes. An inducible form of the enzyme is also present in cells of the innate immune system; its expression is upregulated by endotoxin and pro-inflammatory cytokines.

Phospholipase A_2. The PLA_2 family of enzymes comprises ten low-molecular-weight secreted isozymes, three 85-kDa cytosolic proteins and members of the platelet-activating acetylhydrolase family[65]. The expression of the most extensively studied of the secreted isozymes, PLA_2G2A (Type IIA $sPLA_2$), is induced by lipopolysaccharide and several pro-inflammatory cytokines, including TNF[66]. Elevated levels of circulating PLA_2 have been detected in critically ill patients with peritonitis, and are predictive of the subsequent development of multiple organ failure[67]. Several small-molecule inhibitors of Type II sPLA2 have been developed[68], and a Phase II study of a PLA_2 inhibitor (Ly315920) in sepsis was recently completed; no benefit was found (E. Abraham, 23rd International Symposium on Intensive Care and Emergency Medicine, Brussels, 2003).

Platelet-activating factor. PAF is generated from membrane phospholipids through the action of PLA_2. Receptors for PAF are expressed on platelets and cells of the innate immune system, particularly neutrophils and monocytes[69]. The engagement of its cell-surface receptor by PAF activates multiple intracellular pathways that modulate calcium release, phosphatidylinositol turnover, cAMP and kinase activation, and contribute to acute inflammatory injury.

Two separate strategies have been used to neutralize PAF. First, the binding of PAF to its receptor has been blocked using synthetic antagonists of the PAF receptor: BN 52021 (Ginkgolide B), TCV-309 and BB-882 (lexipafant). Pooled data from six studies that enrolled a total of 1,279 patients and evaluated the efficacy of PAF receptor antagonists demonstrated a statistically nonsignificant reduction in mortality of 3.1% (placebo 51.5%, PAF receptor antagonists, 48.4%)[70–75]. Subgroup effects were seen in several of the trials, although the effects were inconsistent. Dhainaut and colleagues[76] showed a significant improvement in survival for patients with Gram-negative sepsis who received BN 52051, whereas Poeze and colleagues[73] found a beneficial effect on organ failure and disease severity measured by APACHE II SCORES in patients receiving TCV-309.

Lexipafant reduced the new development of organ dysfunction in patients with severe pancreatitis in a Phase II trial[77]; however, the approach was without apparent benefit in a large, as-yet-unpublished Phase III trial. Johnson and colleagues[78], however, reported that lexipafant could reduce rates of subsequent sepsis in patients with pancreatitis.

The second strategy exploits the fact that PAF is inactivated through the enzymatic activity of PAF acetylhydrolase, producing lyso-PAF; levels of the enzyme are reduced in patients with sepsis[79]. Administration of recombinant PAF acetylhydrolase to patients with sepsis resulted in a significant improvement in 28-day all-cause mortality (from 44.2 to 21.4%, p=0.03) in an unpublished Phase II study. A Phase III trial was discontinued for futility following an interim analysis after 1,250 patients had been enrolled (Icos press release Dec. 19, 2002).

Prostaglandins. The cyclooxygenase inhibitor ibuprofen was shown to reduce temperature, heart rate, oxygen consumption and lactate levels, but not to alter mortality, in a multicentre trial of 455 patients[80], a finding replicated by several other small studies[81,82].

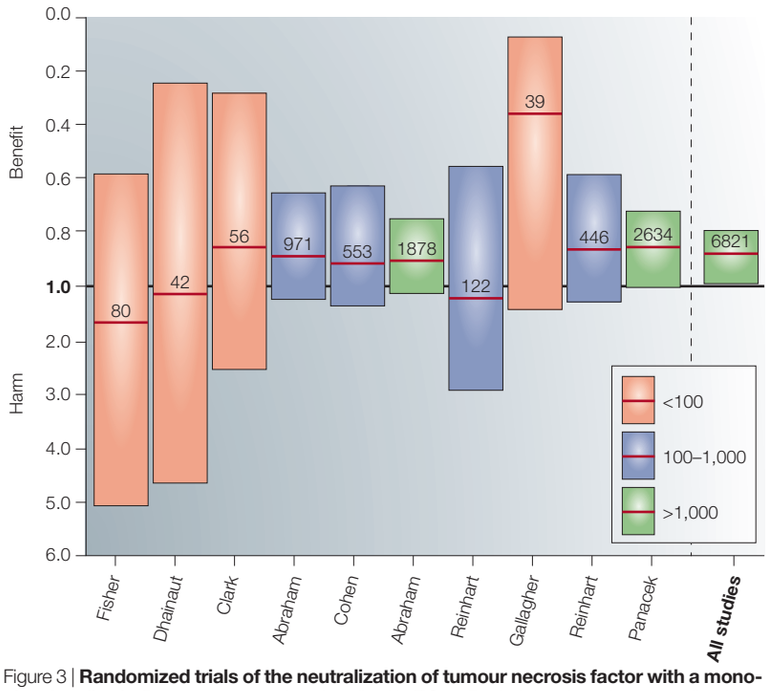

Figure 3 | **Randomized trials of the neutralization of tumour necrosis factor with a monoclonal antibody.** Data are presented as described in FIG. 2. Pooled data from the ten studies that have assessed the effects of neutralization of tumour necrosis factor (TNF) on survival do show a small (3.5%), but statistically significant, benefit for anti-TNF therapy (OR = 0.88, 95% CI 0.80–0.97).

