A prevailing hypothesis is that at low levels cytokines perform important homeostatic actions that regulate immunologic and physiologic events. At higher concentrations, cytokines can potentially exert harmful biologic effects, which range from tissue and organ dysfunction to a life-threatening systemic reaction. Cytokines are "communication proteins," which are essential for cell-to-cell signaling. Cytokines transmit information by binding to specific transmembrane receptors on responding cells in peripheral tissues and initiate a signal-transduction mechanism.

Molecular biologic techniques have made possible explicit definition of the structure of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-4, IL-6, and IL-8. All of these cytokines might be involved in the sepsis syndrome. Cytokine receptors have been described, characterized, and cloned for TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-8. These receptors are all transmembrane glycoproteins which have an extracellular binding domain specific for a particular cytokine and an intracellular signal-transducing domain that initiates signal transduction. It appears to be obligatory that a cell has a surface receptor for a particular cytokine in order for that cytokine to elicit a response. However, the number and ligand affinity of these surface receptors does not necessarily predict the intensity or type of response that can be elicited.

Cytokines appear in the bloodstream of animals experimentally treated with endotoxin and the bloodstream of humans with serious spontaneous Gram-negative bacterial infections. Since cytokines have been implicated as important mediators of sepsis, it seems sensible to devise ways to interrupt or mitigate their actions on target cells. There are two basic strategies that can be employed to reach this goal: inhibiting the cytokine while it is still in the peripheral circulation and blocking receptor-cytokine interaction in the target tissue. Antibodies can be employed to accomplish both goals since they can be directed toward either the cytokine or the cytokine receptor. More recently, other potential therapeutic agents have become available. Under specific physiologic conditions, some of the cytokine receptors shed the extracellular ligand-binding domain into the circulation. Each of these "circulating inhibitors" can bind its cytokine and prevent any biologic response to it.

In addition to circulating inhibitors, cytokineline molecules have been described. These molecules bind to the IL-1 receptor, block IL-1 binding, and are incapable of eliciting signal transduction.

In this review, we will examine the experimental evidence that supports the therapeutic use of three different types of agents: antibodies, circulating inhibitors, and receptor antagonists (Table 1). The potential of these inhibitors to serve as therapeutic agents awaits clarification. Although the biotechnology exists to support these human studies, careful, prospective, placebo-controlled randomized trials in well-defined groups of patients are necessary to precisely define any therapeutic value of these newer and more expensive agents. It should be cautioned that these agents may have unexpected deleterious effects, since cytokines appear to be important in tissue homeostatic and reparatory processes. Preliminary data indicate

<table>
<thead>
<tr>
<th>Table 1 — Potential Therapeutic Agents for Sepsis Syndrome That Block Cytokines</th>
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<tr>
<td><strong>Anticytokine antibodies</strong></td>
</tr>
<tr>
<td>Anti-TNF-α&lt;sup&gt;1,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-IL-6&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td><strong>Anti-receptor antibodies</strong></td>
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<tr>
<td>Anti-IL-1 receptor&lt;sup&gt;4,5&lt;/sup&gt;</td>
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<td><strong>Circulating inhibitors</strong></td>
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<tr>
<td>IL-1 inh&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>IL-2 inh&lt;sup&gt;7,8&lt;/sup&gt;</td>
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<tr>
<td>IL-4 inh&lt;sup&gt;9&lt;/sup&gt;</td>
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<tr>
<td>IL-6 inh&lt;sup&gt;10&lt;/sup&gt;</td>
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<tr>
<td>TNF-α inh&lt;sup&gt;11,12&lt;/sup&gt;</td>
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<tr>
<td><strong>Receptor antagonist</strong></td>
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<td>IL-1 ra&lt;sup&gt;13,14&lt;/sup&gt;</td>
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*From the Department of Veterans Affairs and Department of Medicine, Vanderbilt University, Nashville.
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that endogenous cytokine inhibitors may have important homeostatic regulatory actions. Spinas et al\textsuperscript{24} recently reported that cytokine inhibitors were present in the plasma of six human volunteers who were treated with a bolus intravenous injection of endotoxin at a dose of 4 ng/kg. The authors hypothesized that these inhibitors might regulate the potential deleterious effects of endogenously released TNF-\(\alpha\) and IL-1\(\beta\). Prieur et al\textsuperscript{25} also reported that a circulating IL-1 inhibitor exists in the serum and urine of patients with systemic juvenile chronic arthritis.

