Proinflammatory and Anti-inflammatory Cytokines as Mediators in the Pathogenesis of Septic Shock*

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During infection, the host produces several proinflammatory cytokines that have been implicated as playing a critical role in the pathogenesis of the disease. The production of these cytokines is initiated by the organisms themselves (phagocytosis) or by soluble products of the organisms: for example, the lipopolysaccharide (LPS) endotoxins of Gram-negative bacteria, the protein exotoxins of Gram-positive bacteria, and the cell-wall glycopeptides such as teichoic acids and muramyl peptides. Of course, LPS is by far the most potent soluble product of bacteria that induces cytokine production, and therefore, most information about cytokine induction is derived from studies using LPS in vitro and in vivo. However, it is important to recognize that the cytokine production in septic shock is neither specific nor unique. The cytokines that contribute to pathologic changes in septic shock are not unique to infection. Multiple trauma, ischemia-reperfusion injury, acute transplant rejection, antigen-specific immune responses, and various acute inflammatory states (acute hepatitis and pancreatitis) initiate the same cytokine cascade and result in both systemic and local inflammatory processes. However, special consideration exists for septic shock since no other disease is associated with such a high mortality despite our ability to provide patients with septic shock with appropriate antibiotics and supportive therapy.

Biologically, interleukin-1 (IL-1) and tumor necrosis factor (TNF) are closely related, although the structure and receptors for IL-1 and TNF are clearly distinct. IL-1 and TNF are active in the low picomolar and femtomole ranges. Based on short-term blockade of IL-1 and TNF receptors in humans and animals and recent data on IL-1β and TNF-α-deficient mice, there is no evidence that these cytokines play a critical role in development, or normal homeostasis such as metabolism, hematopoiesis, renal and hepatic function, or regulation of BP. During inflammation, injury, immunologic challenge, or infection, IL-1 and TNF are produced. One concludes from those studies that biological properties of IL-1 and TNF mimic host responses to infection, inflammation, injury, or immunologic challenge. In animal models of systemic inflammation (such as in septic shock), specific blockade of either IL-1 or TNF results in a reduction in the severity of the inflammation. Moreover, IL-1 and TNF act synergistically in nearly every in vitro and in vivo model of local or systemic inflammation. When both cytokines are specifically blocked, the severity of inflammation is reduced further.

**BIOLOGICAL EFFECTS OF IL-1 AND TNF RELEVANT TO SEPTIC SHOCK**

**Local and Systemic Effects**

A distinction is made between the local effects of IL-1 and TNF and the consequences of their systemic levels. If the function of host defense is the elimination of the invading organism or destruction of foreign tissue, inflammation is the price that is paid for an effective defense. Therefore, in systemic inflammation, large amounts of IL-1 and TNF are released into the circulation, inducing hypotension and shock that can be lethal in experimental animals. Humans are particularly sensitive to the pyrogenic and hypotensive properties of IL-1 and TNF; a single IV injection of IL-1 or TNF, 10 ng/kg, induces fever (temperature of 39°C), whereas hypotension is consistently observed at doses of 100 ng/kg; 300 ng/kg is the maximal dose tolerated because of severe fall in BP.1-5

**Synergistic Actions of IL-1 and TNF**

As shown in Table 1, IL-1 and TNF act synergistically. The synergism between IL-1 and TNF is highly consistent and a frequently reported phenomenon. In addition, the synergism between IL-1 and TNF is also observed in vivo, whereas the synergism between IL-1 and IL-6, IL-1 and bradykinin, or IL-1 and the various growth factors is mostly on prostanoid synthesis and primarily an in vitro finding. The mechanism for IL-1 synergism in the synthesis for prostaglandin E2 (PGE2) likely involves the ability of

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one cytokine to release arachidonate and of the ability of IL-1 to stimulate cyclooxygenase type-2 (COX-2) synthesis. The mechanism for synergism may also involve receptor modulation; however, in IL-1 and TNF synergism, receptors for TNF are downregulated by IL-1.6,7 Could the synergism be explained at the level of signal transduction? Although this is an attractive hypothesis, no pathway of IL-1 or TNF signal transduction appears unique to either cytokine at the present time to account for synergism. In fact, since signal mechanisms appear similar, additive rather than synergistic effects should be observed. Like IL-1, TNF also stimulates hydrolysis of phosphatidylcholine, release of ceramide from sphingomyelin following activation of sphingomyelinase,8 and release of arachidonic acid from phospholipids via cytosolic phospholipase A (PLA2) and activation of phospholipase activating protein.9,10 In addition, some of the kinases that are activated by IL-1 are also activated in cells stimulated with TNF.11-14

