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Potential Strategies for Inflammatory Mediator Manipulation: Retrospect and Prospect

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Abstract. Sepsis syndrome and septic shock remain significant causes of morbidity and mortality. To date, clinical trials of novel agents to treat sepsis have failed to demonstrate clinical efficacy despite considerable animal data to suggest a positive therapeutic benefit. This article reviews the recent major clinical trials on sepsis and discusses the hypotheses on which these therapies are based and the critical issues associated with clinical sepsis. Recommendations for future clinical trials on sepsis are made.

Sepsis is a systemic response to infection characterized by alterations in body temperature regulation, tachycardia, tachypnea, decreased systemic vascular resistance, leukocytosis or leukopenia, and evidence of organ dysfunction. Altered mental status, hypotension, hypoxia, oliguria, coagulopathy, and metabolic acidosis are frequently present. Sepsis and its sequela remain a major cause of morbidity and mortality in medical and surgical intensive care units (ICUs). Despite therapeutic and technologic advancements in the care of these patients, the average mortality ranges from 30% to 50%, and the incidence of this disorder continues to increase [1–7]. Between 1979 and 1987 the rate of septicemia increased 139%, from 73.6 to 176.0 per 100,000 persons [3]. Septic shock is the 13th leading cause of death in the United States and is a frequent cause of death in the ICU, resulting in an estimated 100,00 deaths per year [3, 7].

Sepsis is the clinical manifestation of the host-derived systemic inflammatory response resulting from invasive infection. Sepsis begins with a nidus of infection, mainly gram-negative bacilli or gram-positive cocci, which proliferate and either invade the bloodstream (bacteremia) or release various substances into it. The presence of bacteremia or endotoxemia may elicit the production of a cascade of endogenous mediators, resulting in a metabolic and immunologic host systemic inflammatory response. Microbial cellular components such as endotoxin, peptidoglycan, techoic acid, or various exotoxins released by microorganisms are thought to initiate this host systemic inflammatory response by stimulating monocytes or macrophages, endothelial cells, or neutrophils to release the endogenous mediators of sepsis (Fig. 1) [8, 9].

Experimental and clinical evidence has demonstrated the proinflammatory cytokines tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β) to be the most important cytokine mediators in the pathogenesis of sepsis syndrome and septic shock [10–15]; IL-6 increasingly appears to be a marker of the presence of activity of proinflammatory mediators [16]; and IL-8 seems to play a major role in neutrophil activation [17, 18]. These primary mediators stimulate a cascade of secondary mediators, including arachidonic acid-derived prostaglandin I_2 (PGI_2), thromboxane A_2, PGE_2, platelet-activating factor (PAF), bradykinin, histamine, serotonin, and complement (Fig. 1). Therefore sepsis may be viewed as a dysregulation syndrome of these messenger molecules; and once initiated, this clinical syndrome can become self-perpetuating and independent of the original infection. The time course for the appearance of TNFα and IL-1β in the plasma of experimental animals after the administration of live or heat-killed bacteria and in humans after the administration of *Escherichia coli* endotoxin has been well characterized. Typically, when measured, plasma concentrations of TNFα and IL-1β increase early in the course of severe sepsis and septic shock, whereas increases in plasma IL-6 and IL-8 concentrations tend to occur later in the septic process [19–25].

The administration of TNFα and IL-1β, alone or in combination, has been shown to reproduce many of the physiologic and laboratory changes observed in animal models and patients with sepsis syndrome and septic shock, including an increased IL-6 level [26, 27]. Experimental administration of IL-8 produces rapid granulocytopenia followed by granulocytosis with negligible elevation in TNFα, IL-1β, or IL-6 concentrations [18].

Based on our understanding of the pathophysiology of sepsis, clinical trials of interventional immunotherapy in humans have been directed primarily toward blocking the effects of either endotoxin, TNFα, or IL-1 [4, 5, 28–35]. To date, no new therapy has demonstrated sufficient clinical efficacy based on an intent-to-treat, 28-day, all-cause mortality analysis to warrant becoming standard therapy for the treatment of sepsis. This lack of success raises the issue of whether the hypotheses by which these compounds were evaluated in clinical trials were sufficiently sound or the molecules tested were ineffective. This paper reviews the potential strategies for mediator manipulation and examines the hypotheses, molecules, and models used in recent clinical trials.