Nitric oxide

Nitric oxide (NO), which is generated from arginine by nitric oxide synthase (NOS), has a variety of effects in the innate response to injury and infection. The most prominent of these is the activation of guanylate cyclase, which results in the relaxation of vascular smooth muscle, and the vasodilation that is characteristic of septic shock. The different ISOFORMS OF NOS can be inhibited nonspecifically by a methylated arginine analog, L-*N*-monomethyl arginine (L-NMMA) or by *N*-omega-nitro-L-arginine methyl ester (L-NAME). An alternative approach to the antagonism of NO is the inhibition of guanylate cyclase with methylene blue.

NO is a potent vasodilator, and its inhibition in several small studies, using L-NMMA[83], L-NAME[84] or methylene blue[85,86], consistently resulted in an increase in blood pressure and a decrease in the need for vasopressors. However, the blockade of NO is also associated with a reduction in cardiac output and an increase in pulmonary artery pressure, effects that might, in part, account for the *increased* mortality seen in an abortive Phase III clinical trial of L-NMMA, as yet unpublished.

Endogenous immunostimulatory molecules

Sepsis has been conceptualized as a disorder resulting from excessive activation of innate immunity, a model that provides a rationale for targeting pro-inflammatory

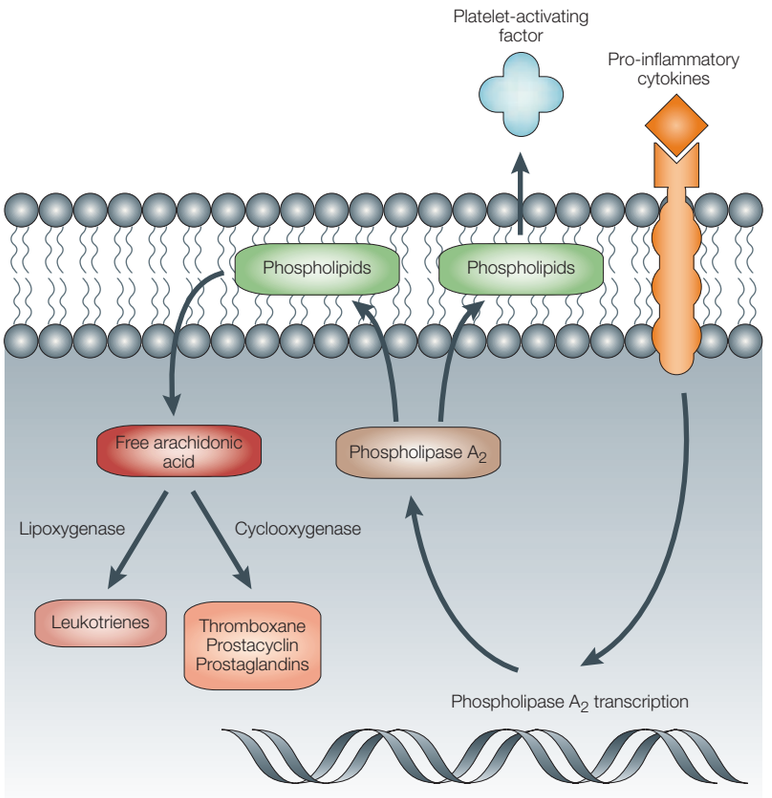

Figure 4 | **Biosynthesis of lipid mediators of sepsis.** Phospholipase A_2 is transcribed in response to pro-inflammatory cytokines, and acts on arachidonic acid in the cell membrane to generate platelet-activating factor. Arachidonic acid can be further processed intracellularly through the action of lipoxygenase to generate leukotrienes and by cyclooxygenase to generate thromboxane, prostacyclin and prostaglandins.

mediators as described above. However, multiple aspects of normal immune responsiveness, including delayed hypersensitivity, lymphocyte blastogenesis, antibody synthesis in response to protein antigens and expression of major histocompatibility complex class I antigens, are reduced in sepsis, leading some to argue that the initial state of excessive systemic inflammation results in immunological exhaustion[87], or, as it has been termed, the compensatory anti-inflammatory response syndrome[88]. So, several strategies whose objective is reversing a state of immune suppression have been evaluated.

