# Anti-interleukin-1 strategies in the treatment of the septic shock syndrome

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**CA DINARELLO. Anti-interleukin-1 strategies in the treatment of the septic shock syndrome. Can J Infect Dis 1994;5(Suppl A):9A-16A.** The septic shock syndrome has recently been termed the systemic inflammatory response syndrome (SIRS). It is an acute illness characterized by generalized activation of the endothelium. The most severe and most common form of the syndrome is found in patients with shock due to Gram-negative sepsis. In this overview, both animal and limited human data are considered for the contribution of the cytokine interleukin-1 (IL-1) to this syndrome. Cytokines are small molecular weight, endogenously produced proteins with multiple biological effects. Laboratory investigations suggest that IL-1 plays a critical role in SIRS by promoting the biochemical and clinical characteristics of SIRS. The biochemical changes induced by IL-1 are similar to those of tumour necrosis factor (TNF) and include increased synthesis of nitric oxide, prostaglandins, platelet activating factor and endothelial cell adhesion molecules. Together, IL-1 and TNF appear to act in a synergistic fashion in a variety of disease models, particularly SIRS. Specific blockade of IL-1 using soluble IL-1 receptors or IL-1 receptor antagonist suggests that blocking this cytokine may be useful in treating human SIRS.

**Key Words:** *Cytokines, Endotoxin, Receptor antagonist, Sepsis, Septic shock syndrome*

## Stratégies à base d'anti-interleukine 1 dans le traitement du syndrome du choc septique

**RÉSUMÉ :** Le syndrome du choc septique a récemment été baptisé syndrome de la réponse inflammatoire systémique (SRIS). C'est une maladie aiguë, caractérisée par une activation généralisée de l'endothélium. La forme la plus grave et la plus fréquente du syndrome s'observe chez les patients qui présentent un choc toxi-infectieux à germes gram-négatifs. Dans cette revue, les données sur l'animal et les données limitées sur l'humain sont étudiées pour identifier le rôle de la cytokine interleukine-1 (IL-1) dans ce syndrome. Les cytokines sont des protéines endogènes de faible poids moléculaire qui exercent de multiples effets biologiques. Les épreuves de laboratoire suggèrent que l'IL-1 joue un rôle central dans le syndrome de la réponse inflammatoire systémique en exacerbant les caractéristiques biochimiques et cliniques de ce syndrome. Les modifications biochimiques induites par l'IL-1 sont semblables à celles du facteur de nécrose tumorale et incluent une synthèse accrue de l'oxyde nitrique, des prostaglandines, du facteur d'activation plaquettaire et des molécules d'adhésion cellulaires endothéliales. Ensemble, l'IL-1 et le facteur de nécrose tumorale semblent agir de façon synergique dans divers modèles pathologiques, particulièrement dans le syndrome de réponse inflammatoire systémique. Le blocage spécifique de l'IL-1, à l'aide de récepteurs solubles de l'IL-1 ou d'inhibiteurs des récepteurs de l'IL-1, suggère que l'inhibition de cette cytokine pourrait être utile dans le traitement du SRIS chez l'humain.

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PATIENTS WITH GRAM-NEGATIVE SEPSIS AND 'SEPTIC SHOCK' continue to be at risk for death despite antibiotics and supportive care. The constellation of hypotension, coagulopathies and organ failure characteristic of 'septic shock' is also present in some patients without infection. A common pathophysiological mechanism(s) which explains the systemic inflammation of these patients is generalized endothelial cell activation. As defined by Bone (1), the term 'systemic inflammatory response syndrome' (SIRS) provides a more inclusive nomenclature. The most severe form of SIRS is the shock syndrome associated with Gram-negative bacteremia, but patients with less severe (noninfectious) forms of the syndrome may benefit from the same therapies. These therapies now include blocking cytokines. Certain cytokines stimulate endothelial cells from their normal anticoagulant state to a procoagulant state with increased adhesiveness for leukocytes and platelets.

There are now over 30 different cytokines, and the names assigned to each reflect a prominent biological property. In some cases where there are multiple biological properties, the term 'interleukin' (IL) is used, followed by a number assigned chronologically. Many cytokines are growth factors such as fibroblast and endothelial cell growth factors, some are hemopoietic growth factors, whereas others possess antiviral activity and are called interferons. Most cytokines are produced primarily in the presence of disease or immunization and contribute to immune responses, inflammation and endothelial cell activation; others are involved in tissue repair.