If the hypotheses were sound, incremental advances in product

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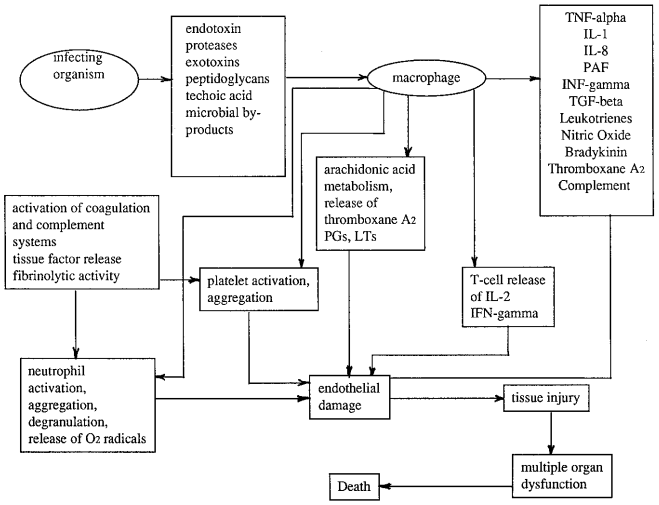

Fig. 1. Network of inflammatory mediators of sepsis.

development and efficacy may be sufficient to lead to positive results in future clinical trials. Alternately, the hypothesis on which these trials have been based may be flawed and therefore would require careful reexamination. Additionally, the current clinical trial's analytic design of evaluating intent-to-treat all-cause mortality may not be the most appropriate model to evaluate this complex disease. In going forward, we must reevaluate our hypotheses, our animal and human study designs, and the safety and efficacy of the molecules we are testing.

Endotoxin Blockade Strategy

Studies have shown that gram-negative bacteremia occurs in approximately 30% of patients with sepsis syndrome [36, 37]. Endotoxin, a lipopolysaccharide (LPS) component of gram-negative bacterial cell walls, causes many of the severe systemic manifestations of gram-negative bacteremia. Considerable basic and clinical evidence supports the hypothesis that the toxic manifestations induced by gram-negative bacteria are largely induced and mediated by endotoxin, particularly by its biologically active innermost component, lipid A [38–41]. Numerous in vitro and in vivo animal experiments have suggested that blocking the effects of endotoxin leads to improved survival. In each of these experiments the challenge was known (e.g., LPS or *E. coli*) and was applied at a known time [19, 26, 42–45]. This condition differs dramatically from most clinical trials where the patients were ill over varying lengths of time from varying challenges and with a host of other underlying diseases. The complexities of human disease makes it difficult to make the transition from animal models to clinical trials.

Immunotherapy with either human polyclonal J5 antiserum derived from normal volunteers immunized with *E. coli* J5, a mutant with only core determinants in its endotoxin, or plasma directed against endotoxin core determinants has been shown in clinical trials to reduce mortality in patients with gram-negative bacteremia [28, 30] and to protect high risk surgical patients from septic shock [29]. Patients enrolled in these clinical trials were carefully screened for documented evidence of gram-negative infection. These observations lead to several clinical experiments using two IgM monoclonal antibodies (MAbs: HA-1A, a predominantly human IgM MAb, and E5, a murine MAb) derived from the same heat-inactivated *E. coli* J5 vaccine that induced polyclonal J5 antiserum. Both HA-1A and E5 have been evaluated in more than one phase III clinical trial.