Interferon-γ. Decreased monocyte expression of the major histocompatability marker HLA-DR is a consistent finding in patients with sepsis. Two small pilot studies have shown that exogenous recombinant interferon-γ (IFN-γ) can restore expression of HLA-DR and increase *in vitro* monocyte cytokine release in response to lipopolysaccharide[89], and increase circulating levels of TNF and IL-6 (REF. 90), although neither study was powered to detect clinical benefit. Similarly, IFN-γ was shown to augment HLA-DR expression on monocytes from trauma patients[91]. The clinical utility of IFN-γ therapy remains unproven.

ZYMOGEN
A proenzyme. Requires enzymatic activation to exert its own effects.

Granulocyte colony-stimulating factor. The growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) act on myeloid cell precursors to stimulate their differentiation into mature monocytes and neutrophils.

G-CSF is an 18-kDa glycosylated protein that is synthesized by macrophages, fibroblasts and endothelial cells in response to pro-inflammatory cytokines[92]. Although it subserves a pro-inflammatory function by virtue of its ability to stimulate neutrophil production, the *in vivo* biology of G-CSF is complex, and includes both enhancement of neutrophil function, inhibition of the release of TNF and IFN-γ and stimulation of the release of anti-inflammatory mediators, such as IL-1ra and soluble TNF receptors[93]. G-CSF has been used to promote neutrophil maturation in patients with neutropenia, and has been found to not only increase neutrophil numbers but to also reduce antibiotic use and total hospital costs[94]. In a study of patients admitted to hospital with community-acquired pneumonia, G-CSF was found to reduce the subsequent occurrence of acute respiratory distress syndrome and disseminated intravascular coagulation[95]. A multicentre study of 480 patients with community-acquired multilobar pneumonia found no evidence of improved survival or reduced organ dysfunction for patients who received G-CSF[96]. Although G-CSF has anti-inflammatory activity when given to critically ill patients, as evidenced by its ability to reduce circulating IL-8 levels and to induce expression of IL-1ra[97], its biological activity in promoting neutrophil release raised concerns that its use could induce or aggravate acute lung injury in patients with sepsis. Although a small pilot study found no evidence of harm in this population[98], a larger multicentre trial that enrolled 701 patients found no evidence of clinical benefit[99].

Targeting coagulation

Microbial products, such as endotoxin, and host-derived inflammatory mediators, such as TNF and IL-1, promote the development of a procoagulant state by simultaneously activating coagulation and inhibiting fibrinolysis (FIG. 5). Endotoxin[100], pro-inflammatory cytokines[101] and engagement of cell-surface integrins[102] induce the expression of tissue factor on monocytes, macrophages and endothelial cells. Tissue factor activates Factor VIIa, and the tissue factor–Factor VIIa complex converts Factor X to Factor Xa. Factor Xa complexes with Factor Va, and catalyses the conversion of prothrombin to thrombin, which, in turn, promotes the conversion of fibrinogen to fibrin.

In health, the activation of coagulation is inhibited through the activity of three endogenous anticoagulant pathways — the protein C pathway, the antithrombin pathway, and the tissue-factor-pathway-inhibitor (TFPI) pathway. The activity of each is deranged in sepsis.

Activated protein C. Protein C is synthesized by the liver and circulates as an inactive ZYMOGEN. Circulating protein C is activated following the interaction of thrombin with thrombomodulin on the endothelial cell to generate activated protein C, a serine protease that complexes

