

A New Paradigm for the Treatment of Sepsis: Is It Time To Consider Combination Therapy?

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Despite the advances in supportive care and the availability of potent antimicrobial agents, mortality from sepsis, a leading cause of death in intensive care units, has not improved. Over the last decade, clinical trials with numerous adjunctive therapies, including antiendotoxin antibodies and inhibitors of the inflammatory response, have yielded disappointing results. Recently, treatment with recombinant human activated protein C reduced mortality 6% compared with controls. Given the likelihood that many processes in the complex pathophysiology of sepsis are simultaneously activated, it is unlikely that therapy directed at any one of them, as has been done in the past, will dramatically improve survival. Rather, a combination of therapies directed at many arms of the septic process, much like the strategy used for cancer and

HIV infection, is required. Given the likelihood that sepsis represents an excessive innate immune response to microbial products, vigorous attempts must be made to develop rapid assays that reflect the level of innate immune activation. Such assays could be used to identify patients who would benefit from therapy and to monitor their response so that overtreatment does not completely abrogate host defense mechanisms and render these patients susceptible to fatal infection. It is now time to test a new therapeutic paradigm based on an improved understanding of the pathophysiology of the septic process and the recognition that we may have reached the limits of adjunctive monotherapy.

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Despite a long series of disheartening failures, researchers continue an aggressive search for a sepsis therapy that could significantly reduce mortality from this disease. Recent clinical trials have built on the experience gained in the design of previous trials and in our understanding of the pathogenesis of this condition. The report that infusions of recombinant human activated protein C (rhAPC) reduced the relative risk for death in septic shock by almost 20% over the control group emphasizes that point (1). While the success of rhAPC in this clinical trial is impressive when measured against previous results with other therapies for sepsis, it is also an important reminder that we need to change our approach to therapy for this leading cause of death in intensive care units.

Natanson and colleagues (2) performed a highly informative meta-analysis of 21 clinical studies that evaluated the efficacy of inflammatory mediator modulation in more than 10 000 patients. They observed that it was impossible to draw any conclusion about treatment effect from studies with small enrollments because of the large confidence intervals. In contrast, studies that enrolled large numbers of patients demonstrated with relatively narrow confidence intervals a marginal improvement in mortality after mediator modulation. After pooling the results of all studies, Natanson and colleagues calculated a small but significant improvement in survival (odds ratio, 1.17).

One can conclude that it is necessary to do a very large study with a highly active compound to measure a small benefit in a single study. Such a study was recently reported (MONARCS [Monoclonal Anti-TNF, A Randomized Controlled Sepsis trial]) (3). This study, the largest clinical trial for sepsis to date, randomly assigned more than 3000 patients to receive one of several doses of a Fab component of a monoclonal antibody against tumor necrosis factor (TNF) or placebo. As predicted by Natanson and colleagues, there was a small improvement in mortality

that mirrored the data presented in their meta-analysis. These observations suggest that it would be unlikely that any single inflammatory mediator modulation would provide the "home run" therapy anticipated.

Similarly, a long-anticipated trial with a different reagent, recombinant bactericidal/permeability-increasing (BPI) protein for treatment of meningococcal sepsis, was reported (4). Unlike the anti-inflammatory mediator strategy, BPI targets a source of mediator induction, the lipopolysaccharide of gram-negative bacteria. Despite heroic attempts at optimizing clinical design, the BPI-treated patients showed only a modest, yet statistically significant, improvement in organ injury scores and no reduction in mortality.

A critique of those earlier studies (that is, MONARCS and the study of BPI) is beyond the scope of this essay, but some of the issues have been addressed (5). In these as well as in the earlier, failed studies (reviewed by Natanson and colleagues), the problem was rarely that the test agent lacked biological activity. Each of the previous agents could modify the activity of the target molecule. Indeed, many of the anti-TNF interventions have become licensed effective products for other disease processes, such as inflammatory bowel disease and rheumatoid arthritis. License applications are pending for additional indications. This suggests that sepsis is a more complex process than many of these other complicated diseases. Again, this complexity demands that we reconsider our approach to therapy for sepsis.

Sepsis, currently defined as a systemic inflammatory response in the presence of infection (6), is increasingly being considered an exaggerated, poorly regulated innate immune response to microbial products (7). Thus, abrogation of the inflammatory response with therapy early in the development of sepsis may render the patient susceptible to infection. Fatal infection necessitated cessation of some therapeutic trials (8, 9). In contrast, a similar abrogation of

that response late in sepsis, when the patient is more in danger from the excessive inflammatory response, may be of benefit. The availability of rapid, reliable assays that could be used to quickly identify the stage or severity of sepsis and to monitor therapy may optimize use of immunomodulatory therapy. Unfortunately, no such assays are available.