On an intent-to-treat analysis, HA-1A did not reduce mortality. The placebo mortality was 43% compared to 39% in the HA-1A-treated group. However, in the subgroup with gram-negative bacteremia (200 patients) mortality was reduced by 39% (p = 0.014). Mortality was reduced by 42% in patients with both gram-negative bacteremia and shock (p = 0.017) [5]. Although the initial results appeared promising and led to licensing of HA-1A in Europe, a confirmatory trial was undertaken at the request of the U.S. Food and Drug Administration (FDA) [46]. The primary endpoint of the second trial was 14-day all-cause mortality in the predefined subgroup of patients with gram-negative bacteremia plus shock present within 24 hours of study entry. The mortality rate was 37% for the placebo group and 41% for the HA-1A group. For patients with gram-negative bacteremia the mortality was 32% for the placebo group and 33% for the HA-1A group [46].

In a similarly designed study using E5, Greenman et al. were unable to demonstrate prospectively a reduction in intent-to-treat all-cause mortality in patients with gram-negative sepsis [4]. On retrospective analysis, E5 reduced mortality in the subgroup with gram-negative infection but without shock from 43% in the placebo group to 30% in the E5-treated group. This result was not repeated in a second trial. The 30-day all-cause mortality rate in patients with gram-negative sepsis and organ dysfunction (n = 139) was 41% in the E5-treated group and 47% in the placebo group (p = NS). Currently, a third trial with E5 is under way focusing on patients with gram-negative sepsis and organ dysfunction or shock (or both).

Using a polyclonal immunoglobulin IgGMA preparation, Schedel et al. enrolled patients only if they had a measurable endotoxin level (> 12.5 pg/ml) and met sepsis criteria. Deaths occurred in 9 of 28 patients (32%) in the placebo group compared to 1 of 27 (4%) in the IgGMA-treated group [30]. Although it was a small, unblinded study, it demonstrates the potential power of using an endotoxin assay as a screening tool to select appropriate patients for clinical trials of antiendotoxin therapy. Presumably, enrolling patients in clinical sepsis trials with a documented presence of endotoxin who meet the clinical criteria of sepsis syndrome would be the most desirable situation for testing the efficacy of an antiendotoxin molecule. Certainly, the larger trials would have been more convincing if a treatment effect was documented in patients in whom endotoxin had been demonstrated to be present. Perhaps HA-1A and E5 have a low affinity for endotoxin. If true, it raises the possibility that a potent endotoxin-binding agent might prevail where these agents have failed. One such possibility exists with bactericidal/permeability-increasing protein (BPI), a naturally occurring cationic protein found in the azurophil granule of the human neutrophil [47]. BPI specifically binds and neutralizes endotoxin in a variety of biologic systems [48–50], has a high binding affinity for the lipid A domain of endotoxin [49, 50], and shares a 44% sequence homology at the amino acid level with LPS-binding protein (LBP). This LBP binds to LPS and stimulates endotoxin-induced inflammatory activity in vitro [49–51], whereas BPI competes with LBP, binds LPS, and

blocks LPS interaction with cells in vitro. Opal et al. have demonstrated that LBP and BPI ratios flip in the presence of infection, suggesting that BPI plays a significant role in primary host defense. Furthermore, BPI has been shown to up-regulate in the face of gram-negative infection [52].

Animal studies of sepsis have demonstrated that BPI provides both protection and salvage [51]. Marra and colleagues have clearly demonstrated the potent endotoxin-binding properties of BPI and the relative weak LPS-binding properties of both HA-1A and E5 [50]. This evidence suggests that BPI is a potent, naturally occurring anti-LPS protein that is part of the primary line of host defense.

The current clinical trial data available do not prove or disprove the endotoxin-induction hypothesis of gram-negative sepsis. If true, the possibility remains that the endotoxin-induced pathway of gram-negative sepsis is valid but has not yet been proved in clinical trials because of inadequacies in either the clinical trial design or the antiendotoxin molecules tested. This situation tends to support the need for additional clinical work, utilizing more potent second-generation endotoxin binding and neutralizing agents.

