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Emerging Evidence of a More Complex Role for Proinflammatory and Antiinflammatory Cytokines in the Sepsis Response

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Since the original discovery and cloning of tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1), our understanding of the underlying role that these and other cytokines play in the sepsis response has greatly evolved. The original concept that the sepsis response is a result of a linear cytokine cascade induced by TNFα and IL-1 has given way to the appreciation that sepsis syndromes often result from a more complex interplay between proinflammatory cytokines, antiinflammatory cytokines, and cytokine antagonists. The proinflammatory cytokine-dominated, systemic inflammatory response syndrome is likely an episodic or transient occurrence; and many septic patients present with a compensatory antiinflammatory cytokine response dominated by the release of antiinflammatory cytokines and cytokine antagonists, leading to immune suppression. There is also growing appreciation that other members of the TNFα superfamily, including Fas ligand (FasL) and glucocorticoids, play an increasingly important role in the loss of immune cells during sepsis through apoptotic processes. The cytokine component of the innate immune response to sepsis plays a complex role not only in the inflammatory response syndrome but also in determining the nature and magnitude of the acquired immune response.

Host Cytokine Response to Sepsis

Tumor necrosis factor-α and IL-1 were initially purified and cloned in 1984–1985.[1–5] Within 5 years convincing animal evidence had demonstrated that an exaggerated TNFα and IL-1 response were the prime cause of mortality in bacteremic and endotoxemic shock.[6–8] Since 1993 there have been more than 15 phase II and phase III clinical trials of TNFα and IL-1 inhibitors in patients with sepsis syndromes. The disappointing results of these clinical trials have been the topic of several outstanding reviews[9–12] and are well beyond the scope of this summary.

There is now growing appreciation that the cytokine response to sepsis is not simply a linear cascade initiated by the exaggerated release of proinflammatory cytokines such as TNFα and IL-1. Early studies in primates suggested that in response to gram-negative bacteria or endotoxin there was an immediate systemic TNFα and IL-1 response that initiated the synthesis and release of more distal proinflammatory cytokines, antiinflammatory cytokines, and cytokine inhibitors. Administration of recombinant TNFα to a healthy organism recapitulated the physiologic[13,14] and cytokine responses to endotoxemic or bacteremic shock,[13,15–18] including the release of IL-1, IL-6, IL-8, IL-10, p55, p75, and IL-1 receptor antagonist (IL-1ra). In primate models where gram-negative bacteria or their products were intravenously administered, blocking the endogenous TNFα response suppressed the release of other, more distal proinflammatory cytokines (e.g., IL-1 and IL-8[19,20]), antiinflammatory cytokines (e.g., IL-6 and IL-10), and cytokine antagonists (e.g., IL-1ra and p55).[16,21,22] Although several rodent studies, using cecal ligation and puncture (CLP) to create sepsis, were unable to confirm that blocking TNFα had a positive effect on either outcome or cytokine production,[23–25] clinical trials were initiated in patients with sepsis syndrome.

During the course of those clinical trials, it became evident that a large proportion of critically ill patients with sepsis syndromes do not sustain an exaggerated proinflammatory response but, rather, exhibit what the late Roger Bone called a compensatory antiinflammatory response syndrome and immunosuppression.[26] Plasma cytokine profiles from patients with sepsis or systemic inflammatory response syndromes suggest that fewer than 10% of patients with sepsis syndrome have elevated levels of proinflammatory cytokines (e.g., TNFα, IL-1β, IL-8) at any given time (Table 16.1). As early as 1992 we reported that few patients meeting the clinical criteria of sepsis syndrome had detectable TNFα bioactivity in their serum;[27] rather, most of these patients had net TNF-inhibitory activity in their plasma. In a retrospective analysis of 83 patients from the IL-1ra phase III clinical trial,[28] fewer than 5% of these patients had detectable TNFα or IL-1β in their circulation.[29] Furthermore, blockade of TNFα in patients with sepsis syndrome does not appear to have a significant impact on the concentrations of other proinflammatory or antiinflammatory cytokines,[30] suggesting that in human sepsis TNFα may not be the early, proximal mediator seen in primate models of endotoxin or bacteremic shock.

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TABLE 16.1. Plasma Proinflammatory, Antiinflammatory, and Cytokine Antagonists in Patients with Sepsis Syndromes.