Expression of Various Genes in Cells Exposed to IL-1 and TNF

A fundamental property of IL-1 and TNF in the pathogenesis of septic shock is the ability to induce a variety of genes that affect the vasculature and the local tissue environment. In most cases, IL-1 and TNF induce new transcripts in cells that express these genes only during disease. There are several examples, but the most dramatic appear to be other members of the cytokine family and inducible enzymes regulating small molecular weight mediators. Mediators such as prostaglandins, leukotrienes, and nitric oxide (NO) require cellular enzymes to covert precursors to active molecules. IL-1 and TNF are potent inducers of these enzymes.

Effects Mediated by Prostanoids

Many IL-1- and TNF-induced changes are mediated by prostaglandins, particularly PGE2. In fact, the use of cyclooxygenase inhibitors for a variety of inflammatory conditions is often a therapeutic strategy to reduce IL-1- and TNF-induced PGE2. Humans injected with IL-1 or TNF experience fever, headache, myalgias, and arthralgias, all of which are reduced by coadministration of cyclooxygenase inhibitors. One of the most universal activities of IL-1 and TNF is the induction of gene expression for type-2 PLA2 and COX-2.

Effects Mediated by NO

The generation of NO in inflammatory disease appears to be a fundamental event.15 Several studies have demonstrated that IL-1 and TNF initiate transcription and translation of the inducible form of NO synthase. This has been observed in a variety of cells, for example, in osteoclasts, murine macrophages, pituitary cells, mast cells, osteoblasts, glial cells, insulin-producing β-cell in the pancreas, smooth muscle cells, chondrocytes, myocytes, and mesangial cells. In mesangial cells, IL-1β-induced NO synthase is augmented by elevated levels of cyclic adenosine monophosphate.16 Like induction of COX-2 and type-2 PLA2, induction of NO likely accounts for a considerable number of biological effects of IL-1 and TNF. In experimental septic shock, the fall in mean arterial pressure and the decrease in systemic vascular resistance are thought to be mediated by the induction of NO from smooth muscle cells. LPS injection into animals increases NO in several tissues and when treated with IL-1 receptor antagonist, there is a 70% decrease in NO.17

IL-1 and TNF Infusion Mimics Septic Shock

Many of the biological effects of IL-1 and TNF are similar to those observed during a septic event; however, recent studies in humans have confirmed data from animal experiments. IL-1α or IL-1β have been administered to humans in phase I trials. Systemic administration of IV IL-1 from 1 to 10 ng/kg has produced fever, sleepiness, anorexia, generalized myalgias, arthralgias, and headache. However, the most dramatic biological response to IL-1
was observed at doses of 100 ng/kg or higher. In those patients, a rapid fall in BP takes place. Because of these results, the dose-limiting toxicity for IL-1 of hypotension has been set at 300 ng/kg. In some patients receiving 1 µg/kg, stage 4 hypotension was reported. The subcutaneous route is associated with less side effects.

TNF infusion into humans is similar to that observed for IL-1. However, TNF also induces a coagulation cascade that has not been observed in humans injected with IL-1. In addition, low doses of TNF in humans induce a neutrophilia, whereas higher doses result in leukopenia.

In the rabbit, a single IV injection of 10 µg/kg of recombinant human IL-1β resulted in a shock-like state with hypotension, neutropenia, and thrombocytopenia. This has been confirmed in studies using human IL-1α in baboons. The mechanism for the hypotensive effect of IL-1 appears to be due to the generation of at least three small molecular weight mediators: cyclooxygenase products, platelet activating factor, and NO. The fall in circulating leukocytes and platelets is thought to be due to the stimulation of endothelial adhesion molecules. The effects of IL-1 in inducing a shock-like state are potentiated by coinfusion of TNF. The potentiation of IL-1 and TNF has been observed in anesthetized as well as in conscious rabbits. In the conscious rabbit, coinjection of IL-1 and TNF induced a fall in mean arterial pressure, onset of lactic acidosis, and glucose intolerance. Many effects of IL-1 and TNF are synergistic in a variety of models in vitro and in vivo.