There are many steps leading to the development of SIRS. As discussed above, generalized endothelial cell activation is a common pathological process in SIRS. Two cytokines play a major role in the pathogenesis of the syndrome: tumour necrosis factor (TNF) and IL-1. There is also a role for interferon-γ in that this cytokine increases the production of and sensitivity to TNF and IL-1 (2). All three cytokines activate endothelial cells. Tissue inflammation associated with SIRS likely involves another cytokine, IL-8. IL-8 is an example of the family of chemotactic peptides, which attracts neutrophils, monocytes and lymphocytes to pass from the circulation into tissues, stimulates the release of enzymes and histamine from phagocytes and mast cells, induces platelet activating factor (PAF) (3) and contributes to local tissue damage (4).

Nearly each biological property of TNF has also been observed with IL-1. These include fever (5), sleep, hemodynamic shock (6), the induction of prostaglandin (PG) and collagenase synthesis (7), bone and cartilage resorption, inhibition of lipoprotein lipase (8) and increased synthesis of hepatic acute-phase proteins (9). The shock-like responses to TNF and IL-1 likely reflect their effects on the vascular endothelium. Figure 1 illustrates the effects of IL-1 and TNF on endothelial cells.

The production of TNF and IL-1 is important in SIRS, especially shock due to severe infection, ie, sepsis. The evidence is derived from studies showing: first, that injection of IL-1 or TNF into animals or humans causes a fall in systemic blood pressure and coagulopathy often seen in SIRS (6,8,10-14); second, that blood levels of these cytokines are often elevated in patients with the syndrome (8,11); and third, that specific blockade of the action of these cytokines in animals with a shock-like syndrome reduces mortality (8,15-17).

Numerous studies imply a role for TNF and IL-1 in the pathogenesis of disease. However, a distinction should be made between the local action of these cytokines and their systemic effects. For example, whereas TNF and IL-1 cause hypotension, IL-8 does not. IL-8 attracts and activates neutrophils, which are responsible for tissue injury. The ultimate function of the host defence system is the elimination of the invading organism, foreign antigen or neoplastic cell, whether by phagocytosis and antibody formation as is the case in most infections, or the induction of cytotoxic T cells for elimination of malignant and virally infected cells.

Inflammation is a consequence for an efficient host defence system. TNF, IL-1 and the IL-8 family are often grouped together and called 'proinflammatory cytokines' to distinguish them from 'anti-inflammatory cytokines'. These latter cytokines are IL-4, IL-10 and transforming growth factor-β. They are considered 'anti-inflammatory' because when administered to animals with infection or inflammation, they reduce the severity of disease and reduce the production of IL-1 and TNF (18).

When injected into experimental animals, either TNF or IL-1 induces hypotension and shock; however, when injected together, this combination acts synergistically (6). Synergism between IL-1 and TNF has been documented in several models of disease, for example, destruction of the insulin-producing beta cells in the islets of Langerhans (19). A single intravenous injection into patients with cancer of TNF (10) or IL-1 (14,20) induces a sudden fall in blood pressure often requiring treatment. Healthy volunteers receiving TNF also respond to it in a similar fashion (12,21).

## PRODUCTION OF TNF AND IL-1 IN DISEASE

Several studies have shown that in patients with SIRS, there is a positive correlation between increasing circulating TNF and IL-1 levels and the severity of disease. Interferon-α or -γ does not correlate with disease severity (22). However, systemic levels of TNF or IL-1 are not always elevated in these patients compared with other cytokines (2), for example, the cytokine IL-6. Plasma IL-6 levels correlate positively with disease severity, particularly in sepsis (23), despite the fact that this cytokine does not produce a shock-like syndrome in animals or humans (24).