TNF Blockade Strategy

Tumor necrosis factor α is thought to play a major role in shock, organ failure, and the systemic manifestations of sepsis and septic shock [9–15, 19, 26]. Injection of TNFα into animals and humans induces hypotension, activation of the clotting cascade, and organ dysfunction. Considerable experimental evidence suggests that blocking TNFα may prevent the sequelae of and reduce the mortality due to septic shock. Anti-TNFα antibodies protect animals from death caused by endotoxin or gram-negative or gram-positive bacteria. Although TNFα is part of the endogenous host defense, it has been demonstrated that excess TNFα is deleterious. Thus it has been postulated that blocking TNFα in this setting may be beneficial [53–57].

In a small phase II trial using a murine monoclonal anti-TNF antibody [16], no overall survival benefit was observed in the 80 patients enrolled. However, in 35 patients with increased circulating TNFα concentrations ($>$ 50 pg/ml) present at study entry, those receiving the high-dose anti-TNF antibody treatment appeared to benefit (86% with a 28-day survival) [32]. It is of interest that study entry circulating TNFα concentrations were highest in patients with gram-negative bacteremia, particularly those who were in shock at study entry [32].

Abraham and colleagues [35] reported no reduction in 28-day all-cause mortality in 35 patients with sepsis syndrome treated with an anti-TNFα MAb compared to those given placebo [101 of 323 patients died (31.3%) in the 15 mg/kg group, 95 of 322 patients (29.5%) in the 7.5 mg/kg group, and 108 of 326 (33.1%) in the placebo group]. Of the 478 patients with shock present at study entry, the 28-day all-cause mortality was 61 of 162 patients (37.7%) in the 15 mg/kg group, 59 of 156 patients (37.8%) in the 7.5 mg/kg group, and 73 of 160 patients (45.6%) in the placebo group. These differences are not statistically significant. However, among shock patients these authors observed a reduction in mortality 3 days after infusion of the study agent: 44% (15 mg/kg group) and 48.7% (7.5 mg/kg group) compared with placebo (p = 0.01 and p = 0.004, respectively) [35]. A follow-up study is under way to evaluate the efficacy of a murine monoclonal anti-TNFα IgG monoclonal antibody in patients with sepsis syndrome and shock present at study enrollment. If these results can be reproduced prospectively, it would lend support to the TNFα-mediated hypothesis of septic shock.

Two phase II clinical trials have been completed using Fab′2 murine anti-TNFα monoclonal antibodies, which are highly human-specific. Although both trials were small and not designed to demonstrate efficacy, when the data were combined and stratified based on IL-6 concentrations of $>$ 1000 pg/ml at study entry, the following 28-day all-cause mortalities were observed. In the placebo group 10 of 11 patients died (91%) compared to 9 of 11 dead (82%) in the 0.1 mg/kg group, 9 of 17 dead (53%) in the 0.3 mg/kg group, 4 of 15 dead (27%) in the 1.0 mg/kg group, and 1 of 4 dead (25%) in the 3.0 mg/kg group (C.J. Fisher, unpublished data; K. Reinhart, personal communication). These data tend to support the TNFα-mediated hypothesis of septic shock and are particularly interesting because of their use of IL-6 concentrations, which we and others have advocated using as a marker of cytokine activation in clinical trials of immunotherapy for septic shock.

It is thought that TNFα mediates its effects through the 60- and 80-kDa cell-surface TNF receptors. The extracellular portion of the receptor is shed in vivo, binding circulating TNFα before it can signal the target cell [58–62]. Potentially, these soluble receptors may be an alternate approach to anti-TNFα monoclonal antibodies for binding TNFα. A recombinant TNF receptor Fc fusion protein formed by linking the extracellular portion of two p80 TNF receptors with the Fc portion of IgG (rhu TNFR:Fc, Immunex), has been tested in patients with septic shock with disappointing and worrisome results. In that clinical trial, significantly increased mortality (p = 0.016) was observed in the treatment groups with increasing doses of rhu TNFr:Fc 86. If these disconcerting findings cannot be explained by imbalance or some as yet unrecognized toxicity of rhu TNFr:Fc, it raises the question of whether removal of TNFα is deleterious in patients with septic shock. Although these observations do not disprove the TNFα-mediated septic shock hypothesis, they certainly are sobering observations regarding how we should approach blocking endogenous host defenses.