Proinflammatory cytokines
TNFα
IL-1α, IL-1β
IL-8
IL-12 heterodimer
IL-18
IFNγ
IL-6
Antiinflammatory cytokines
IL-6
IL-10
LIF
Cytokine antagonists
IL-1ra
p68 (sIL-1RII)
p55 (sTNFR I)
p75 (sTNFR II)

TABLE 16.2. Known Antiinflammatory and Immunosuppressive Properties of Interleukin-10.

Antiinflammatory properties
Suppresses TNFα, IL-1, IL-8, IL-12, GM-CSF, MIP-1, and IFNγ expression
Increases IL-1ra release, shedding of p55 and p75 receptors
Inhibits antigen presentation by macrophages and dendritic cells
Suppresses effector functions of macrophages, T and NK cells
Blocks phosphorylation of IκB
Immunosuppressive properties
Suppresses T cell proliferation to mitogen such as concanavalin A
Suppresses Th1 cell development, reducing cell-mediated immune responses
Promotes Th2 cell development and antibody formation
Downregulates IFNγ induced and constitutive expression of MHC class II
May stimulate lymphocyte apoptosis
In vivo responses
Exogeneous administration prevents endotoxin-induced shock and lethality
Endogenous production reduces inflammatory response and improves outcome to cecal ligation and puncture and pancreatitis
Endogeneous production after cecal ligation and puncture increases lymphocyte apoptosis and decreases innate immune responses in the lung to *Streptocococcus* and *Pseudomonas*
Endogeneous production after a burn injury responsible for T cell energy
Tolerance to endotoxin is IL-10-dependent

In a large proportion of patients, the clinical manifestations of the sepsis syndrome may not represent a systemic proinflammatory cytokine response but, rather, a refractory antiinflammatory response that leads to anergy and immunosuppression. Indeed, Bone[26,31] argued convincingly that a significant proportion of patients with the sepsis syndrome may be manifesting an unbalanced, compensatory antiinflammatory response that represents the opposite end of the biologic continuum from the proinflammatory response. Indeed, elevated levels of the antiinflammatory cytokine IL-10 have been reported in a large proportion of patients initially with septic shock,[32] and these elevated levels appear to correlate with adverse outcome.[33–35] The apparent immunosuppression seen in some patients with sepsis syndrome has led to the proposition of restoring macrophage deactivation by exogenous administration of interferon-γ (IFNγ).[36,37]

In addition, there is clear evidence that plasma concentrations of cytokine antagonists are elevated in patients with sepsis syndrome; and unlike TNFα and IL-1β, their concentrations remain elevated for sustained periods. In response to an endotoxin or gram-negative bacteremic challenge, TNFα and IL-1β concentrations peak within 1–3 hours and disappear within 4–8 hours,[8,38] whereas IL-1ra, p55, and p75 concentrations remain elevated for up to 24 hours.[39–41]

Interleukin-10

Interleukin-10 has attracted a great deal of attention lately, but its role in the sepsis response remains unclear and complex. Of all the principal antiinflammatory cytokines, IL-10 appears most frequently in the circulation; and modulation of its activity has been shown to have both positive and adverse effects on outcome. IL-10 is produced by macrophages and T cells in response to a variety of stimuli, including endotoxin.[42] The plasma appearance of IL-10 in response to endotoxin stimulation is delayed relative to the proinflammatory cytokines IL-1 and TNFα;[16] IL-10 mRNA transcripts appear 8 hours after endotoxin challenge and peak at 24 hours.[43] In addition, both TNFα[16] and IL-1[44] induce expression of IL-10; and IL-10 has been shown to downregulate its own expression from endotoxin-activated monocytes in an autocrine fashion.[43] IL-10 exhibits direct antiinflammatory and immunosuppressive properties against a variety of cell types including macrophages, endothelial cells, and tissue fibroblasts[42] (Table 16.2). Specifically, IL-10 has been shown to inhibit TNFα, IL-1α, IL-1β, IL-6, IL-8, and macrophage inflammatory protein-1 (MIP-1) expression by endotoxin-stimulated monocytes and neutrophils.[36,37,45,46] In addition, IL-10 promotes shedding of the TNF receptors (p55 and p75) and expression of IL-1ra.[36] In vivo, both Howard et al.[48] and Gerard et al.[49] reported that pretreating mice with IL-10 attenuated the plasma TNFα response and lethality associated with endotoxemic shock. Additionally, intravenously administered IL-10 was shown to block ex vivo endotoxin induction of IL-1 and TNFα in a dose-dependent fashion in normal volunteers, with the effects lasting up to 48 hours.[50,51] These data, in toto, suggest that IL-10 is a natural component of the inflammatory response and serves to shorten its duration and reduce its magnitude. This conclusion is supported by the observation that IL-10 knockout mice and normal mice treated with anti-IL-10 antibodies manifest an exaggerated inflammatory response in response to endotoxin or peritonitis.[52,53]