**Anticytokine Antibodies**

Although the efficacy of anticytokine and anticytokine receptor antibodies has not been established in human sepsis, there is considerable experience utilizing cellular and animal models of sepsis. Beutler et al\textsuperscript{26} first reported that anti-TNF-\(\alpha\) antibodies protected against the lethal effect of endotoxemia in mice. Tracey et al\textsuperscript{27} used anti-TNF-\(\alpha\) monoclonal antibodies to passively immunize baboons prior to treatment with live *Escherichia coli*. In their study, pretreatment with antibodies for 2 h completely protected against shock and multiple-system organ failure in three baboons. Fong et al\textsuperscript{28} showed that intravascular infusion of a lethal number of *E coli* organisms into baboons resulted in increased circulating levels of TNF-\(\alpha\), IL-1\(\beta\), and IL-6. These authors could attenuate this release of cytokines by passive immunization of the animals with TNF-\(\alpha\) antibodies. Bagby et al\textsuperscript{29} reported that administration of TNF-\(\alpha\) antibodies improved survival from 8 percent to 75 percent in rats that were treated with intravenous endotoxin. Grau et al\textsuperscript{30} reported that TNF-\(\alpha\) antibodies could diminish the lethality of TNF-\(\alpha\) infusion in mice. These authors noted that there was not a clear relationship between the *in vitro* binding of the TNF-\(\alpha\) antibody to TNF-\(\alpha\) and the *in vivo* efficacy and recommended extensive *in vivo* testing of potential therapeutic antibodies.

There is very little information regarding the effects of anticytokine antibodies in humans. Exley et al\textsuperscript{31} reported preliminary observations in a phase I study of a murine monoclonal antibody to human rTNF-\(\alpha\) in 14 patients with severe septic shock. This treatment appeared to be safe and was associated with a sustained rise in mean arterial blood pressure in spite of withdrawal of vasoactive agents.

Fewer *in vivo* data are available from studies utilizing antibodies directed against other cytokines. Recently, Starnes et al\textsuperscript{32} reported that anti-IL-6 antibodies protected mice against the lethal effect of an intraperitoneal injection of live *E coli* organisms and intravenous administration of TNF-\(\alpha\). In their study, treatment with IL-6 antibodies paradoxically increased levels of circulating TNF-\(\alpha\), and treatment with anti-TNF-\(\alpha\) antibodies resulted in a 70 percent decrease in serum IL-6 levels. Thus, it appears from these studies that treatment with anti-TNF and anti-IL-6 antibodies at least partially abrogates the *in vivo* effects of Gram-negative bacteremia. Furthermore, these preliminary data provide a fascinating suggestion that TNF-\(\alpha\) increases the production of IL-6 and that IL-6 suppresses the production of TNF-\(\alpha\).

**Anticytokine Receptor Antibodies**

Anticytokine receptor antibodies would be expected to bind to the surface of cytokine-sensitive responding cells and prevent the binding of cytokines. One group of investigators has reported experiments with an IL-1 receptor antibody.\textsuperscript{33-37} Chizzonite et al\textsuperscript{38} showed that monoclonal and polyclonal antibodies specific for the receptor blocked binding of \textsuperscript{125}I-labeled IL-1 to human T cells, fibroblasts, and epithelial cells. These same antibodies did not block IL-1 binding to bone marrow cells, pre-B cells, and macrophages. These data indicate that there are two types of IL-1 receptors with different immunologic epitopes, which these authors refer to as types I and II. Rivier et al\textsuperscript{39} showed that anti-IL-1 receptor antibodies are effective in blocking endotoxin stimulation of stress hormones in mice. Neta et al\textsuperscript{40} reported that anti-IL-1 receptor antibody blocks IL-1 stimulation of IL-6. The study by McIntyre et al\textsuperscript{41} demonstrated that anti-IL-1 receptor antibodies are effective in preventing IL-1-elicted elevation in peripheral blood neutrophil counts. The same study showed that anti-IL-1 receptor antibodies blocked acute endotoxin-induced peritonitis. Gershonwald et al\textsuperscript{42} showed that anti-IL-1 receptor antibodies prevent turpentine-induced chronic weight loss in mice and are associated with a decrease in plasma levels of IL-1. All of these studies appear to indicate that the physiologic effects of IL-1 can be effectively blocked by an antibody directed against the IL-1 receptor. To our knowledge, there are no studies using antibodies directed at other cytokine receptors.

**Circulating Inhibitors**

Soluble cytokine inhibitors recently have been described for IL-1\textsuperscript{10} IL-2,\textsuperscript{43-46} IL-4,\textsuperscript{47} IL-6,\textsuperscript{48} and TNF-\(\alpha\).\textsuperscript{48-50} These proteins have been shown to be structurally similar to the extracellular binding domain of the surface receptors. These agents appear to be capable of binding to cytokines that are still in the circulation. Seckinger et al\textsuperscript{51} and Engelmann et al\textsuperscript{52} described 27- and 33-kd TNF-\(\alpha\) inhibitors, respectively; these inhibitors, when incubated with TNF-\(\alpha\), blocked binding to the TNF-\(\alpha\) receptor. Giri et al\textsuperscript{53} identified a soluble IL-1 inhibitor with biochemical characteristics consistent with the extracellular binding domain of the IL-1 receptor. Robb and Kutyn\textsuperscript{54} reported that activated lymphocytes release a soluble form of the IL-2 receptor, which appears to be the result of proteolytic
cleavage of the extracellular domain of the IL-2 receptor. Rubin et al\textsuperscript{41} and Narcon et al\textsuperscript{42} reported a soluble fragment IL-2 receptor which functions as an IL-2 inhibitor. Mosley et al\textsuperscript{43} reported a secreted form of the IL-4 receptor. Novick et al\textsuperscript{44} reported a soluble extracellular fragment of the IL-6 receptor which functions as a soluble IL-6 inhibitor.