IL-1 Blockade Strategy

Interleukin 1 is an important mediator in the sepsis syndrome and septic shock [14, 15, 20, 27, 63–66]. Infusion of IL-1β produces hypotension in experimental animals and functions synergistically with TNFα to induce the hemodynamic features found with gram-negative sepsis and endotoxin-induced shock [20, 27, 63, 65]. There are several possible approaches to IL-1 blockade, including soluble IL-1 receptors, antibodies directed against IL-1 receptor, inhibition of IL-1 converting enzyme, and IL-1 receptor antagonist (IL-1ra). Of these possibilities, only IL-1ra has been tested to date in clinical sepsis trials [33, 34]. IL-1ra is produced by macrophages and other cells in response to IL-1, endotoxin, and other microbial products; it recognizes and binds to both type I and type II IL-1 receptor yet possesses no IL-1 agonist activity [66–68].

Recombinant IL-1ra (rhIL-1ra) infusion results in a dose-dependent decrease in the mortality due to endotoxin-induced shock in rabbits and the decreased mortality due to *E. coli*-induced septic shock in primates [69, 70]. Similarly, in a small

phase II clinical trial (99 patients), mortality decreased in a dose-dependent manner with increasing doses of rhIL-1ra [33]. These observations were not reproduced in a large clinical trial testing two doses of rhIL-1ra (1.0 and 2.0 mg/kg) versus placebo [34]. A significant increase in survival time was not observed in either all patients who received rhIL-1ra ($n = 893$; $p = 0.22$) or only those who presented with shock at study entry ($n = 713$; $p = 0.23$). Secondary analysis from this trial suggested an increase in survival time with rhIL-1ra treatment in patients with more than one organ dysfunction present at study entry ($n = 563$; $p = 0.009$) [34]. A follow-up trial was initiated to study patients with shock and organ failure. At the time of this writing, the trial has been stopped, based on a interim analysis suggesting that efficacy would be difficult to prove. There was no evidence to suggest a deleterious effect. These data neither prove or disprove the IL-1-mediation hypothesis of sepsis.

Blockade of Other Mediators

Platelet-activating factor (PAF), a potent phospholipid mediator involved in sepsis [71–73], is produced by a variety of cells, including endothelial cells, platelets, leukocytes, monocytes, and lymphocytes. PAF leads to the release of inflammatory mediators [74, 75], and infusion of PAF reproduces many of the signs of sepsis [71, 75]. PAF concentrations are increased following endotoxin challenge [76, 77]. In a phase III study infusion of a PAF antagonist did not significantly reduce mortality.

Bradykinin antagonist has also been examined in a large phase II study. No overall treatment benefit was observed [78].

Ongoing Clinical Trials

On-going clinical trials in sepsis include inhibition of cyclooxygenase, inhibition of nitric oxide, anti-TNF MAb in patients with septic shock, Fab′2 anti-TNF MAb in patients with sepsis and elevated IL-6 concentrations at study entry, and sTNFR:FC-IgG (Table 1). Other approaches being evaluated for clinical trials include antiendotoxin approaches using more potent endotoxin binding and neutralizing drugs, inhibition of coagulation, granulocyte colony-stimulating factor, and TNF-binding protein.

Future Directions in Sepsis Research

Expertise in the design and conduct of clinical trials on sepsis has grown enormously since the early trials with steroids [79]. Current trials are prospective, randomized, double-blind, and placebo-controlled with a priori definitions and analytic plans. Despite using optimal clinical trial designs, we still struggle with a heterogeneous patient population. We continue to refine our definitions in an effort to make the patients enrolled in clinical trials more homogeneous and therefore provide a better opportunity to test our hypotheses. The addition of biologic markers such as IL-6, IL-8, TNF, IL-1 sTNF:rc, IL-1ra, endotoxin, and others during patient screening and enrollment may further refine the study patient population. For example, IL-6 and endotoxin have both been demonstrated to be good markers of disease activity and have both been used in clinical trials. Would it not be prudent to measure the presence of endotoxin in patients prior to enrolling them in an antiendotoxin clinical trial or to use IL-6 concentrations as an index of disease severity for entry and duration of therapy.