Experimental studies from our laboratory in the murine visceral ischemia and reperfusion model have examined the potential therapeutic role of IL-10.[54] Pretreatment with human recombinant IL-10 (0.2–20.0 μg) prior to visceral ischemia resulted in a dose-dependent reduction in the resultant lung injury as measured by neutrophil infiltration. Notably, lung injury was reduced with the lower dosages (10–250 μg/kg body weight), but IL-10 was ineffective at the higher dosages

(1–10 mg/kg). Furthermore, no TNFα was detected in the plasma during reperfusion after pretreatment with the lower IL-10 dosages. Similar findings have been reported from our laboratory after pretreatment with IL-10 prior to skeletal muscle ischemia and reperfusion injury in a rat model.[55]

The antiinflammatory properties of IL-10 cannot be distinguished from its immunosuppressive properties, and there is accumulating evidence to suggest that the immune suppression seen after sepsis or thermal injuries is frequently associated with reduced T cell mitogenic responses and increased lymphocyte apoptosis. Kelly et al.[56] demonstrated that systemic release of IL-10 can explain the observed T cell anergy after thermal injury in an experimental model of burn injury. Additionally, Song and colleagues demonstrated that the splenic T cell proliferative responses to concanavalin A were reduced after CLP, due to an endogenous IL-10 response.[57] Similarly, Steinhauser and colleagues demonstrated that after CLP intratracheal instillation of *Pseudomonas* produced an IL-10-dependent increase in mortality.[58] These authors concluded that the septic response substantially impairs the lung's innate immunity to *Pseudomonas*, and this effect is mediated primarily by endogenously produced IL-10. Furthermore, Van der Poll et al.[59] demonstrated that exogenous IL-10 increased the susceptibility to *Streptococcus pneumoniae*-induced acute respiratory distress syndrome (ARDS) in a murine model.

Therapeutic use of IL-10 as an antiinflammatory agent in patients with sepsis and the systemic inflammatory response syndrome (SIRS) remains a highly controversial topic. IL-10 has been or currently is in clinical trials in patients with rheumatoid arthritis, inflammatory bowel disease, human immunodeficiency virus (HIV) infection, hepatitis C infections, and other chronic inflammatory diseases[60,61] (Table 16.3). The safety profile has been good; and despite chronic administration for periods of several months, an increased frequency or severity of infections has not been reported. The primary complications associated with its administration have been minimal and include injection site inflammation, lethargy and headache. There are at present two clinical trials with IL-10 in patients with ARDS and following intestinal ischemia/reperfusion injury after thoracoabdominal aortic injury repair, which will be reported in the near future. The goal of these studies is to determine whether IL-10-mediated suppression of the inflammatory response to ARDS or ischemia/reperfusion injury results in reduced severity and frequency of multisystem organ failure. These studies should go a long way to answering the question of whether the antiinflammatory properties of IL-10 are outweighed by its immunosuppressive properties and if IL-10 has a clinical role as a therapeutic agent in sepsis syndrome.

TABLE 16.3. Diseases for Which IL-10 Therapies Have Been Used and Their Outcome.