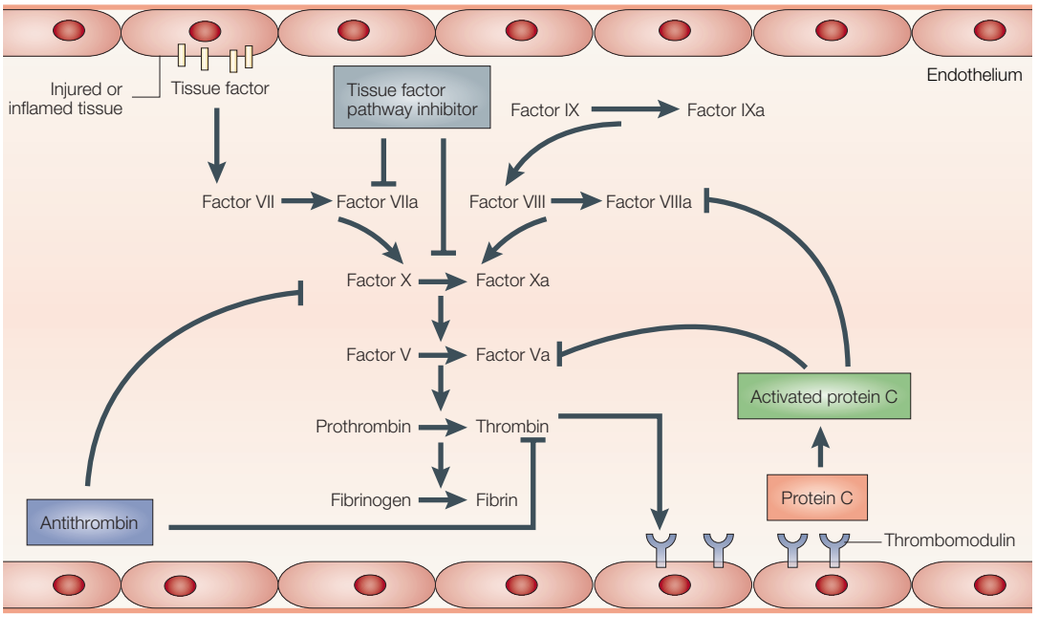

Figure 5 | **The coagulation cascade in sepsis.** Coagulation is initiated through the interaction of tissue factor, upregulated by inflammatory stimuli, with Factor VII, which generates activated Factor VII (Factor VIIa). The tissue factor–Factor VIIa complex then catalyses the conversion of Factor X to Factor Xa, leading sequentially to the activation of Factor V, the conversion of prothrombin to thrombin, and the conversion of fibrinogen to fibrin. Three key anticoagulant pathways can inhibit this process. Protein C is activated through its interaction with cell-surface thrombomodulin, and inhibits the activities of Factors V and VIII. Antithrombin blocks the activation of Factors XII, XI, IX, and X, whereas tissue factor pathway inhibitor interferes directly with the tissue factor–Factor VIIa complex.

with protein S to inhibit Factors V and VIII, and blocks the further generation of thrombin[103]. Both protein C and activated protein C (APC) can bind to the endothelial cell protein C receptor (EPCR), an interaction that accelerates the generation of APC and signals cellular responses that are anti-inflammatory in nature. The activation of protein C, therefore, plays a crucial role in regulating thrombin activity *in vivo*: the multiple abnormalities of sepsis impair this normal anticoagulant activity and promote a net pro-thrombotic state. Protein C synthesis by the liver is reduced in sepsis, and the shedding of endothelial cell thrombomodulin prevents the normal thrombin–thrombomodulin interaction that activates protein C. EPCR expression is downregulated by cytokines such as TNF, and levels of free protein S, a co-factor for APC activity, are reduced[103]. Finally, acute-phase reactants, such as α_1-antitrypsin, whose circulating concentrations increase in sepsis, accelerate the degradation of APC.

Protein C concentrate has been evaluated in several small case series of paediatric patients with meningococcaemia[104,105]. The activation of the protein C zymogen is impaired in sepsis, and so protein C has been expressed in a fully activated, recombinant form, and its activity studied in human sepsis. In a multicentre Phase III trial enrolling 1,590 patients with severe sepsis, the administration of recombinant APC (known generically as drotrecogin alpha activated) resulted in a significant reduction in 28-day all-cause mortality from 30.8 to 24.7% ($p = 0.006$)[4]. Subgroup analyses suggested that the greatest clinical efficacy occurred in patients with more significant degrees of organ dysfunction at the time of study entry, and greater severity of illness as measured by APACHE II scores[106]. Although robust conclusions about subgroup efficacy could not be drawn, other populations that seemed to show the greatest benefit were patients with pneumonia and patients with coagulopathy at study entry. Bleeding complications occurred more frequently in patients receiving drotrecogin alpha activated (3.6 versus 2.0%, $p = 0.06$), an observation that is consistent with the anticoagulant activity of the agent. Drotrecogin alpha activated has received regulatory approval in both the United States and Europe. An open-label Phase IIIb study has just been completed, and a large multicentre trial to assess its efficacy in patients with less severe illness is in progress.

Antithrombin. Antithrombin (also known as antithrombin III) inactivates thrombin by forming thrombin–antithrombin complexes that are cleared by the liver. Antithrombin exerts additional anticoagulant activity as a serine protease inhibitor, and binds and inactivates Factors XIIa and XIa, as well as Factor IXa, and prevents activation of Factor Xa . The antithrombin molecule contains a heparin-binding site, and its interaction with heparin amplifies antithrombin activity[107]. Antithrombin exerts anti-inflammatory effects that are independent of its anticoagulant effects, and triggers the release of prostacyclin from endothelial cells. Moreover, its ability to inhibit thrombin blocks the pro-inflammatory consequences of the interaction of thrombin with its receptor, including NF-κB activation and NO release[108].