Comparison With IL-6

The most consistent correlations of clinical severity in inflammatory, autoimmune, or infectious disease with plasma cytokine levels are clearly those with IL-6, not IL-1 or TNF. The best correlation of plasma cytokine levels with mortality from septic shock has been made with IL-6. IL-6 levels but not TNF-α levels were found to predict a fatal outcome in patients with septic shock. Therefore, one can conclude that elevated levels of IL-6 in patients with septic shock represent the net effect of biologically active IL-1 and TNF.

It is important to emphasize that unlike IL-1 and TNF, there is no evidence that IL-6 is itself an inflammatory cytokine. IL-6 does not induce PGE₂ but rather suppresses IL-1-inducible cyclooxygenase. IL-6 does not cause shock in mice or primates regardless of the amount given either alone or with TNF. In humans, IV administration of IL-6 at 30 µg/kg has not produced hypotension, whereas at 100 ng/kg, IL-1 induces a fall in BP in nearly all patients.

IL-6 has been given to humans in very high concentrations (100 µg/kg) without hypotension. The only sign was headache and fever. Thus, human and animal experiments support the concept that IL-6 does not have a causal role in septic shock.

Reducing Production of IL-1 and TNF

Inhibition of IL-1 and TNF Processing Enzymes

IL-1β is unique in that the precursor lacking a leader sequence is barely active and remains in the cytosol until cleavage and release. TNF-α has a weak leader sequence, appears to be associated with the Golgi, and exists in a cell membrane form before being cleaved and released. The precursors for IL-1β and TNF-α undergo myristoylation on lysines which is thought to contribute to membrane localization. Although the primary N-terminal amino acids for extracellular IL-1β and TNF-α have been known for several years, how the respective precursors are cleaved and transported out of the cell was poorly understood. The IL-1β-converting enzyme (ICE) is a constitutively produced intracellular cysteine protease that appears to be the sole enzyme for cleaving precursor IL-1β between aspartic acid and alanine. ICE is stored in cells in an inactive form but becomes enzymatically active by the same cell stimuli that induce the synthesis of IL-1β.

Serine proteases cleave the 26,000-d TNF-α precursor between alanine (76) and valine (77) yielding the 17,000-d mature TNF-α. Unlike ICE, a putative, specific TNF-α-converting enzyme is present, although it appears to be in the general class of metalloproteinases with a zinc binding motif of HEXGH. In vitro, metalloproteinase inhibitors and zinc chelators suppress the processing of TNF-α from human blood monocytes and murine macrophages but neither affect the release of lymphotxin-α from T lymphocytes nor the release of other cytokines. These metalloproteinase inhibitors did not affect production of IL-1β or IL-6 in whole human blood incubated with LPS. Even membrane-associated cytokines such as macrophage colony stimulating factor and transforming growth factor-α were unaffected. The protease inhibitors used in those studies are not specific since they can cleave other proteins. However, when administered to rats or mice, these metalloproteinase inhibitors reduced circulating levels of LPS-induced TNF-α.

Cytokine-Suppressing Anti-inflammatory Drugs

Recent studies have taken advantage of pyridinyl-imidazole compounds that block the synthesis
of IL-1β and TNF-α without affecting transcription or their steady-state levels of messenger RNA. Recent studies on the mechanism by which these drugs reduce IL-1 and TNF translation appear to be due to their ability to bind to and inactivate two related mitogen activating protein (MAP) kinases.32 Like most MAP kinases, the novel kinases are serine-threonine kinases. These kinases phosphorylate proteins required for translation of cytokine messenger RNAs into their respective proteins.32 These MAP kinases also have the same nucleotide sequences as that of the IL-1 and TNF signal-associated MAP kinase p38.33,34 The p38 MAP kinase is a homologue of the yeast HOG-1 gene. The cytokine synthesis-inhibiting drugs bind and inactivate these MAP kinases in cells stimulated with LPS or hyperosmolarity.33,34 A HOG-1 gene-related p38 MAP kinase is part of the IL-1 and TNF signal transduction phosphorylation cascade.11,13