In patients with acute infection, particularly bactere-

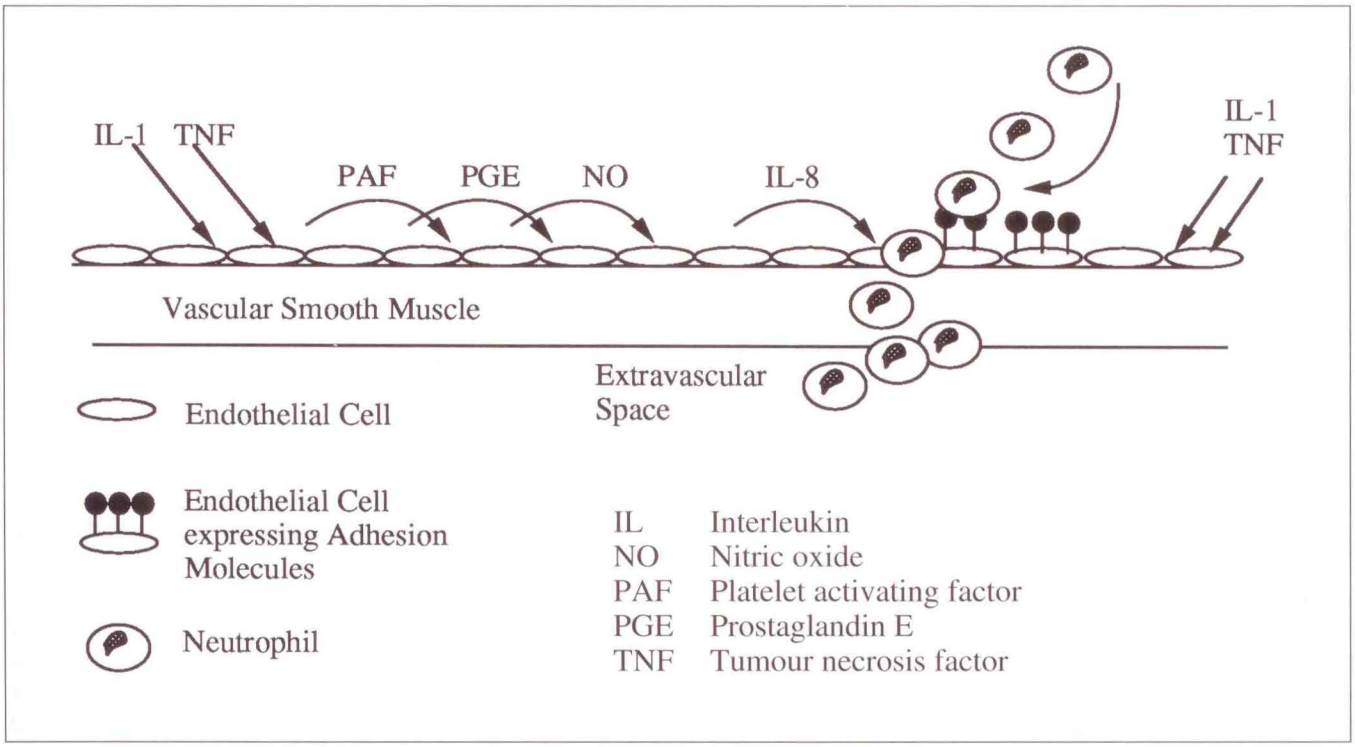


**Figure 1)** *IL-1 and TNF stimulate PAF, PGE and NO synthesis, resulting in vasodilation and hypotension. Hypotension leads to poor organ perfusion, acidosis and organ failure. IL-1 and TNF also stimulate the synthesis of endothelial adhesion molecules, which facilitate the adherence of circulating neutrophils to its surface. IL-1 and TNF induce IL-8 from the endothelium; this cytokine attracts neutrophils adhering to the endothelial surface to emigrate into the extravascular space, where they release enzymes and oxygen reactive radicals, leading to tissue damage*

mia, circulating IL-1 and TNF can sometimes be below the detection limits of the assays. Why are circulating levels of these cytokines not high even in the presence of severe disease? The most likely reason is that TNF and IL-1 are only transiently elevated. For example, in humans given an intravenous injection of endotoxin (25,26), levels of TNF are elevated for 60 to 120 mins despite a 6 h duration of fever. IL-1 is minimally elevated for only 60 mins. Therefore, a single blood sampling may miss the elevation of either cytokine in the circulation. In severe SIRS in some individuals, the levels of IL-1 fall as the disease worsens (27).