The inability to identify the patients at risk is a problem both for patient enrollment in studies and for establishing guidelines for therapy. In the rhAPC and anti-TNF studies, the interleukin-6 level failed to identify patients who would benefit from therapy. In the former study, however, the Acute Physiology and Chronic Health Evaluation score did identify patients who benefited most from therapy and was used for product labeling. The serum procalcitonin level has also been proposed as a useful indicator of severity (10). In the near future, it may be possible to accurately predict the likelihood of response to immunomodulatory agents through gene chip technology. This may allow for specific, early interventions tailored to the patient's individual needs, based on the knowledge of a patient's particular single nucleotide polymorphism, which may be associated with a more vigorous inflammatory response.

Microbes and microbial products (for example, lipopolysaccharide, peptidoglycan, DNA) stimulate cells of the immune system, including neutrophils, endothelial cells, and macrophages, and initiate coagulation, kinin, and complement cascades (Figure). Microbial products are recognized by members of a family of pathogen-associated molecular pattern receptors that shares homology with the Toll proteins on *Drosophila* that participate in fruit fly development and antimicrobial defense; consequently, they are called "Toll-like receptors" (11). Through these and other surface receptors, microbial products stimulate intracellular signaling pathways that generate inflammatory mediators (including cytokines [such as TNF- α and interleukin-1], free radicals [including oxidants and nitric oxide], and arachidonate metabolites [including phospholipid mediators such as platelet-activating factor]). When properly regulated, these host responses form effective antimicrobial defenses. In excess, these responses devolve into sepsis.

The complexity of this response affords ample targets for therapy, as indicated in the Figure. Clinical trials with single agents have been or are being conducted with inhibitors of TNF- α , interleukin-1, kinins, platelet-activating factor, coagulation factors, nitric oxide generation, lipopolysaccharide receptors (E5564, anti-CD14), and lipopolysaccharide itself. In addition, evidence suggests that a longer (>2 to 3 days) course of low-dose corticosteroid therapy may be of benefit, particularly in septic patients with poor adrenal reserve as measured by the corticotropin stimulation test (12). Early aggressive maintenance of blood pressure and oxygen saturation may also improve outcome from sepsis (13). Finally, subgroup analysis of patients entered into the rhAPC and antithrombin III

studies indicates that the use of low-dose (<15 000 U/d) unfractionated heparin may merit further study. Experimental therapies have been evaluated in models of sepsis with other agents shown in the Figure, including inhibitors of intracellular signaling proteins such as tyrosine kinase and mitogen-activated protein kinases.

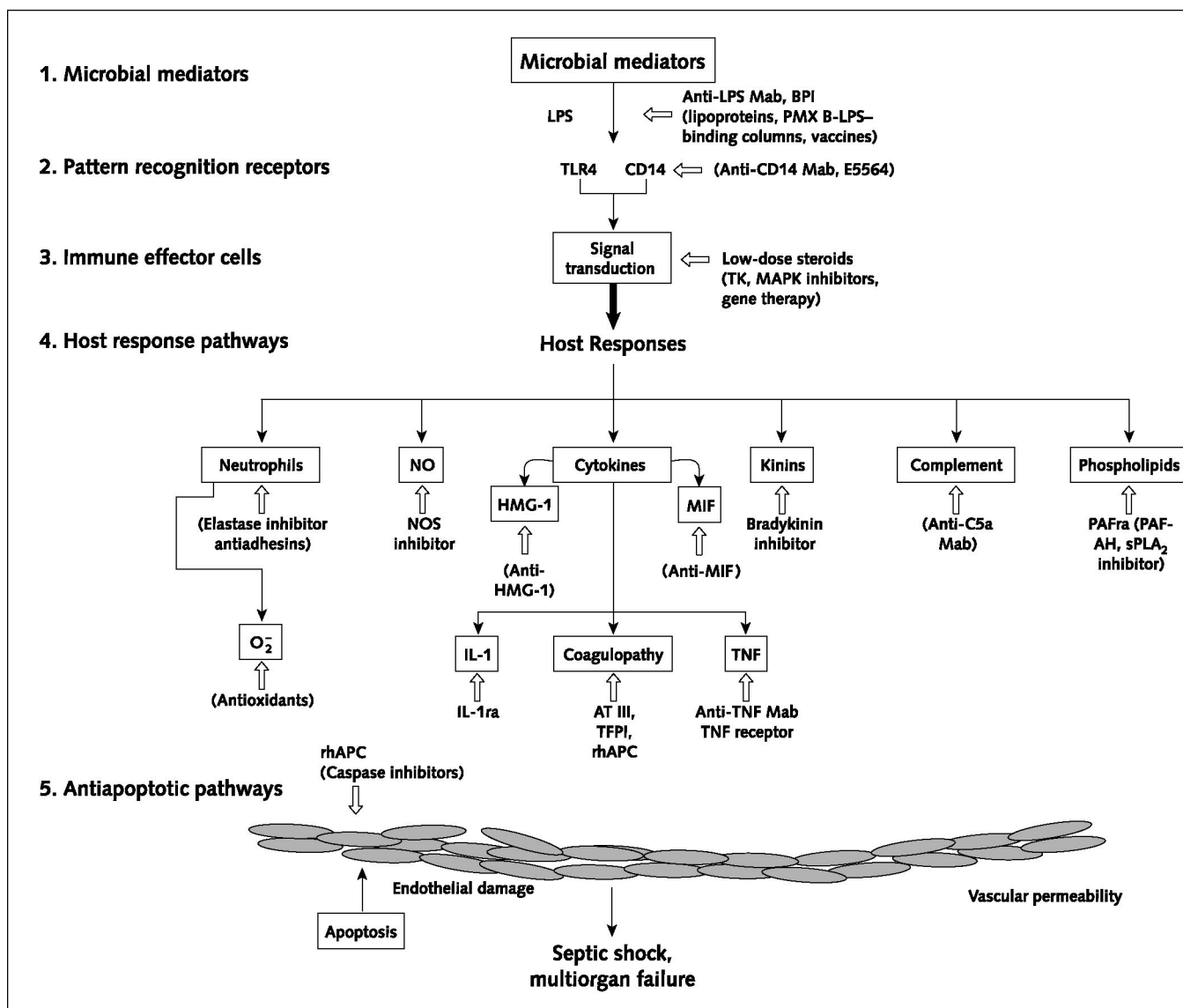
At the time of diagnosis of sepsis, an entire network of cellular responses and septic cascades is probably already activated. Consequently, it is unlikely that therapy aimed at only one process (for example, recognition of microbial products by immune-competent cells; generation of inflammatory mediators; coagulopathy) would by itself have the highly significant impact on sepsis mortality that has been sought.