Box 2 | **Sepsis syndrome and SIRS**

Sepsis syndrome[146]
- Proven or suspected infection, plus
  - Temperature ≥ 38.3 or ≤ 35.6°C
  - Heart rate > 90 beats per minute
  - Respiratory rate > 20 breaths per minute, or mechanical ventilation
  - Evidence of altered organ perfusion

Systemic inflammatory response syndrome (SIRS)[145]
- Temperature > 38.0 or < 36.0°C
- Heart rate > 90 beats per minute
- Respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg
- Neutrophils < 4,000 or > 12,000 cells per microlitre, or > 10% bands

Antithrombin levels are reduced in sepsis[109], and antithrombin supplementation has been evaluated in a number of small clinical trials. A meta-analysis of three randomized controlled trials recruiting 122 patients suggested a 23% reduction in 30-day all-cause mortality[110]. However, an international multicentre Phase III trial that recruited 2,314 patients failed to show any overall evidence of benefit for antithrombin replacement, although there was a suggestion of benefit for patients who were not concomitantly receiving heparin[111].

Tissue factor pathway inhibitor. Tissue factor pathway inhibitor (TFPI) is a 42-kDa glycoprotein synthesized primarily by endothelial cells; synthesis is increased in response to injury, shear stress or pro-inflammatory cytokines[112]. TFPI is a serine protease inhibitor that exerts its anticoagulant effects through direct inactivation of the tissue factor–Factor VIIa complex in the presence of Factor Xa.

An unpublished Phase II study of 210 patients showed a trend towards reduced mortality for TFPI-treated patients[113]; however, a larger Phase III trial was unable to reproduce this effect. Striking differences were seen in placebo mortality rate and an apparent response to therapy in the results of the first interim analysis when compared with those of the second half of the study population. The explanation for this discrepancy is unclear.

Other anticoagulant strategies. Other strategies for modulating the coagulation cascade in sepsis include the direct inhibition of tissue factor by antibody and the antagonism of Factors VIIa and Xa. Active site-inhibited Factor VIIa was found to block thrombin and fibrin generation in human volunteers receiving a bolus of endotoxin[114]. A small-molecule inhibitor of Factor Xa has undergone preliminary evaluation, but results are unavailable. The results of the Phase III study of antithrombin raise the possibility that heparin alone might exert benefit in sepsis[115]; however, this hypothesis has not been directly tested.

Corticosteroids

Cortisol, released from the adrenal cortex in response to the secretion of adrenocorticotrophic hormone (ACTH) by the anterior pituitary gland, exerts potent anti-inflammatory activity through various mechanisms, including the prevention of the dissociation and nuclear translocation of NF-κB and inhibition of PLA_2 (REF. 116). Cortisol secretion is increased in response to a variety of physiological stresses, including infection and tissue injury. Although adrenal haemorrhage or necrosis are rare in sepsis, a number of observational studies have established that functional adrenal insufficiency and an inability to augment adrenal cortisol release in response to ACTH stimulation are common, and associated with adverse outcome[117,118]. So, the administration of exogenous corticosteroids, both to correct an inadequate stress response and to blunt an exaggerated inflammatory response, has held a pervasive attraction for investigators[119].

Early work by Schumer suggested that the administration of large doses of exogenous corticosteroids to septic patients could improve survival[120]. A small randomized trial failed to show survival benefit for corticosteroid therapy, but did demonstrate a more rapid reversal of septic shock[121]. However, two subsequent studies of high-dose corticosteroid therapy in sepsis suggested that such therapy was without benefit[122,123], a conclusion that was supported by a meta-analysis of all trials of steroid therapy completed before the early 1990s[124].

The potential role of corticosteroid replacement therapy has been revisited more recently, through studies evaluating the utility of smaller doses of glucocorticoids in patients with refractory septic shock[125]. In a randomized multicentre trial of 297 patients with septic shock, Annane and colleagues documented a significant reduction in mortality for patients who were non-responders to a short corticotropin test. For these patients, comprising three quarters of the study population, the combination of hydrocortisone at a dose of 50 mg four times daily and fludrocortisone 50 μg daily reduced mortality from 63 to 53% ($p = 0.02$), and increased the percentage of patients in whom vasopressor therapy could be successfully weaned[5]. Subsequent work has confirmed that the administration of exogenous corticosteroids increases circulating cortisol levels[126] and reduces circulating levels of the pro-inflammatory cytokines IL-6 and IL-8 (REFS 127, 128), which provides biological support for the presumed mechanism of action. Two multicentre European studies of corticosteroid replacement therapy in sepsis are presently in progress.

Other strategies and therapeutic targets

The list of other potential therapeutic targets in sepsis is extensive, although clinical efficacy is by and large unproven. A study of 504 septic patients found no overall benefit for the inhibition of bradykinin, but did indicate mortality reduction for the subset of patients with Gram-negative infection[129]. Complement inhibition with C1 inhibitor[130] reduced serum creatinine levels in a study of 40 septic patients, and treated

Box 3 | **A rational approach to the evaluation of novel sepsis therapies**

The complexity of modulating an adaptive host response by targeting the right process in the right patient at the right dose and time suggests the need for an explicit and sequential process of evaluation of a new therapy:

1. Does the drug show efficacy in a panel of animal models?
The use of a carefully considered panel of animal models, including endotoxaemia, bacterial challenge in the lung and peritoneum, fungal challenge, complex peritonitis, studies in immunocompromised animals, and large-animal studies to define physiology not only confirm drug activity, but can point to potential populations who might experience maximal benefit. Both pretreatment and post-insult treatment should be evaluated. Endotoxaemia in human volunteers provides further insights into the domains of drug efficacy and the response of potential markers of therapy.