Bioassays for these cytokines can be misleading since naturally occurring inhibitors of each cytokine interfere with activity. For TNF these are soluble TNF receptors (28); for IL-1 these can be inhibitors (29), receptor antagonists (30) or soluble receptors (31). TNF can be bound to its soluble receptors and not be detected by either bio- or immunoreactive assays (32). In general, the role for either TNF or IL-1 as mediators of disease is not derived from studies on circulating levels in disease, but rather from specific blockade of either cytokine in animal models of disease.

The local effects of TNF and IL-1 appear to mediate neutrophil emigration, lower pain threshold and release secondary lipid-derived mediators such as PAF, PGS and leukotrienes. The IL-8 family of neutrophil and monocyte chemotactic peptides is important in inflammation, and IL-1 and TNF are potent inducers of IL-8 synthesis from monocytes, fibroblasts and endothelial cells. Concentrations of TNF or IL-1 as low as 1 to 10 pg/mL will induce IL-8 production from cultured cells (33). Therefore, because of the potency of TNF and IL-1, local inflammation is likely mediated, in part, through the induction of IL-8. IL-8 stimulates the release of enzymes from neutrophils and these enzymes, in turn, break down tissues (4). In addition, IL-8 activates neutrophils to generate reactive oxygen radicals, leading to tissue damage. The endothelium becomes 'leaky' after exposure to IL-8-activated neutrophils.

## TNF AND IL-1 AS MEDIATORS IN INFECTION

A major teaching is that microorganisms bring about hypotension in sepsis by their direct effect on the vasculature. Most bacterial invasion is associated with the production of toxins that, upon entrance into the circulation, cause hypotension, decrease perfusion of vital organs, acidosis and death. These toxins can be lipopolysaccharide endotoxins from Gram-negative bacteria and a large number of various exotoxins from Gram-positive and Gram-negative bacteria.

As recently reviewed (2), endotoxins and exotoxins can directly contribute to hypotension by activating complement. Cell wall materials other than lipopolysaccharide from bacteria and fungi can also activate complement. Activated complement induces the release of

**TABLE 1**
**Strategies for reducing the effects of IL-1 in disease**

| Substance | Mechanism (reference) |
|---|---|
| **Nonspecific** | |
| Corticosteroids | Reduced transcription (70) |
| TGF-β; IL-4*;IL-10* | Reduced transcription and synthesis (71-73) |
| **Specific** | |
| Soluble receptors (sIL-1RI)* | Neutralizes IL-1 (62) |
| Antireceptor antibodies | Blocks IL-1 receptors (61) |
| Receptor antagonists (IL-1Ra)* | Blocks IL-1 receptors (30) |
| Inhibition of IL-1 converting enzyme (none present) | Prevents formation of active IL-1 (50,51) |

**In clinical trials; IL Interleukin; Ra Receptor agonist; RI Type I receptor; TGF Transforming growth factor*

PAF, PGs and nitric oxide, each a potent vasodilator. In addition, microorganisms and their toxins can indirectly cause hypotension by inducing TNF and IL-1 which, in turn, induce PAF (34,35), PG (36) and nitric oxide (37).

The best example of toxin-mediated SIRS is 'toxic shock syndrome' associated with special strains of *Staphylococcus aureus*. This organism produces a potent exotoxin. However, this 'toxin' is not, in the traditional sense, damaging to cells, but rather exhibits 'toxicity' due to its ability to induce TNF and IL-1 (38). Injections of the toxin into animals results in a shock-like state with elevated levels of TNF and IL-1. Patients dying with the staphylococcus toxic shock syndrome rarely have evidence of bacteremia. Streptococcal toxic shock syndrome is also due to an exotoxin that induces TNF and IL-1 production (39).

The first evidence that bacteremias or toxemias (either Gram-negative or Gram-positive) were lethal via the production of cytokines came from a study showing that neutralizing antibodies to TNF prevented death in mice following a lethal injection of endotoxin (40). That experiment clearly established that specifically blocking a cytokine could prevent a host-mediated self-destructive process. The conclusion from that study was that infectious organisms (or their toxins) induce the host to make a lethal amount of TNF. Subsequent studies showed that blocking TNF with monoclonal antibodies reduced deaths in baboons given a lethal injection of *Escherichia coli* organisms (41), or prevented endotoxin-induced hypotension in rabbits (42). Similar data show that blocking IL-1 also prevents lethal shock in mice, rabbits or baboons (43-46).