THE ANTI-INFLAMMATORY CYTOKINE NETWORK IN SEPSIS

IL-4, IL-10, IL-13, and Transforming Growth Factor-β

IL-4, IL-10, IL-13, or transforming growth factor-β (TGF-β) each suppress gene expression and synthesis of IL-1, TNF, and other cytokines. In vitro, these cytokines can reduce endotoxin-induced gene expression and synthesis of IL-1 and TNF as much 90%, and when given to mice or rats, can reduce lethal endotoxemia. As such, they are potentially useful in some clinical situations. IL-10 appears to be particularly useful because unlike IL-4 or TGF-β, IL-10 has no clinical side effects. A randomized, double-blind, placebo-controlled trial (phase I) in healthy human volunteers demonstrated the absence of clinical toxic reactions and also studied the effect of a single IV injection of IL-10 on cytokine production.35 Blood was removed before and 3, 6, 24, and 48 h after the injection, incubated in vitro with endotoxin, and the amounts of IL-1β, TNF-α, IL-6, IL-8, IL-1Ra, and TNF soluble receptor p55 were measured. At doses of 10 and 25 μg/kg, there was a 90% reduction in IL-1β, TNF-α, and IL-6 production in blood taken 3 and 6 h after the injection; at 25 μg/kg, a 50% reduction IL-1β, TNF-α, and IL-6 production was present after 24 and 48 h. In contrast, there was no suppression of IL-1Ra or TNF soluble receptor p55.

IL-4 and IL-13 also suppress LPS-induced IL-1 and TNF gene expression and synthesis. In addition, they increase IL-1Ra production.36 IL-4 and IL-13 share the same receptor complex on monocytes, and hence, similar biological effects for both cytokines are often observed. There are, however, few if any receptors for IL-13 on T lymphocytes, and the immunologic suppressive effects of IL-4 and IL-10 are not observed for IL-13. Similar to IL-4, IL-10, and IL-13, TGF-β suppresses gene expression and synthesis of IL-1 and TNF and also increases IL-1Ra production.37 However, TGF-β, which has profound immunosuppressive effects, is a growth factor for normal and neoplastic cells.

NATURALLY OCCURRING INHIBITORS OF IL-1

IL-1 Receptor Antagonist

IL-1Ra is produced primarily from macrophagic cells as a 22-kd glycosylated protein. IL-1Ra binds to IL-1RI with nearly the same affinity as IL-1α or IL-1β but does not trigger a response.38 The cytokine is thus the naturally occurring inhibitor of IL-1. IL-1Ra has nearly the same affinity (approximately 200 pM) for the IL-1RI as that of human IL-1α and IL-1β.39-41

In animal studies, administration of IL-1Ra reveals that IL-1 plays an important role in the pathogenesis of inflammatory and immunologically mediated disease, including animal models of septic shock.42 Only a few IL-1RI need be occupied to trigger a biological response, and therefore, it is necessary to sustain a high level of IL-1Ra to block unoccupied receptors. When exogenous IL-1Ra is injected into animals, high plasma levels (10 to 20 μg/mL) are needed before a reduction in disease is observed. In humans, similar levels of IL-1Ra are needed to block the hematologic response to LPS.43

IL-1Ra Production in Sepsis

It is not unusual to measure high and more sustained levels of IL-1Ra than IL-1β in patients with septic shock.44 For example, in healthy volunteers injected IV with a low dose of Escherichia coli endotoxin, circulating IL-1Ra levels are at a 100-fold molar excess (peak level of 6,000 to 7,000 pg/mL) to those of IL-1β (70 to 80 pg/mL) and are significantly elevated above the baseline levels for >24 h.45 In patients with septic shock, juvenile rheumatoid arthritis, or inflammatory bowel disease, a similar ratio can be observed and elevated IL-1Ra levels can correlate with the severity of disease.46 In patients with thermal burns, levels of IL-1Ra correlated with the burn surface area and the highest levels of IL-1Ra were measured in nonsurvivors.47 The IV injection of 30 ng/kg of IL-1α into humans induces 25 to 30 ng/mL of IL-1Ra,48 which is fourfold higher
than that induced by LPS. Injection of IL-1β into humans results in an 86-fold increase in plasma IL-1Ra after 1 h.49 In humans, endogenous TNF production during endotoxemia contributes to IL-1Ra production.50

**IL-1Ra in Experimental Endotoxemia in Humans**

IL-1Ra given IV to healthy volunteers is without side effects or changes in biochemical, hematologic, or endocrinologic parameters, even when peak blood levels reach 30 μg/mL and are sustained above 10 μg/mL for several hours.51 To evaluate the effect of IL-1 receptor blockade on clinical disease under controlled experimental conditions, healthy volunteers were challenged with IV endotoxin and administered an infusion of 10 mg/kg of IL-1Ra at the same time. There was no effect on endotoxin-induced fever, although blood levels of IL-1Ra were not significantly elevated until 1 h after the bolus injection of endotoxin. In animal studies, peripheral endotoxin induces fever by triggering IL-1 induction of IL-6 synthesis in the CNS.52 Since IL-1Ra does not cross the blood-brain barrier, this may account for the inability of IL-1Ra to diminish endotoxin fever.53 However, there was a 50% reduction in the endotoxin-induced neutrophilia and a reduction in the circulating levels of granulocyte colony-stimulating factor compared to subjects injected with endotoxin plus saline solution.43