2. Is the drug biologically active in the target population?
Early, intensive studies in critically ill patients can determine whether the drug alters levels of its putative target or downstream mediators in the potential population of interest, and can define variability in pharmacokinetics.

3. Does the drug alter patient physiology during administration?
Similar early studies in a potential target population can assess whether the agent demonstrates the physiological and biochemical effects that would be predicted on the basis of its biological activity. Such changes could also suggest appropriate entry criteria, for it would be expected that an active agent would accelerate the reversal of study entry criteria during its administration.

4. Does the drug alter acute morbidity attributable to its target?
In addition to reversing study entry criteria, an active agent would be expected to prevent new organ dysfunction, or more rapidly hasten the resolution of dysfunction in organs whose pathological derangements arose from the activity of the target of interest.

5. Does the drug adversely affect the potentially beneficial effects of its target?
Because sepsis is an adaptive response, it is important to determine whether therapy adversely impacts innate immunity, for example, through the study of *in vitro* cell function or *in vivo* susceptibility to infection or complications such as bleeding.

6. Does the drug impact short-term morbidity and mortality?
It would be anticipated that the most compelling evidence of mortality improvement would be seen during, and in the next few days to week following, treatment. Organ-dysfunction assessment can provide an estimate of the effects on short-term morbidity.

7. Does the drug impact intermediate morbidity and mortality?
The classical assessment of 28-day all-cause mortality provides a measure of intermediate efficacy. Organ-dysfunction scores and failure-free days reflect intermediate morbidity.

8. Does the drug improve long-term outcomes and quality of life?
Because the natural history of survivors of sepsis differs from that of an age-matched population, it is important to assess the impact of therapy on mortality at 90 days and 6 months, as well as on post-hospital quality of life.

patients showed a trend towards diminution of organ dysfunction[131]. Blockade of the adhesion molecule E-selectin with a monoclonal antibody was without apparent benefit[132].

Non-pharmacological approaches to the treatment of sepsis have recently shown utility. A study of aggressive goal-directed fluid resuscitation initiated in the emergency department showed that this strategy resulted in a 16% absolute mortality reduction, and underlined the crucial role of early pre-emptive measures in reducing the morbidity and mortality of patients with sepsis[133]. The tight control of serum glucose has been found to improve survival in an heterogeneous population of critically ill patients, although the extent to which efficacy will be seen in the distinct population of septic patients[134] remains unknown. And for those who despair about the complexity of the disease and the futility of attempts to treat sepsis, a recent report has suggested that prayer can alter its clinical course[135].

Mediator-directed therapy in sepsis: the future

It will be evident from the preceding summary that attempts to alter the clinical course of sepsis by targeting its molecular mediators have been largely disappointing, and that the therapeutic promise apparent in animal models has not been realized when evaluated in populations of critically ill patients. Much has been written about the ongoing disappointments of clinical research in sepsis[2,136–140], and from the failures of the past arise insights that should yield success in the future. It is very clear from the body of completed work that lack of evidence of efficacy does not constitute evidence of lack of efficacy. The reasons for failure are many.

Absence or loss of the agent's biological activity. Recombinant proteins are challenging to produce, and their apparent biological activity *in vitro* or in a simple animal model is not always replicable in human sepsis. The initial evaluation of the monoclonal antibody HA-1A indicated utility in patients with Gram-negative infections[24], an effect that could not be reproduced in a follow-up trial[25]. Further *in vitro* work showed that although the antibody bound endotoxin, it did not neutralize its capacity to evoke a cellular response[141], and that it failed to provide *in vivo* protection in a canine model of Gram-negative infection[142]. A monoclonal antibody to TNF that yielded a striking survival benefit in a primate model of Gram-negative infection[143] proved ineffective in a large Phase III study of septic shock[54]. Bioassays of plasma from patients enrolled in that trial showed that although the antibody could be detected in treated patients, levels of TNF bioactivity were identical in both populations during the treatment period (S. Porter, personal communication).