Table 1. Summary of clinical trials with proinflammatory mediator antagonists.[a]

Mediator	Improved outcomes[b]		Year of trial or publication	Reference
	Phase II	Phase III		
LPS antibodies				
J5	Yes	No	1982, 1985 1988, 1992	28, 29, 80–83
E5	NA	No	1990	4, 84
HA-1A	NA	No	1990	5, 85
TNF				
MAb	Yes	No	1991, 1993	32, 35
sTNFR:Fc-IgG	No	ND	1994	86
IL-1 RA	Yes	No	1993, 1994	33, 34
PAF antagonist	Yes	No	1993, 1994	73
Cyclooxygenase: ibuprofen	Yes	ND	1991	87, 88
Bradykinin: bradycor	No	ND	1994	78
Corticosteroids	Yes	No	1984, 1987	36, 37, 89

NA: Not applicable; ND: data not available.
[a]Published and unpublished data.
[b]Results compared with controls.

Clinical trials must address intrinsic differences in patient populations such as those that exist between medical, surgical, and trauma patients. Stratification for these and other characteristics, such as center and country differences, must be addressed. Furthermore, the intervention is usually for a short period, yet our test is a 28-day all-cause mortality. This setup is different from that in the animal models and perhaps is an unrealistic expectation.

Animal models must be refined to more closely approximate the course of sepsis in humans. These refined animal models will be invaluable for answering such questions as when to introduce mediator blockade, duration of therapy, and the role of combination immunotherapy. Serious consideration should be given to the appropriateness of 28-day mortality as the definitive endpoint versus resolution of organ failure or reduction of biologic markers of disease such as IL-6.

Recommendations for Future Clinical Trials on Sepsis

The therapeutic rationale should be well worked out in animal models. Ideally, the mechanism of action of the agent should be understood and efficacy established in at least two animal models. Biologic markers such as endotoxin and IL-6 should be seriously considered for entry criteria and for monitoring efficacy during and after therapy. Patient populations should be stratified for site and country and for medical, surgical, or trauma patients. Power calculations should be conservative and the sample size large enough to answer the question being asked. Antibiotics should be standardized. Clinical centers selected for enrolling patients into clinical trials should be selected for their demonstrated expertise in the study and management of septic patients, not on a patient accrual basis only. Ideally, patients enrolled in clinical trials should be carefully selected so the hypothesis and the molecule can be adequately and fairly tested.

Résumé

Le syndrome de sepsis et le choe septique sont une cause non négligeable de morbidité et de mortalité. Jusqu'à présent, les essais cliniques testant les nouveaux agents en matière de sepsis n'ont pas démontré d'efficacité réelle clinique en dépit d'un nombre considérable d'information suggérant un bénéfice thérapeutique réel chez l'animal. Cet article résume les essais récents concernant le sepsis, discute les hypothèses sur lesquelles ces attitudes thérapeutiques sont basées et les problèmes inhérents aux essais thérapeutiques en matière de sepsis. Des recommandations pour les essais thérapeutiques à venir sont données.

Resumen

El sìndrome sèptico y el shock sèptico se mantienen como causa significativa de morbilidad y mortalidad. Hasta la fecha, los ensayos clìnicos con noveles agentes en la sepsis han fallado en cuanto a demostrar eficacia clìnica, a pesar de considerable informaciòn proveniente de animales experimentales que sugiere un beneficio terapèutico positivo. El presente artìculo revisa los recientes ensayos clìnicos mayores en la sepsis, discute las hipòtesis sobre las cuales se fundamentan tales terapias y los aspec tos crìticos asociados con la sepsis clìnica. Finalmente, se hacen recomendaciones acerca de futuros ensayos clìnicos en la sepsis.

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