

CRITICAL CARE ROUNDS™

May/June 2001
Volume 2, Issue 3

AS PRESENTED IN THE GRAND ROUNDS OF
PARTICIPATING HOSPITALS ACROSS CANADA

Modulation of the systemic inflammatory response in sepsis: Current status, future prospects

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Sepsis is a complex disease process that arises through the activation of a systemic response to infection. It afflicts some 750,000 North Americans each year and is directly responsible for more than 200,000 deaths annually, a figure that equals the number of deaths from myocardial infarction.¹ Until quite recently, sepsis management has been limited to resuscitation and physiological support, surgical control of the foci of ongoing infection, and administration of systemic antimicrobial agents. Work over the past two decades has established conclusively that sepsis morbidity occurs, not through the direct cytopathic effects of invading micro-organisms, but indirectly, through activation of an inflammatory response by the host.² This response is enormously complex, involving over 200 different mediator molecules that serve to activate, expand, modulate, and ultimately resolve the acute inflammatory response. After a series of disappointing attempts to improve outcome through the selective manipulation of these mediators, the past year has witnessed the first convincing evidence of this principle and treatment is on the verge of becoming a clinical reality. This review will highlight the biological rationale, the clinical frustration, and the promise of mediator-targeted therapy for sepsis.

A highly simplified schema summarizing the components of a systemic inflammatory response is outlined in Figure 1. Bacterial products, such as endotoxin from the cell wall of Gram-negative bacteria, or peptidoglycan from Gram-positive bacteria, activate cells of the innate immune system (predominantly macrophages and neutrophils). As a result, the host cell is activated to synthesize pro-inflammatory mediators (ie, tumour necrosis factor [TNF] and interleukin-1 β [IL-1]), regulatory cytokines (ie, IL-6), and counter-inflammatory molecules (ie, interleukin-10, transforming growth factor beta [TGF β], and the interleukin-1 receptor antagonist). Release of these molecules, in turn, triggers an extraordinary cascade of effector substances, including non-protein mediators (ie, platelet activating factor, prostaglandins, nitric oxide, acute phase proteins, and late mediators of inflammation [HMG-1]). Activation also induces a series of physiological effects, including microvascular thrombosis, increased capillary permeability, reduced systemic vascular resistance, and the programmed cell death or *apoptosis* of epithelial and endothelial cells. Altered regional blood flow and microvascular thrombosis render tissues ischemic; the response to local injury further results in increased amplification of the inflammatory cascade. In the experimental animal, disruption of any component of this complex network can improve outcome;³ however, the results in human clinical trials are more variable.

Neutralization of microbial toxins

The microbial pathogens responsible for clinical sepsis exert their effects by eliciting an inflammatory response in the host. Complex lipid and carbohydrate molecules in the bacterial cell wall serve as triggers to induce these responses, and include lipo-polysaccharide (endotoxin) and bacterial lipoprotein from Gram-negative bacteria; lipoteichoic acid and peptido-



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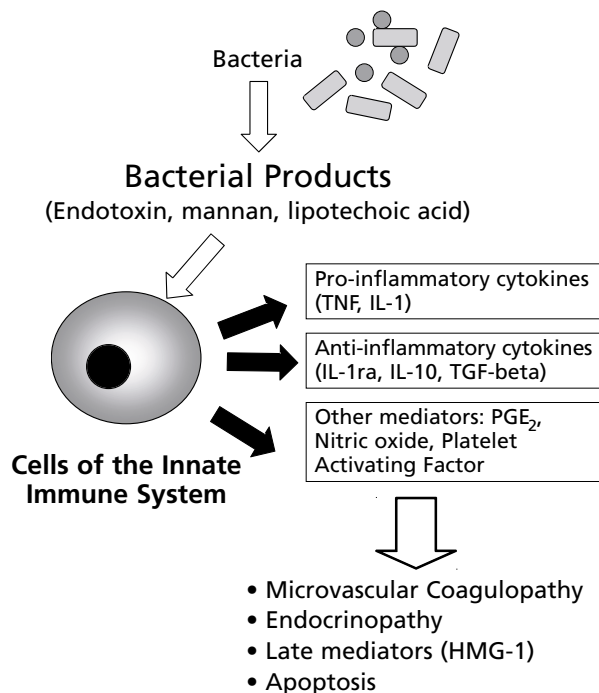
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Figure 1: The Inflammatory Cascade. The innate host response to an infectious challenge is complex and multidimensional; potential therapeutic targets exist at the level of the microorganism, its products, the early host inflammatory response, and its late homeostatic sequelae.