Inappropriate dose, duration or timing of therapy. For most therapies whose efficacy has been evaluated in clinical trials, the optimal dose and duration of therapy is unknown. It is standard practice in the intensive care unit (ICU) to titrate therapy to optimize clinical efficacy, for example, to target the dose of a vasopressor agent to achieve a predetermined level of blood pressure, or the dose of insulin to achieve a target blood-sugar level. For the most part, titration of therapy in sepsis trials has not been possible; in one trial in which an inhibitor of NO biosynthesis was titrated to a mean arterial pressure between 70 and 90 mm Hg, the target pressure might have been inappropriately high. Similarly, treatment in sepsis trials is typically given for an arbitrary and set duration of time, rather than for as long as a particular constellation of physiological or biochemical abnormalities is present, and re-treatment with the agent is not permitted. As clinical efficacy is evaluated as mortality at 28 days, the implicit assumption is that a therapy administered early during the course of a disease will result in an early and sustained reversal of the disease process. In

practice, however, ~40% of patients who survive sepsis remain in the ICU at day 28, indicating that the course of the disease is both prolonged and recurrent.

The optimal time to initiate therapy is usually not known. Intervention is grounded in the implicit assumption that the process the therapy targets is both present and amenable to modulation. Administered too late, a treatment might be ineffectual at modifying the course of a disease whose progression is no longer dependent on the target of therapy. Conversely, when therapy is administered too early, the target might not yet be present. Goal-directed fluid resuscitation results in a striking survival improvement when it is initiated early in the emergency department[133]; used later following admission to the ICU, it is without significant effect[144]. APC, on the other hand, seems to be more useful when used later in patients with more significant degrees of organ dysfunction.

Heterogeneity of the target population. The physiological criteria for the systemic inflammatory response syndrome (SIRS)[145] or sepsis syndrome[146] (BOX 2) were established through an arbitrary and imperfect consensus process with the objective of defining an appropriate population for enrolment into a clinical trial at an early time point, before the results of microbial cultures or other investigations were available. They are nonspecific[147,148], and delineate a very heterogeneous population with respect to both clinical setting and levels of circulating pro-inflammatory mediators[149]; indeed, it can be readily argued that the disappointing results of trials using these criteria says more about the limitations of the patient selection criteria than the inherent biological shortcomings of the therapeutic strategy. Equally, the clinical criteria for sepsis exclude significant numbers of patients who might benefit from a particular therapy. For example, there is no *a priori* reason to believe that an intervention that targets coagulation will have greater efficacy in a population of patients with coagulopathy resulting from documented or suspected infection than from any of the other causes of coagulopathy in critical illness.

Several recent conferences have highlighted the need to develop better methods of identifying appropriate patient populations for sepsis trials[138,150–152]. By analogy to the TNM STRATIFICATION SYSTEM that is widely used by oncologists, a template for a staging system for sepsis, known as the PIRO model, has recently been proposed[151]. The proposal in its present form is a concept — short on specific details, widespread acceptance or empirical support — but hopefully the harbinger of a new philosophy that successful descriptive models must reflect the complexity of the disease and the modalities available to treat it.

In the PIRO model, P denotes predisposing factors that can render a patient more or less likely to benefit from a particular therapy. Genetic factors, for example, have a significant impact on the probability of adverse outcome following infection[153]. Polymorphisms in genes involved in innate immunity are common, and their presence predicts illness severity. A polymorphism in the promoter region of the TNF-α gene (an A→T substitution at position –308) is associated with augmented release of TNF, higher circulating TNF levels in sepsis and increased mortality[154,155]. Similar polymorphisms have been identified in genes for TLRs, CD14, IL-1 and IL-10, whose expression contributes to the evolution of sepsis. Predisposing factors are not limited to genetic variability, but might also include pre-morbid illnesses, or religious or cultural attitudes that can impact on therapeutic decision-making.

The I of PIRO describes the insult; in the case of sepsis this is, by definition, infection. However, because innate immunity is activated by other factors, such as tissue injury and ischaemia, the appropriate stratification by insult depends on the therapy to be used. If the therapy is an anti-Pseudomonal agent, then the insult is infection with *Pseudomonas*. But if we are studying an anti-endotoxin strategy, the I becomes endotoxaemia, and if the intervention targets TNF, then it is best studied in patients facing any insult that evokes TNF release.

R denotes the response of the host, and can be defined physiologically or biochemically. Activated protein C, for example, seems to be most efficacious in patients with significant physiological derangements, as reflected by an elevated APACHE II score, and in patients with coagulopathy[4,106]. Anti-TNF therapy might be more effective in patients with elevated levels of IL-6 (REF. 56). Optimal measures of the endogenous host response are not well-defined, but it is intuitively obvious that, just as blood-sugar levels guide therapy with insulin, they will be invaluable in identifying appropriate patients for therapy and in evaluating the response to treatment.

Finally, the O of PIRO denotes the degree of organ dysfunction, and is readily quantifiable using one of several published scores, such as the Multiple Organ Dysfunction (MOD) score[156] or the Sequential Organ Failure Assessment (SOFA) score[157]. The greatest therapeutic response to activated protein C is seen in patients with greater degrees of organ dysfunction. On the other hand, the efficacy of an antibody to TNF is greatest in patients who do not have significant organ dysfunction at the time of study entry[158].