TNF-α has been injected into human subjects, and many of its systemic effects, such as fever, leukopenia and hypotension, which were first reported in animals, have also been observed in humans (10,12,21,47,48). When human volunteers are injected intravenously with endotoxin, the rapid fall in blood leukocyte counts correlates directly with the peak level of plasma TNF (25). TNF induces an immediate fall in circulating neutrophils in healthy humans, followed by a leukocytosis (21). A single intravenous injection of TNF results in activation of several clotting parameters, including an increase in plasminogen activator inhibitor levels (12). These and other aspects of the human response to TNF are similar to those observed in acute infection and systemic inflammation.

IL-1 has been administered to human patients with melanoma in phase 1 trials. Intravenous administration of IL-1 from 10 to 300 ng/kg has produced fever, anorexia, generalized myalgias, arthralgias, headache and hypotension (14,20,49). Moderate to severe hypotension has been reported with doses over 30 ng/kg. The subcutaneous route is associated with fewer side effects. These clinical studies confirm animal experiments showing the neutrophil-inducing property of IL-1. An increase in circulating platelets has also been observed in humans following IL-1 administration (49).

## PREVENTING IL-1 ACTIVITY

**Inhibition of synthesis or processing of IL-1:** The strategies being investigated in attempts to reduce the production or action of IL-1 are shown in Table 1. A number of drugs, cytokines and other substances inhibit IL-1 and TNF production, but in a nonspecific fashion since the production of other cytokines is inhibited as well. Inhibition of the cleavage step required to generate an active form of IL-1 is a potential mechanism for reducing IL-1 activity (50,51).

**Specific receptor blockade using IL-1 receptor antagonist:** Naturally occurring substances that specifically inhibit IL-1 have been detected in various human body fluids. Of these substances, the most studied and well characterized has been the 'IL-1 inhibitor' described by Jean-Michel Dayer and William Arend. The IL-1 inhibitor competes with the binding of IL-1 to its cell surface receptors (30). Because of this mechanism of action, 'IL-1 inhibitor' was renamed IL-1 receptor antagonist (IL-1Ra).

Administration of IL-1Ra to animals significantly reduces the severity of diseases, including those associated with infections. This topic has recently been reviewed (15,52). In mice, rabbits or baboons injected with lethal doses of endotoxin or *E coli*, prior administration of IL-1Ra reduces death (13,43-45). IL-1Ra also blocks the shock-like state due to *Staphylococcus epidermidis* (53). In many of these disease models, local inflammation mediated by IL-8, particularly in the lung, plays a key role. The ability of IL-1Ra to block endotoxin-induced IL-8 production (33) may be a major component of the anti-inflammatory property of IL-lRa.

**Administration of IL-1Ra to humans:** In a phase 2 trial, patients with SIRS being treated with antibiotics and supportive care received either a placebo infusion or an infusion of IL-1Ra (133 mg/h) for 72 h. The 28-day mortality was 44% in the placebo group and 16% in the IL-1Ra group ($P<0.015$) (54). A recent double-blind