glycan from Gram-positive bacteria; mannan from fungi; and bacterial DNA from disrupted bacterial cells.⁴ Endotoxin is the best known of these, and the biologic processes through which it activates host cells have been extensively characterized.⁵ Endotoxin is present as an integral component of the cell wall in all Gram-negative bacteria, accounting for approximately 10% of the cell weight. Free endotoxin is transported in the blood by lipopolysaccharide-binding protein (LBP). The LBP-endotoxin complex binds to CD14 and the interaction of this complex with a member of the toll-like family of receptors, Tlr4, mediates the process of transducing the LPS (endotoxin) signal to the interior of the cell, and so inducing the synthesis of inflammatory cytokines.

The family of toll-like receptors consists of 10 distinct cell-surface receptors that recognize specific microbial patterns and comprise the pathogen recognition mechanism of the innate immune system.⁶ Endotoxin binds to Tlr4, while Tlr2 recognizes bacterial lipoprotein and certain cell wall constituents of Gram-positive bacteria. Tlr9 responds to bacterial DNA and it has recently been reported that Tlr5 binds flagellin. The toll-like receptors represent, in a sense, the antigen-recognition site on cells of the innate immune system.

Endotoxin is released through the lysis of Gram-negative bacteria; however, it is also detectable in clinical circumstances where viable bacteria are not cultured (ie, cardiopulmonary bypass, burns, and intestinal ischemia).⁷ As a common and important proximate trigger of septic response, endotoxin has been an appealing therapeutic target.

Ziegler and colleagues demonstrated that an antiserum used against the core polysaccharide of endotoxin

significantly improved the survival of patients with sepsis secondary to Gram-negative infection, particularly those with septic shock at the time of diagnosis.⁸ These encouraging findings stimulated the development of a monoclonal antibody to endotoxin known as HA-1A. In a phase III trial of 543 patients with sepsis, HA-1A treatment was associated with a significant increase in survival for the 200 patients with documented Gram-negative infection and for patients with septic shock secondary to Gram-negative infection. The lack of an effect in patients without Gram-negative infection resulted in the mortality benefit for the entire population being statistically non-significant (4%).⁹ Concerns about minor irregularities in the conduct of the trial and recognition of the significant economic impact that this new therapy would have if it were licensed, prompted American regulatory authorities to mandate a second, confirmatory study of HA-1A. To the dismay of the study sponsor and the surprise of the investigators, this study showed HA-1A to be without efficacy for patients with Gram-negative infection and even suggested that therapy might be harmful for patients with Gram-positive infections.¹⁰ Further development and testing of HA-1A was thus curtailed.

A variation on this scenario occurred with a second anti-endotoxin monoclonal antibody, E5. The initial clinical evaluation of this agent suggested that it could improve the outcome for patients with Gram-negative infection who were not in shock at the time of therapy.¹¹ A subsequent larger trial; however, failed to show any convincing evidence of therapeutic efficacy.¹²

Another strategy to neutralize endotoxin has been evaluated in children with meningococemia, a disease associated with high levels of circulating endotoxin. Bactericidal permeability increasing protein (BPI) is a neutrophil product with endotoxin-neutralizing properties. A recombinant peptide derived from BPI – rBPI21 – has been shown to reduce tissue loss in children with meningococemia.¹³ It has not been licensed as a therapeutic agent because the mortality benefit associated with therapy was minimal.

Other strategies targeting endotoxin have been studied, including an antibody to enterobacterial common antigen, HDL, a modified form of the antibiotic polymyxin B, and endotoxin removal with an extracorporeal column. To date, none of these strategies has shown unequivocal evidence of clinical efficacy.