Neutralization of endotoxin (through the use of BPI), inhibition of TNF activity (through the use of anti-TNF monoclonal antibody), and arrest of coagulopathy leading to multiorgan failure (through the use of rhAPC) may have been effective in large clinical trials, but only modestly affected total mortality (for example, a 6% absolute reduction in mortality for rhAPC). Furthermore, the disappointing results from the recent phase III trials with similar endogenous anticoagulants (high-dose antithrombin [14] and tissue factor pathway inhibitor [15]) have tempered enthusiasm for the hypothesis that inhibition of thrombus formation in the microcirculation during sepsis may prevent organ failure and thereby improve outcome. Rather, recent studies have suggested alternative mechanisms for the efficacy of rhAPC (16, 17). While neutralization of some underappreciated inflammatory mediator or of one of the newly described late-acting cytokines (such as migration inhibitory factor and high mobility group 1 protein) (18, 19) may provide the single-therapy "Holy Grail" of sepsis intervention, it is more likely that survival from this condition will improve only through combination therapy directed at multiple stages of the sepsis process.

Combination therapy directed at a single process (for example, interleukin-1 and TNF cytokine generation) would not be expected to be any more successful than effective single-dose therapy against that one process. In fact, experimental evidence suggests that inhibition of both interleukin-1 and TNF increases the septic risk by blocking so much of the cytokine response that none is left for normal, effective host defenses (20). Furthermore, an animal model of sepsis shows that therapy directed against the bacteria, the circulating endotoxin, and the induced cytokines is far more effective in preventing death than any one therapy or combination of two therapies (21).

Until recently, it has been considered unethical to conduct a trial of combination therapy since any single arm of that trial would have no expected benefit. Now, with the completion of large studies demonstrating the safety and modest effectiveness of rhAPC and perhaps BPI and anti-TNF antibody therapy, testing combination versus single-agent therapy for sepsis seems reasonable. A similar approach of combination therapy has been highly successful

Figure. Novel interventions for the treatment of severe sepsis.



The five potential intervention points are directed toward 1) microbial mediators, 2) pathogen-associated molecular patterns, 3) signal transduction mechanisms of immune effector cells, 4) host response mediator networks, and 5) antiapoptotic pathways. The solid arrows are activation sites, and the open arrows are inhibitory sites. Interventions that have gone through phase III tests are listed, along with experimental agents that are under investigation (in parentheses). AT III = antithrombin III; BPI = bactericidal/permeability-increasing protein; HMG-1 = high mobility group 1 protein; IL-1ra = interleukin-1 receptor antagonist; LPS = lipopolysaccharide; Mab = monoclonal antibody; MAPK = mitogen-activated protein kinase; MIF = migration inhibitory factor; NO = nitric oxide; NOS = nitric oxide synthase; PAF-AH = platelet-activating factor acetyl-hydrolase; PAF_ra = platelet-activating factor receptor antagonist; PMX B = polymyxin B; rhAPC = recombinant human activated protein C; sPLA₂ = soluble phospholipase A₂; TFPI = tissue factor pathway inhibitor; TK = tyrosine kinase; TLR = Toll-like receptor; TNF = tumor necrosis factor.

in improved treatment for HIV infection and many neoplastic diseases.

In summary, we have learned much from the extensive experience in evaluating biological therapies for sepsis. The most recent studies have shown that these can be given safely and have small but demonstrable clinical efficacy. Yet these well-controlled studies have also suggested that a dramatic breakthrough with monotherapy is unlikely. It is now time to test a new paradigm based on an improved understanding of the pathophysiology of the septic process

and the recognition that we must step beyond single-agent therapy. While this poses practical problems (for example, joint development of a product by separate companies), these are not insurmountable. It is time to begin.

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