Disease	Outcome
Rheumatoid arthritis	Positive results in phase II clinical trials; studies continuing
Inflammatory bowel disease	Positive results in phase II clinical trials; studies continuing
Hepatitis C	Combination therapy with interferon ongoing
ARDS	Results of blinded, randomized phase II trial pending in 1999
Ischemia/ reperfusion syndrome	Interim analysis of phase 1A safety trial pending in 1999
OKT3 treatment	Only modest effectiveness
Human volunteers	Antiinflammatory and immunosuppressive properties defined
Psoriasis	Some modest beneficial effects in open label study
Jarisch–Herxheimer reaction	Only modest effectiveness

TNFα and Members of Its Superfamily

TNFα is only one member of an increasingly large superfamily of proteins with structural and functional homology. As shown in Table 16.4, there are multiple members of this family, many of which have functions that are still unknown. Known members of the TNF family [including TNFα, FasL, and apolipoprotein I (Apo I) ligand], and TNF-related apoptosis-inducing ligand (TRAIL, or Apo II ligand) play at least two critical roles in intercellular communication and tissue homeostasis. These proteins signal the presence of inflammation through NFκB-dependent processes and initiate removal of unnecessary or potentially dangerous cells by apoptosis.[62] The proinflammatory properties of TNFα are well known, but it is only during the past few years that the proapoptotic properties of TNFα have been studied, especially in regard to sepsis and SIRS. At present it is unclear why so many distinct members of this cytokine family exist; and the relative contributions played by each in specific inflammatory processes, such as endotoxemic shock and bacterial peritonitis, is uncertain.

The most well known member of the TNF family is TNFα, in part because it is the only member of the TNF family that is readily secreted in a biologically active form. Although 17-*k*Da TNFα is presumed to play a central role in the vascular changes associated with bacteremic/endotoxemic shock, there is increasing evidence that membrane-associated TNFα also plays a

TABLE 16.4. Known Members of the TNF Superfamily.

Tumor necrosis factor-α (TNFα)
Fas ligand (FasL)
TNF-related apoptosis-inducing ligand (TRAIL)
Lymphotoxin-α, Lymphotoxin-β
Nerve growth factor (NGF)
CD40 ligand, CD27 ligand, CD30 ligand
OX-40 ligand, 4-1BB ligand
Osteoclast differentiation factor (ODF)
TNF-related activation-induced cytokine (TRANCE)
Receptor activator of NFκB ligand (RANKL)
LIGHT
TWEAK
APRIL (a proliferation-inducing ligand)

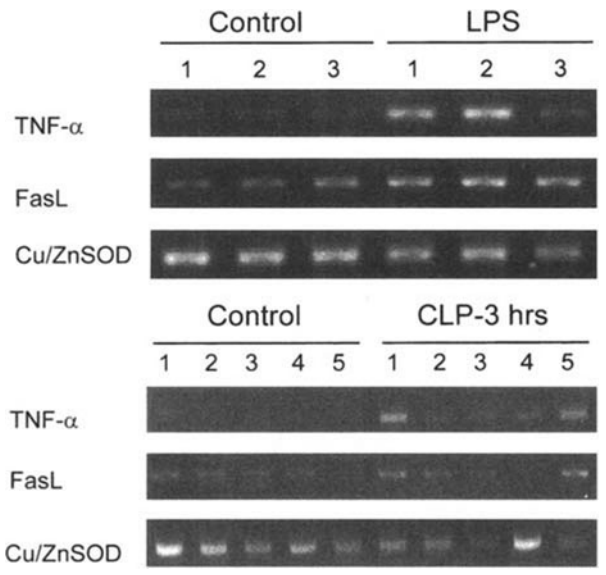

FIGURE 16.1. Reverse transcriptase-polymerase chain reaction (RT-PCR) determination of TNFα and FasL expression in endotoxinemic shock (LPS) and cecal ligation and puncture (CLP). Samples were obtained from mice 2 hours after endotoxemic shock and 3 hours after cecal legation and puncture. (Modified from Tannahill et al.,[69] with permission).

critical role in inflammation and apoptosis associated with hepatitis and rheumatoid arthritis.[63–65] Other members of the TNF family, including FasL and TRAIL, appear to be almost exclusively membrane-associated, although the adamolysins (which process membrane-associated TNFα) may also process FasL.[66] However, secreted FasL may not be bioactive; rather, it may be an inhibitor of Fas/CD95 signaling.[67]

Members of the TNF family are therefore primarily cell-associated and communicate in a paracrine fashion,[62,68] so it is unlikely that we would detect these proteins in the circulation. Because members of these protein groups often share overlapping biologic activities, the role other members of this family play in the host response to sepsis or SIRS remains unknown. This is particularly the case in rodent CLP models, where the immunologic dissonance and mortality are not dependent on TNFα. In a recently reported study we employed a sensitive, semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) technique to quantitate the changes in mRNA levels that occur in response to these inflammatory stimuli[69] (Fig. 16.1). TNFα, FasL, TRAIL, CD30L, and CD40L expression were all evaluated in organs of the reticuloendothelial system in both endotoxemic shock and CLP models.