Disruption of intracellular signalling pathways

Within the cell, new gene expression in response to endotoxin, or to proinflammatory cytokines, requires the activation of intracellular signalling cascades. These pathways are common to a variety of extracellular stimuli and are attractive targets for therapeutic manipulation. To date, however, these have not been extensively evaluated in humans.

A family of proteins known as mitogen-activated protein (MAP) kinases is critical in the response to inflammatory stimuli. Each kinase is activated through the addition of a phosphate molecule to the inactive enzyme, a process known as tyrosine phosphorylation. Inhibitors of tyrosine kinases improve survival in animal models of sepsis,¹⁴ but have yet to be studied in humans. Inflammatory gene expression also requires the transcription factor,

NFκB. NFκB exists in quiescent cells in an inactive form, complexed with an inhibitor, IκB. Activational signals cause the release of NFκB from this complex; free NFκB translocates to the nucleus, where it binds to the promoter sequence of a variety of proinflammatory genes and initiates gene transcription. NFκB can be inhibited by a variety of compounds, including acetylsalicylic acid (ASA), and corticosteroids. Two multicentre trials evaluated high-dose corticosteroids in patients with sepsis and failed to show any evidence of therapeutic efficacy; mortality was not reduced and rates of secondary infection were higher in steroid-treated patients.^{15,16}

Neutralization of proinflammatory mediators

Activation of macrophages by endotoxin or other microbial products triggers the synthesis and release of a family of proinflammatory proteins known as cytokines. Tumour necrosis factor (TNF) and interleukin-1 are among the first wave of cytokines released from the cell. Through paracrine and endocrine interactions with other cells, they induce the release of a cascade of mediators producing the clinical manifestations of sepsis.

Studies in a variety of animal models, including non-human primates, revealed that neutralization of TNF could prevent mortality following a challenge with live bacteria, even in the absence of systemic antibiotics.¹⁷ Two strategies have been used to neutralize TNF in human sepsis.

- Neutralizing antibodies to TNF have been evaluated in Phase II and Phase III clinical trials. In aggregate, these led to a statistically significant 3.5% reduction in 28-day all-cause mortality;¹⁸ however, only one study had sufficient power to document a mortality benefit. The North American study of a murine monoclonal antibody to TNF evaluated 2634 patients with sepsis. It demonstrated a significant reduction in mortality from 35.9% in the placebo group to 32.3% in patients treated with the antibody. However, the *a priori* target group for the study was a subset of 998 patients with elevated IL-6 levels, a marker of an exaggerated inflammatory response. Because of a slight baseline imbalance in severity of illness, the final mortality difference between the 2 study populations was only 4% and only when this imbalance was corrected by logistic regression did the adjusted mortality of 6.9% attain statistical significance. It is unclear whether this agent, known as afelimomab, will be licensed for therapy.

- A second strategy targeting TNF has been the creation of soluble constructs of the TNF receptor. TNF binds to 1 of 2 receptors — designated TNFR1 (p55) and TNFR2 (p75) — on the surface of its target cells. It was hypothesized that a chimeric protein, consisting of the extracellular portion of the TNF receptor fused to the Fc portion of an immunoglobulin molecule, would bind TNF with greater force than an antibody, and would therefore, be more effective in neutralizing its biological activity. A phase II study of the p75 receptor construct revealed that mortality was *increased* in treated patients,¹⁹ and further development of this agent as a treatment for sepsis was curtailed. It has found a therapeutic role, however, in the treatment of rheumatoid arthritis. A phase II study of a p55 receptor construct suggested therapeutic efficacy, but

this was not confirmed in a larger phase III trial, and further development of this agent has been abandoned.

Activity of the proinflammatory cytokine IL-1 is selectively inhibited through the release of an endogenous inhibitor, the interleukin-1 receptor antagonist (IL-1ra). IL-1ra has been evaluated in three randomized trials.¹⁸ The first of these, a phase II study, showed a dramatic, dose-dependent mortality reduction, from 45% in placebo-treated patients to 16% for patients receiving IL-1ra.²⁰ However, the treatment effect observed in the two subsequent trials was much smaller and the results failed to achieve statistical significance. Pooled data from these 3 studies evaluating IL-1ra show a significant mortality benefit of 4.9%.¹⁸ Unfortunately, further developmental work on this agent as a therapy for sepsis has been discontinued.