Although the PIRO model is at present simply a concept, and not a validated and reproducible descriptive system, it does provide a sensible basis for designing future trials of mediator-targeted therapy. A study of an anti-endotoxin therapy, for example, would seek to recruit patients with documented endotoxaemia who do not have significant organ dysfunction at study entry. A study of an agent targeting TNF might recruit patients with the TNF2 genetic polymorphism and elevated IL-6 levels who do not have significant degrees of organ dysfunction. Finally, a trial of a novel anticoagulant might selectively recruit patients with coagulopathy, independent of cause, who do have evidence of evolving organ dysfunction.

Limitations of outcome measures. The conventional endpoint used in sepsis research has been 28-day all-cause mortality, based on the recognition that sepsis is a lethal disease process, and that therapy should reduce that mortality, rather than simply prolong the process of dying by several days. However, although survival is unquestionably important to patients, mortality measures fail to reflect the global burden of illness of sepsis, and

TNM STRATIFICATION SYSTEM
The TNM (tumours, nodes, metastasis) system, widely used in cancer clinical trials, characterizes patients by variables that not only correlate with prognosis, but also reflect a differential potential of response to therapy.

underestimate the importance to patients of non-mortal outcomes[159]. Moreover, mortality as an outcome measure is not particularly informative, for it is insensitive to small changes in clinical status that might be used to titrate therapy, to refine patient-selection criteria, to evaluate the interactions of two or more interventions, or to permit real-time decisions about the success or failure of a particular therapeutic approach in a given patient.

Alternative or complementary outcome measures for use in sepsis trials are not well developed[160]. Organ-dysfunction scales[156,157] have been used to measure both the organ-system-specific and global burden of the physiological derangement seen in sepsis. Although they offer greater insight into the effects of therapy, they have proven to be only slightly more sensitive to therapeutic effects, and can be criticized because they measure physiological derangements rather than the clinical effects of these for the patient.

The morbidity of sepsis is substantial, and is reflected in the use of prolonged life-support measures, each having their own potential for further complications. Such interventions include mechanical ventilation, vasoactive and inotropic drugs, haemodialysis, sedative medications, antibiotics, parenteral nutrition, blood component therapy and other aspects of care in the ICU. However, the use of each of these is variable, and there is no validated mechanism for quantifying that use as an outcome measure in a clinical trial. The measurement of 'organ-failure-free' days has been incorporated into a number of studies in an effort to integrate the impact of mortality and significant clinical morbidity, and quantifies the number of days a patient is both alive and not receiving a particular intervention, such as mechanical ventilation, vasoactive therapy or intensive care. However, studies using failure-free days as an outcome have generally not found it to be more sensitive to clinically important effects than the simple measure of all-cause mortality. There is an unmet need for the development of sensitive and responsive outcome measures that can facilitate better evaluation of the course of sepsis and its modulation by novel therapies.

Conclusions

Sepsis is the clinical expression of a highly conserved biological response that evolved to protect multicellular organisms against a hostile environment. It results in a disease that is paradoxical and complex: the biological processes that contain a threat become the forces that, following resuscitation and the initiation of intensive care, are responsible for substantial morbidity and mortality. In fact, the syndrome as it is encountered in the critically ill patient is truly an iatrogenic disorder, arising only in patients who, without medical intervention, would have died a rapid death; it evolves in large part because of the inadvertent effects of the interventions required to sustain life.

Our understanding of the fundamental biology of the septic response has advanced rapidly over the past quarter of a century; the translation of these insights into clinical therapy has proven much more difficult. The reasons for this divergence between biological understanding and clinical efficacy are many, and include the enormous complexity of the disease, the inadequacies of simple animal models, the intrinsic heterogeneity of the patient population, and the confounding influence of the many interventions used to treat critically ill patients. The drive to move rapidly from biological understanding to clinical utility has no doubt led to the premature abandonment of therapies, discarded because simplistic models for drug development have proven inadequate to the challenge at hand. Success in the future will hinge on the adoption of a more deliberate, hypothesis-driven and evidence-based investigative programme (BOX 3).

On the other hand, the biological processes that mediate sepsis — the host response to infection — are the same processes that cause tissue injury following trauma, ischaemia and a host of other acute threats, and the ultimate potential for transforming the clinical course of acute life-threatening illness is enormous ... truly the stuff that dreams are made on.

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Online links

DATABASES

The following terms in this article are linked online to:

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink

BPI | IFN-γ | LBP | PLA_2G2A | TFPI | TLR4 | TNFR1 | TNFR2

FURTHER INFORMATION

Encyclopedia of Life Sciences: http://www.els.net

Septicaemic shock

Access to this interactive links box is free online.