**Clinical Trials of IL-1Ra in Septic Shock**

IL-1Ra has been given to patients with septic shock. The initial (phase II) trial was a randomized, placebo-controlled, open-label study in 99 patients. Patients received either placebo or a loading bolus of 100 mg followed by a 3-day infusion of 17, 67, or 133 mg/h IL-1Ra.54 A dose-dependent improvement in 28-day mortality was observed; mortality was reduced from 44% in the placebo group to 16% in the group receiving the highest dose of IL-1Ra (p=0.015). In that study, there was a dose-related fall in the circulating levels of IL-6 24 h after the initiation of IL-1Ra infusion. This fall in IL-6 levels is consistent with the well-established control of cirulating IL-6 levels by IL-155 and the correlation of disease severity and outcome with IL-6 levels.22 The mean plasma level of IL-1Ra was 25 to 28 μg/mL in the high-dose group and this order of magnitude of circulating IL-1Ra concentration is measured in animals that benefit from IL-1Ra during experimental shock.56

A large phase III trial in 893 patients revealed a trend but without a statistically significant reduction in 28-day mortality.57 However, a retrospective analysis of 563 patients with a predicted risk of mortality of 24% or greater revealed a significant reduction in 28-day mortality (45% in the placebo group and 35% in patients receiving 2 mg/kg/hr for 72 h, p=0.005).57 Similar improvement was observed when patients were scored based on organ failure at entry. Circulating levels of thromboxane B2, prosta-glandin I2, and leukotrienes C4, D4, and E4 were attenuated (p<0.05) at 72 h in patients receiving the high dose of IL-1Ra, whereas in patients receiving the placebo, these eicosanoids were increased at 72 h.58 A second phase III trial using 10 g of IL-1Ra infused over 3 days was undertaken but terminated during an interim analysis because a reduction in overall 28-day mortality would not likely reach statistical significance.

### Reducing the Activity of TNF

#### Soluble TNF Receptors

Unlike IL-1, a naturally occurring receptor antagonist to TNF has not been found. However, soluble receptors to TNF are present in the circulation of healthy humans and may act as naturally occurring inhibitors of TNF activity. The situation is similar to that of soluble IL-1 receptors. There are two cell surface TNF receptors: p55 and p75.60-63 The extracellular domains of each TNF receptor are shed from the cell surface by a serine protease associated with cell activation7 and are found in the circulation of healthy humans. The concentration of the p75 is approximately 300 pM and is threefold greater than that of the p55 form.64,65 However, increases in the circulating levels of soluble receptors to TNF during disease states appear greater than those for soluble IL-1 receptors. For example, endotoxemia induces the release of both TNF receptors into the circulation and the increase is several-fold over that of the concentration in healthy subjects.66,67 TNF itself induces the release of its soluble receptors.68 Soluble TNF receptors are also elevated in patients with cancer and the levels correlate with the tumor burden or extent of the metastases.69 Other studies have documented the presence of soluble TNF receptors in the circulation or joint fluid in a variety of autoimmune and inflammatory diseases.

The soluble receptors for IL-1 inhibit the action of IL-1 in a dose-dependent fashion. In contrast, soluble TNF receptors can act as “carriers” of TNF in certain experimental models. This phenomenon was first shown by adding increasing amounts of soluble TNF receptors to cells exposed to TNF. The biological activity of TNF was enhanced at low molar ratios of receptor to ligand.70 At higher molar ratios of soluble receptor to TNF, the activity was decreased and there was dose-dependent inhibition of
TNF activity. Therefore, at low molar ratios, the soluble TNF receptors protect the TNF from degradation or destabilization. One likely mechanism for the stabilization of TNF by the soluble receptors is to maintain the trimer structure of TNF since monomeric TNF is biologically inactive. The amount of natural inhibition or natural “stabilization” of TNF by the soluble receptors during inflammation is unclear. Mice deficient in the p55 TNF receptor do not manifest increased susceptibility to infection or inflammation. However, the p75 receptor is thought to function as the natural carrier of TNF compared to the p55 receptor.