Blockade of downstream mediators

Platelet activating factor (PAF) is a complex phospholipid that exerts potent proinflammatory activity when released from cell membranes through the action of phospholipase-A2. Attempts to improve survival in pancreatitis and sepsis using synthetic antagonists of the PAF receptor have been disappointing. Recently, however, a striking survival advantage has been documented in a phase II trial of recombinant platelet-activating factor, acetylhydrolase, the enzyme responsible for degrading PAF. In a study of 240 patients following trauma or sepsis, the recombinant enzyme reduced mortality from 44.2% to 21.4% for patients with sepsis (unpublished). A phase III trial of this agent is currently in progress.

A randomized trial of inhibition of prostaglandin-E2 using ibuprofen failed to show a beneficial effect on mortality and similar disappointing results were obtained in a trial evaluating antagonism of bradykinin.

Nitric oxide is released from vascular endothelial cells in response to the upregulation of the enzyme, nitric oxide synthase, by bacterial products and proinflammatory cytokines. A phase II trial of a competitive inhibitor of nitric oxide synthase showed a beneficial effect on the resolution of septic shock; unfortunately, mortality was significantly increased in a follow-up phase III study, and further development of this compound has been suspended.²¹

The coagulation cascade

Bacterial products and proinflammatory mediators are potent activators of the coagulation cascade and extensive clinical and laboratory data show that intravascular coagulation contributes prominently to the pathogenesis of the sequelae of sepsis.²² A variety of strategies with the objective to reverse the procoagulant state, have been evaluated as therapies for sepsis.

The most successful approach to date has been the administration of recombinant human activated protein-C (APC). Protein-C is a naturally occurring anticoagulant that is produced by the liver and circulates in an inactive form. It is activated by the interaction of thrombomodulin on the endothelial cell with thrombin, generating a serine protease that inhibits Factors Va and VIIIa, and binds to a cell-surface receptor whose engagement blunts the expres-

sion of the proinflammatory cytokines, IL-6 and IL-8. A recent, phase III, multi-centre study of recombinant activated protein-C reported a significant reduction in 28-day all-cause mortality from 30.8% to 24.7% ($p=0.005$), a relative mortality reduction of almost 20%.²³ It is anticipated that this agent will be licensed for clinical use later this year, although the costs of a course of therapy are likely to be high.

Expression of tissue factor on the cell surface triggers the extrinsic coagulation pathway and tissue factor can be inhibited by tissue factor pathway inhibitor (TFPI). Treatment with recombinant TFPI in sepsis was associated with a trend towards decreased mortality in a small phase II study. However, a large, international, phase III study is in progress and will likely be completed in the near future.

Yet another anticoagulant strategy that has undergone clinical evaluation is the administration of anti-thrombin III, a potent, naturally occurring inhibitor of thrombin. Antithrombin III is known to be deficient in sepsis, and early trials suggested that its replenishment could improve clinical outcome. However, a phase III trial that recruited more than 2300 patients failed to confirm clinical efficacy, possibly because the pharmacological action of the compound was inhibited in a large number of patients by the concomitant use of heparin (Opal, personal communication).

Corticosteroids as adrenal replacement therapy

Finally, recent success has been reported with the use of adrenal corticosteroids in sepsis, not as agents that might non-selectively blunt the inflammatory response, but rather as replacement therapy for an acquired state of adrenal insufficiency. Studies by Annane showed that the combination of elevated basal cortisol levels and reduced adrenal responsiveness to ACTH stimulation predicted an increased risk of ICU mortality.²⁴ More recently, this same group demonstrated that correction of adrenal insufficiency with pharmacological doses of steroids (50 mg hydrocortisone q6h), in conjunction with fludrocortisone, can result in a statistically significant 15% absolute mortality reduction in patients with prolonged vasopressor-dependent septic shock.²⁵ In contrast to the considerable expense associated with the use of recombinant proteins in sepsis, glucocorticoid therapy is both effective and inexpensive. It appears that the patients most likely to benefit are those with impaired responsiveness to ACTH stimulation, in conjunction with prolonged requirements for vasoactive therapy.