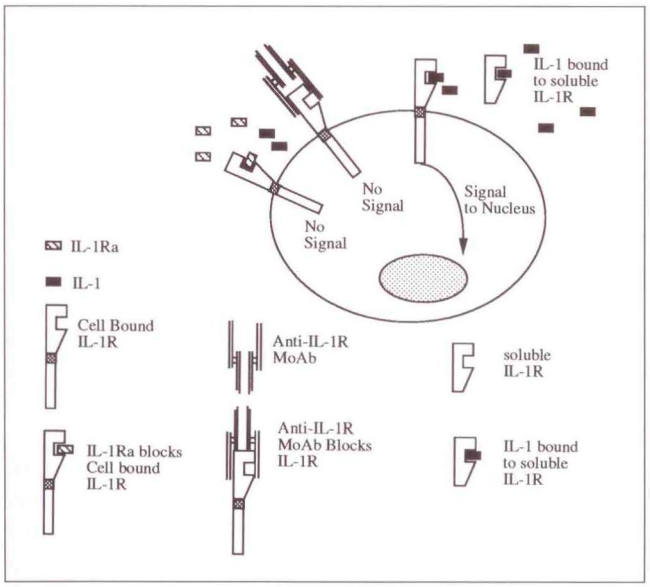


**Figure 2)** *Cell-bound receptors for interleukin (IL)-1 are blocked by monoclonal antibodies (MoAb), which prevent binding of IL-1 to the extracellular portions of the receptors. No signal is transduced to the nucleus. In the presence of excess IL-1 receptor antagonist (IL-1Ra) IL-1 binding sites on the receptors are occupied, thus preventing bona fide IL-1 from binding to the same sites. Soluble IL-1 receptors (IL-1R) (extracellular portions of IL-1 receptors) bind IL-1 and prevent the cytokine from interacting with cell-bound receptors. Soluble IL-1 receptors act as antibodies to IL-1*

phase 3 trial of IL-1Ra in 901 patients with SIRS has been completed. The overall mortality in patients receiving placebo was 34%, and 29% in the patients receiving a high dose IL-1Ra (2 mg/kg/h for 72 h). However, in severely ill patients entering the study with a predicted mortality of 24% or greater, the 28-day mortality was 44.6% in the placebo group and 35% in patients receiving high dose IL-1Ra (P=0.032 by Wilcoxon) (55). These latter data are similar to those reported in the phase 2 trial, and suggest further that blocking IL-1 with high dose IL-1Ra is effective in reducing deaths due to SIRS as the severity of the disease worsens. Assuming that this and other trials of IL-1Ra show improvement in this patient population, blocking IL-1 may become an accepted therapeutic modality in some patients with SIRS and other severe acute diseases.

Animal experiments demonstrate that despite an excess of 100 molecules of IL-1Ra to one molecule of IL-1, increasing this ratio from 1000 or even 10,000 of IL-1Ra to one of IL-1 results in improved survival rates (43,44). In animal (43,45,46) and human (56) studies, the concentration of IL-1Ra that is effective is 20 to 25 μg/mL. These high levels of IL-1Ra are needed to block nearly all IL-1 receptors. In vitro studies show that occupancy of as few as 5% of IL-1 receptors by IL-1 is sufficient to trigger a biological response (18). Therefore, increasing the plasma concentration of IL-IRa to 20 μg/mL insures nearly complete blockade of endothelial cell IL-1 receptors.

**TABLE 2**
**Reduction in severity of animal disease activity**

| Disease model | Cytokine antagonism (reference) |
|---|---|
| Death due to sepsis | Anti-TNF; IL-1Ra (32,41,45,46,69) |
| Death due to endotoxin | IL-1Ra; anti-TNF; sTNFR (43,44,74) |
| Shock due to *Escherichia coli* | Anti-TNF; IL-1Ra; sTNFR (32,45,46) |
| Shock due to staphylococcus | IL-1Ra (53) |
| Systemic inflammation | Anti-IL-1R (61) |
| Lung injury | IL-1Ra; anti-TNF (75) |
| Allograft rejection | sIL-1R (62) |
| Hypoglycemia | IL-1Ra (76) |
| Graft-versus-host disease | IL-1Ra; anti-TNF (77) |

*IL Interleukin; R Receptor; Ra Receptor agonist; s Soluble; TNF Tumour necrosis factor*

**Balance of IL-1 and IL-1Ra production:** During experimental endotoxemia in humans or in patients with SIRS, levels of IL-1Ra are 100-fold greater than levels of IL-1 (57,58). It is unclear whether these endogenous levels of IL-1Ra are effectively blocking IL-1 since blood levels in humans being treated with IL-1Ra are 20 μg/mL, whereas the concentrations of IL-1Ra that circulate in human disease states are rarely over 1.0 to 2.0 ng/mL – a 10,000-fold difference. Nevertheless, the balance between the agonist, IL-1, and the antagonist, IL-1Ra, may determine outcome in some diseases. For example, leukemic cells from patients with acute myelogenous leukemia do not express the gene for IL-1Ra, whereas there is spontaneous expression of IL-1 in these same cells (59). High levels of IL-1Ra in the joint fluids of patients with Lyme arthritis correlate with a shorter duration of inflammation compared with patients with low levels of the antagonist (60).