The results were clear-cut. Of the five members of the TNFα superfamily examined, only expression of TNFα and FasL were increased in both models (Table 16.5). FasL expression was increased in both liver and lung following endotoxinemia and CLP and paralleled the increases in expression of TNFα. Surprisingly, TRAIL expression appeared to be constitutive and was generally unaffected by the presence of inflammation, although expression may have decreased in the lung. CD30L and CD40L expression were highly variable in the organs but did not seem to be affected by either endotoxemia or CLP.

TABLE 16.5. Summary of Cytokine Expression in Various Organs after CLP or Endotoxinemia.

Cytokine	Lung	Liver	Kidney	Spleen
TNFα	++++	++++	+/−	−
FasL	++++	++++	+/−	−
TRAIL	++/−	+/−	+/−	+/−
CD40L	+/−	+/−	+/−	+/−
CD30L	nd	+/−	nd	+/−

CLP, cecal ligation and puncture.
++++, significant increase in expression following CLP and endotoxinemia; −, significant decline in expression after CLP; ++/−, increase in lung expression transiently after endotoxinemia but a progressive decline 24 hour after CLP; +/−, no consistent change in expression; nd, samples were not analyzed.

These data suggest that TRAIL, CD30L, and CD40L expression likely do not contribute to organ apoptosis or an inflammatory response after CLP or endotoxinemia. However, TRAIL bioactivity may not be regulated at the level of TRAIL expression but, rather, at the level of receptor signaling. TRAIL can bind to either of two functioning receptors (TRAIL-R1 or TRAIL-R2) or to two decoy receptors (DcR1 and DcR2), which do not transduce a signal.[70] It is the distribution of these receptors that appears to determine the differential responsiveness of normal and malignant cells to TRAIL-mediated apoptosis.

The current findings should help focus research attention on the possible roles played by TNFα and FasL in the host response to acute bacterial infections and endotoxemia. There is growing appreciation that although an exaggerated TNFα response contributes to the organ failure and death associated with bacteremic or endotoxemic shock TNFα does not play a significant contributory role in mortality after CLP. We know, for example, that TNFα through p55 receptor signaling can induce apoptosis in lymphoid, epithelial, and endothelial cells. However, Hiramatsu et al. demonstrated that blocking an endogenous TNFα response had no effect on the increased apoptosis seen in thymus, spleen, lung, and gut.[71] Similarly, Ayala et al. reported increased thymic apoptosis in mice subjected to CLP that was unaffected by TNFα blockade.[72] Although increased TNFα expression appears to be a ubiquitous response to gram-negative bacterial infections and endotoxemia, the findings suggest that other mediators, not TNFα, must contribute to these apoptotic and inflammatory changes.

The current observation that both FasL and TNFα are concordantly increased in liver and lungs from mice following CLP suggests that increased FasL expression may be an additional mediator contributing to the apoptotic processes present in these tissues or organs. We have previously shown that FasL and TNFα expression are concordantly increased in the livers of mice with concanavalin A hepatitis, a T cell-mediated injury, although inhibiting FasL activity with a soluble Fas immunoadhesin had only modest effects.[68]

The role FasL plays in the response to endotoxemia and CLP remains unclear. Nagata's group showed that FasL contributes

to the hepatic injury secondary to overexpression of hepatitis B virus (HBV) antigens or endotoxin in a *Corynebacterium parvum*-primed mouse,[73] but that has not been our experience. We demonstrated that FasL played a role in the apoptotic liver injury following administration of concanavalin A only when the processing of FasL was inhibited by a matrix metalloproteinase inhibitor.[68] We could not demonstrate a role for FasL in the apoptotic injury that accompanied concanavalin A administration or after endotoxin-induced shock (unpublished observations).