Current status of mediator-directed therapy for sepsis

Intensive care physicians are, for the first time, faced with a new series of therapeutic decisions to determine which patient should receive adjuvant therapy and which agent should be used (Table 1). Clinical experience is essentially limited to popula-

tions enrolled in clinical trials, however, additional considerations of the differential biology of the agents, and the inferences that might be gleaned from subgroup analyses of completed trials, may assist in the decision-making process.

Therapy with recombinant proteins is expensive. Based on the use of other recombinant proteins, and on the use of anticytokine therapy for indications other than sepsis, the cost of a course of treatment with any of these agents is likely to be many thousands of dollars. From the perspective of cost, availability, and clinical efficacy, corticosteroid therapy is the most promising first-line therapeutic agent. Its role appears to be that of endocrine replacement therapy, and its use should be limited to those patients in whom that deficiency can be documented. The typical clinical profile is of a patient with prolonged dependence on vasopressors, often having a very high, non-spiking temperature. If at all possible, an ACTH stimulation test should be performed to establish the diagnosis of reduced responsiveness to stimulation. Although both hydrocortisone and fludrocortisone were used in the original study, often only hydrocortisone is administered at a dose of 50 mg every six hours. A reduction in temperature and in vasopressor requirements is typically seen within 12 to 24 hours; the optimal duration of therapy is unknown.

Activated protein-C is currently being reviewed for licensure by the FDA in the United States and it is anticipated that this agent will be available for clinical use by the end of this year. It is likely to be very expensive. Since the agent targets the coagulation cascade, it will probably be most useful in those patients with sepsis and disseminated intravascular coagulation (DIC), meningococemia being a particular attractive indication. APC was shown to reduce levels of D-dimers, thus this may be a useful marker

Table 1: Emerging options in adjuvant therapy for sepsis

Agent	Reference	Comments
Hydrocortisone	Annane ²⁴	15% absolute mortality reduction (21% RRR) for patients with persistent vasopressor-dependent septic shock and deficient response to ACTH stimulation; given for 7 days
Activated protein-C	Bernard ²³	6.1% absolute mortality reduction (19% RRR) for patients with severe sepsis and 3/4 SIRS criteria; FDA approval anticipated later this year
Anti-TNF monoclonal antibody	Panacek (Submitted)	4% absolute mortality reduction, 6.9% adjusted, 14% RRR for patients with severe sepsis and elevated IL-6 levels; licensure pending

of the patient most likely to benefit. APC is associated with an increased risk of bleeding, and so must be used with caution in the patient at risk for bleeding.

The status of the anti-TNF monoclonal antibody, afelimomab, is uncertain, despite recent evidence that it can improve survival and attenuate the severity of organ dysfunction. Subgroup analyses demonstrated the greatest efficacy in those patients with sepsis, but with only modest degrees of organ dysfunction at the time of drug administration. This observation is entirely consistent with the biological role of TNF as an early mediator of the inflammatory cascade.

Future challenges

There have been a great many disappointments in the more than 60 clinical trials that have evaluated strategies to modulate the inflammatory response in sepsis.¹⁸ The fact that we have only recently achieved some success is a testimonial to the difficulties of successfully intervening in a complex biological process. Indeed, a critical review of the course of sepsis research to date suggests that the negative trials of the past were not so much negative as indeterminate; the reasons for the lack of a strong therapeutic signal have important implications for both research and clinical decision-making (Table 2).

Virtually all studies performed over the last 15 years have used as their entry criteria, the physiological abnormalities of alterations in temperature, respiratory rate, heart rate, and white-cell count that are deemed to define sepsis syndrome²⁶ or the systemic inflammatory response syndrome (SIRS).²⁷ In fact, neither of these are truly syndromes, for they encompass a tremendously heterogeneous population of patients, with differing premorbid conditions, differences in the site, bacteriology, or even presence of infection, and different profiles of circulating inflammatory mediators.²⁸ Such heterogeneity dilutes any therapeutic signal that might be present in a trial.