In several animal models of sepsis and inflammatory disease, administration of recombinant forms of soluble p55 TNF receptor has reduced inflammation or prolonged survival. The subject has recently been reviewed. Although a chimeric receptor to TNF p75 has been found in human sepsis, the survival at the higher doses was without benefit. This may be due to prolongation of the half-life of TNF due to trimer stabilization. In general, clinical trials for soluble forms of the p55 TNF receptor are presently underway for sepsis, inflammatory bowel disease, and rheumatoid arthritis. Using soluble forms of the p55 TNF receptor in these clinical situations is based on the beneficial effects of monoclonal antibodies to TNF in several trials. Therefore, it is anticipated that soluble forms of the p55 TNF receptor will be used to treat acute and chronic graft rejection, sepsis, graft-vs-host disease, and a variety of inflammatory diseases.

Neutralizing Antibodies to TNF

From a historical viewpoint, the first experiment that implicated the importance of endogenous cytokines in the pathogenesis of septic shock was the demonstration that neutralizing antibodies to TNF (also called cachectin) reduced the lethality of LPS in mice. This study was then expanded to primates and employed live E. coli organisms and in rabbits using LPS. A protective role was again observed. Furthermore, anti-TNF antibodies had a dramatic effect in reducing the circulating levels of IL-1 and IL-6. Following these studies, many reports confirmed a role for TNF in the lethality of endotoxin shock in animals. Only a few studies, namely using cecal ligation as a model, have shown that anti-TNF antibodies do not affect outcome. Nevertheless, animal studies formed the basis for using anti-TNF antibodies in humans with septic shock. Clinical trials have yielded mixed results, not dissimilar to those reported in patients treated with IL-1Ra.

A large, randomized, placebo-controlled, double-blind multicenter study of a murine antihuman TNF-α monoclonal antibody was performed in 971 patients with the “sepsis syndrome.” There were two doses of antihuman TNF-α, a single infusion of 7.5 mg/kg, or 15 mg/kg. There was no overall benefit in 28-day all-cause mortality in patients receiving the antibody. However, in a subset of 478 patients who had septic shock upon entry into the study, there was reduction in 3-day all-cause mortality compared to matched placebo control subjects (44% reduction at 15 mg/kg, p=0.01; and 48% reduction at 7.5 mg/kg, p=0.004). At 28 days, there were no differences in mortality in patients treated with either dose of the antibody. Similar results have been reported in smaller studies. Short-term benefits on left ventricular function have been observed in patients with septic shock treated with anti-TNF.

Why do these trials fail to show overall efficacy in reducing the mortality in these patients and only benefit for a segment of the patients? The topic of heterogeneity of patients as well as of disease causation has been used to explain these results. Is there anything wrong with the concept that blocking (or reducing) TNF activity or production should reduce the mortality of patients with septic shock as has been observed in the vast majority of animal studies? The best test for efficacy of an anti-TNF in terms of patient heterogeneity are studies in patients with rheumatoid arthritis. This patient group is not as heterogeneous as is the group with septic shock. In a multicenter, placebo-controlled study, monoclonal anti-TNF reduced the severity and the biochemical markers of rheumatoid arthritis. Therefore, one may conclude that in order for patients with septic shock to show a benefit with anti-TNF treatment, a better set of entry criteria needs to be selected. Using plasma IL-6 levels as the surrogate marker of biologically active TNF (and IL-1) may provide a better selection criterion. An alternate approach is to use patients in whom the underlining disease does not contribute to 28-day mortality other than that due to the sepsis episode. This approach, however, will require a far greater amount of time to complete a large pivotal study.

References

4. van der Poll T, Bueller HR, ten Cate H, et al. Activation of
12 Guesdon F, Waller RJ, Saklatvala J. Specific activation of β-casein kinase by the inflammatory cytokines interleukin-1 and tumor necrosis factor. Biochem J 1994; 304:761-68
27 Stevenson FT, Bursten SL, Cantor F, et al. The 31-kDa precursor of interleukin-1α is myristoylated on specific lysines within the 16-kDa N-terminal propiece. Proc Natl Acad Sci USA 1993; 90:7245-49
36 Vannier E, Miller LC, Dinarello CA. Coordinated anti-inflammatory effect of IL-4: IL-4 down regulates IL-1 synthesis but up regulates IL-1ra production. Proc Natl Acad Sci USA 1992; 89:4076-80
42 Dinarello CA. Biological basis for interleukin-1 in disease. Blood 1996; 87:2065-2147