**Antibodies to IL-1 receptors:** There are two IL-1 receptors (IL-1R) on cells: type I receptor (IL-1RI) is found on endothelial cells, hepatocytes, fibroblasts, keratinocytes and T lymphocytes, whereas the type II receptor (IL-1RII) is found on B lymphocytes, monocytes and neutrophils. Neutralizing antibodies to the IL-1RI on mouse cells have been used to block IL-1 effects in murine models of disease. For example, during systemic inflammation in mice, administration of anti-IL-1RI antibodies reduces anorexia, weight loss (lean and fat loss) and IL-6 production (61). There are no clinical trials of anti-IL-1 receptor antibodies at present.

**Soluble IL-1 receptors:** Soluble receptor is another term to describe the extracellular portion of a receptor. The extracellular portion of the IL-1RI binds and neutralizes IL-1 in a fashion similar to antibodies to IL-1. When soluble IL-1 type I receptors were given to mice undergoing allograft heart transplantation, survival of mice was increased (62). The effects of the soluble type I receptor are likely due to decreased inflammation

rather than decreased immunoresponsiveness. Soluble human IL-1RI is in phase 2 clinical trials for SIRS. Figure 2 illustrates the various mechanisms for blocking IL-1. Table 2 lists the effects of IL-1 antagonism in animal models.

## IS THERE AN ADVANTAGE TO BLOCKING IL-1 AND TNF AT THE SAME TIME?

Although IL-1 and TNF act in a synergistic fashion, blocking either TNF or IL-1 reduces the severity of acute disease in a variety of animals. Limited in vitro studies suggest that blocking IL-1 and TNF at the same time is even more effective than blocking each separately; however, this dual blockade has not been studied in animal models and thus it remains speculative whether blocking both cytokines at the same time will improve outcome over those of blocking IL-1 or TNF separately. There are no clinical studies at present to examine the effectiveness of combined therapy.

In the case of SIRS, blocking IL-1 or TNF may not be beneficial in some circumstances. First, once these cytokines are produced and once they trigger their respective receptors, it will be too late to reduce their effects. For example, after IL-1 or TNF stimulate PAF, PG or nitric oxide, blocking the cytokines will not reduce the expression of these critical small mediator molecules. Second, in the case of complement activation, the induction of PAF, PG and nitric oxide could be direct and bypass a requirement for IL-1 and TNF mediation. Finally, it is possible that both IL-1 and TNF need to be blocked at the same time for a benefit to be observed.

## DOES BLOCKING IL-1 ENDANGER HOST IMMUNE AND NATURAL DEFENCE SYSTEMS?

Is it possible that blocking these cytokines in some patients worsens their disease? Administering IL-1Ra or anti-TNF antibodies to healthy animals has not revealed evidence of decreased immunological function. In a phase 1 clinical trial, healthy volunteers received a infusion of IL-1Ra for 3 h, and there was no evidence of impaired host defence mechanisms (56). Similar findings have been reported for monoclonal anti-TNF (63). Thus, short term blockade of TNF or IL-1 appears to be safe.

The remaining question is whether a more prolonged blockade will interfere with fundamental host defence mechanisms, particularly during disease states. Antibodies to TNF have a longer half-life in the circulation than TNF soluble receptors have and, therefore, may have greater effects on host defence. There is a large body of evidence showing that either IL-1 or TNF, particularly at low concentrations, increases natural resistance in animals (64,65). In some animal models, administration of antibodies to TNF has not been effective in reducing severity of disease (66), and blocking TNF suppresses immune functions (67). Similarly, blocking IL-1 in some animal models increases mortality (68,69). Therefore, contrary to most animal data discussed above, some models reveal a worsening of disease when blocking TNF or IL-1. If blocking IL-1 or TNF in disease impairs host defence systems, then clinical trials in patients with SIRS may show an increase in mortality associated with this therapeutic approach. It has been reported in the lay press that in large scale studies of patients with SIRS, a subgroup of patients (those without bacteremia) apparently have increased mortality when treated with either anticore endotoxin (HA-1A) or monoclonal anti-TNF antibodies, compared with patients treated with placebo. At present, there are no rapid, predictive tests to determine which patients will benefit and which patients might worsen during cytokine blockade.

**ACKNOWLEDGEMENTS:** This study was supported by NIH grant AI15614.

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