The role of these endogenous agents in nature has not been defined, and to our knowledge there are no reports of in vivo data which show that these circulating inhibitors can be used to treat Gram-negative sepsis in humans or animals. Yet there is enormous potential for these agents as specific cytokine blockers, and there is substantial in vitro evidence of efficacy. These inhibitors may also be important tools for deciphering the role of a single cytokine during a complex multiple-cytokine-mediated response, such as human sepsis syndrome.

**Receptor Antagonists**

A receptor antagonist is a cytokine-like molecule that can bind to a cytokine receptor but does not elicit a response. At the time of this publication, only an IL-1 receptor antagonist has been described.\textsuperscript{54,51-54} The IL-1 receptor antagonist appears to be a distinct gene product that is highly homologous to IL-1 and has been detected in nature. This molecule is biologically active in a recombinant form and can be used to block IL-1-mediated events. It is possible that receptor antagonists exist in nature for other cytokines.

Seckinger et al\textsuperscript{55} purified an IL-1 inhibitor from urine of febrile rabbits. The inhibitor had an apparent molecular weight of 18 to 25 kD and bound to the IL-1 receptor. It completely blocked binding of authentic IL-1. The action of this inhibitor appeared to be specific since it did not block the action of TNF-\alpha.

Hannum et al\textsuperscript{56} showed that this IL-1 inhibitor bound to the IL-1 receptor but lacked IL-1 biologic activity. Studies by Dripps et al\textsuperscript{57} confirmed that the inhibitor bound to the IL-1 receptor but did not initiate IL-1 signal transduction. Eisenberg et al\textsuperscript{58} isolated the cDNA, and the sequence demonstrated that this protein shares 26 percent homology with IL-1\beta and 19 percent homology with IL-1\alpha.

Rochemonteix et al\textsuperscript{59} reported that an IL-1 receptor antagonist is produced by alveolar macrophages.

Ju et al\textsuperscript{60} have provided some preliminary data that support a hypothesis that a spectrum of cytokine agonists-antagonists exists. These authors have created an analogue of the IL-1 receptor antagonist by site-specific mutations which resulted in the substitution of a single amino acid. This had the effect of transforming the IL-1 antagonistic properties of this molecule so that it became an IL-1 agonistic agent. One can speculate from this observation that it is possible to produce synthetic proteins that would have the antagonistic or agonistic effects of a particular cytokine.

The existence of preliminary data showing the in vivo efficacy of IL-1 receptor antagonists might support future trials in human sepsis syndrome. Zahedi et al\textsuperscript{61} cloned a homologous IL-1 receptor antagonist from a murine monocytic cell line. They showed that hepatic murine IL-1 receptor antagonist mRNA could be induced in vivo by the subcutaneous injection of azocasein, a protein that induces chronic inflammation. Henricson et al\textsuperscript{62} reported that the IL-1 receptor antagonist was effective in blocking the induction of colony stimulating factor by endotoxemia in mice. Ohlsson et al\textsuperscript{63} reported that at a dose of 4 mg/kg, human IL-1 receptor antagonist could block IL-1\beta-induced hypotension and changes in the peripheral leukocyte counts in rabbits. The same authors reported that at a dose of 100 mg/kg, the IL-1 receptor antagonist could improve the seven-day survival of endotoxin-induced shock from 20 percent to 90 percent in rabbits. Wakabayashi et al\textsuperscript{64} showed that the IL-1 receptor antagonist could block the hemodynamic and hematologic effects of E. coli infusion in rabbits. These authors also reported that the IL-1 receptor antagonist could block E. coli-induced acute lung inflammation. In contrast to these effects, the IL-1 receptor antagonist was not effective in blocking the appearance of TNF-\alpha and IL-1\beta in the circulation of E. coli-treated rabbits.\textsuperscript{65}

**Summary**

Blocking the effects of cytokines is a potential new therapeutic avenue for the treatment of Gram-negative sepsis. Three classes of agents are currently being evaluated: antibodies, circulating inhibitors, and receptor antagonists. Data in the current literature support the consideration of these agents as potential therapeutic agents in Gram-negative sepsis. The clinical utility of these agents is contingent on the results of well-designed, prospective, randomized, placebo-controlled clinical trials in well-defined clinical populations. These trials will require the cooperation of clinical and basic scientists. At this time, preliminary and early clinical trials are in progress utilizing IL-1 and TNF-\alpha circulating inhibitors, IL-1 receptor antagonists, and monoclonal antibodies to TNF-\alpha and the IL-1 receptor.

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