Efficacy in these studies has been measured by the impact of the intervention on 28-day all-cause mortality. Mortality is a relatively insensitive measure of therapeutic effect in a population where a substantial component of the mortality risk is defined by severity of illness at the onset of the study and by co-morbidities that independently impact on survival, as well as, the extent to which aggressive supportive measures will be applied.²⁹

Equally importantly, the optimal duration of therapy and dosages of the agent is unknown, and generally established by protocol in a somewhat arbitrary manner. Titration of therapy to physiological response, a hallmark of ICU practice, is not permitted, nor is reinstatement of the study agent after the initial course, a situation not dissimilar to undertaking a study of a novel vasopressor that is administered in a single dose, and discontinued, regardless of the blood pressure. Indeed, there is some evidence that inappropriate doses, rather than intrinsic toxicity, account for

Table 2: Why has anti-inflammatory therapy had so little impact in clinical trials?

Issue	Challenges
Inferences from pre-clinical studies	<ul style="list-style-type: none"> <input type="checkbox"/> Responses often model- or species-specific <input type="checkbox"/> Models not representative of ICU patient-healthy animals, no concomitant support
Study population	<ul style="list-style-type: none"> <input type="checkbox"/> Non-specific entry criteria (Sepsis syndrome and SIRS) <input type="checkbox"/> Heterogeneity in site and bacteriology of infection <input type="checkbox"/> Heterogeneity in clinical response <input type="checkbox"/> Significant life-threatening co-morbidity <input type="checkbox"/> Genetic variability
Outcome measures	<ul style="list-style-type: none"> <input type="checkbox"/> Mortality is insensitive measure <input type="checkbox"/> Co-morbidities exert significant influence on survival <input type="checkbox"/> Lack of widely-accepted measures of morbidity or quality of life
Therapeutic agents	<ul style="list-style-type: none"> <input type="checkbox"/> Optimal dose and duration of therapy unknown <input type="checkbox"/> Repeat dosing not permitted <input type="checkbox"/> Unanticipated loss of biologic activity in vivo
Concomitant care	<ul style="list-style-type: none"> <input type="checkbox"/> Significant variability in placebo survival, approaches to practice, adequacy of care, and attitudes towards limitation of care

the unexpected increased mortality seen in the study of nitric oxide inhibition described above.²¹

The therapeutic efficacy may also be weakened by differences in concomitant care between centres, such as the surgical control of infections, antibiotic choices, ventilation strategies for ARDS, and other ICU treatments. Indeed, the differences between centres in the mortality rates in the placebo arms of several large phase III sepsis studies have been striking.

There is also evidence that some of the agents used in large, phase III, clinical trials were not biologically active. HA-1A, for example, has not been convincingly shown to neutralize endotoxin, and at least one preclinical study suggested that it was without effect.³¹ An anti-TNF antibody evaluated in approximately 2000 patients in the Norasept II trial, did not neutralize TNF bioactivity in the serum of the patients to whom it was administered (personal communication).

Finally, pre-clinical models demonstrate that a given strategy can have either beneficial or detrimental effects on survival, depending on the study model. Disruption of the inflammatory response may render a patient more susceptible to infection, and data from both the early corticosteroid trials,¹⁵ and a large trial of antiendotoxin antibody therapy¹⁰ suggest that the net effect of intervention was harm, at least in a subgroup of patients.

Clearly, a need for greater sophistication in diagnosing, classifying, and monitoring patients with sepsis has been demonstrated in work done to date, and the need will only become more acute as these expensive agents become available for clinical use.

The situation is not unlike that faced by oncologists three decades ago when the first effective agents for the adjuvant therapy of cancer became available. Future success will depend on the ability to apply an equally rigorous approach to the development, evaluation, and introduction of this potent new approach to modulating the course of critical illness.

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