Role of Cytokines in Organ Apoptosis after Sepsis and Burns

There has been increasing awareness that apoptosis occurs after sepsis and burn injury, but little is known about the organs and cell types affected. We and others have demonstrated that after CLP, endotoxinemia, or a thermal injury increased apoptosis is limited primarily to lymphoid tissues.[71–77] Increased apoptosis is not observed in parenchymal cells of the liver or in epithelial cells of the kidney, although there are modest increases in the lungs. Rather, the increases in organ apoptosis are primarily seen in lymphoid-rich organs (spleen, thymus, small intestine). Hotchkiss et al.[78] also reported increased lymphocyte apoptosis in the lungs of mice after CLP. Such increased apoptosis of lymphoid cell populations, frequently seen following a variety of inflammatory insults, is proposed to contribute to the immune suppression that often results. This increased apoptosis in thymus and spleen is seen in both mature and immature T and B cells; and it appears to be dependent primarily on activation of caspase-3-dependent pathways. We have shown that treatment of mice with a synthetic inhibitor of caspase-3 completely prevented the increased apoptosis seen after a burn injury.[74] Similarly, Hotchkiss et al. reported that mice overexpressing BCL-2 had attenuated apoptosis and increased survival after CLP.[78]

Interestingly, Hotchkiss and colleagues have examined organ apoptosis, caspase-3, and BCL-2 activity in lymphoid organs from patients who had immediately expired from sepsis.[79] Using histologic criteria, these investigators demonstrated that almost 50% of these patients had increased apoptosis and caspase-3 activity in lymphoid cells from spleen and intestine. In contrast, apoptosis was a rare event in patients expiring from nonseptic events. This is the first demonstration of increased apoptosis in lymphoid organs from patients expiring from sepsis and suggests that increased apoptotic loss of immune cells is a real event in human sepsis.

Caspase-3- and BCL-2-dependent apoptosis are hallmarks of TNFα- and FasL-mediated apoptosis, signaling via the p55 and Fas/CD95 receptors, respectively.[62] Activation of the death domains of these two receptors results in concatamerization of death effector molecules, such as TNF-Receptor associated death domain (TRADD) and Fas-associated death domain (FADD), and activation of caspase 8, Mast-associated CED3 Homologue, *MACH* 1, or *FLICE*, FADD-like IL-1-beta converting enzyme. Activation of this early member of the caspase cascade leads to catalysis and autoactivation of caspase-3, a primary effector arm of apoptosis.

Despite convincing evidence that this increased lymphoid apoptosis is caspase-3-dependent, the current evidence to date suggests that neither TNFα nor FasL is responsible for the increased apoptosis in spleen, thymus, and bone marrow. The only exception appears to be in splenic B lymphocytes. Ayala et al. reported that the increased apoptosis of mucosal B lymphocytes seen in mice following bacterial peritonitis was secondary to FasL, as it was markedly reduced in mice expressing defective FasL.[77]

Rather, the data suggest that increased corticosteroid release can explain this increase in apoptosis seen with thermal injuries and CLP, presumably through a caspase-3-dependent process (Table 16.6). There are, in fact, two lines of evidence to suggest a primary role for glucocorticoids in mediating this response. When dexamethasone was administered to healthy mice, similar transient increases in apoptosis were seen in spleen and thymus.[80] Nakamura et al. similarly observed that during glucocorticoid-induced thymocyte death most apoptotic cells aggregated to form clusters being phagocytosed by macrophages.[80] This histologic feature is similar to that observed in thymus and spleen after burn injury. Caspase-3 activity also increases during thymocyte apoptosis induced by dexamethasone; and pretreatment with a caspase-3 inhibitor prevents apoptosis due to corticosteroid administration.[81,82]

Second, blocking endogenous glucocorticoids with mifepristone reduced not only apoptosis in both organs but also caspase-3 activity. We have previously shown that treatment of mice with a caspase-3, but not a caspase-1, inhibitor prevented apoptosis after a burn injury in both spleen and thymus.[74] Treating mice with mifepristone also blocked the increased apoptosis seen in thymus and spleen after a burn injury. These findings are therefore consistent with previous work demonstrating that the increased apoptosis observed in thymus after CLP appeared to be due to glucocorticoids alone.[72] Because suppression of glucocorticoid responses after sepsis is not a viable therapeutic alternative, recent efforts have been directed at blocking organ apoptosis through inhibition of caspase-3 pathways. Synthetic inhibitors of caspase-3 as well as novel pox viral proteins (Crm A and SPI-2) are currently in preclinical testing as a means to reduce the apoptosis of lymphoid organs that accompanies sepsis.

Cell-Associated TNFα Signaling

Although it has been known since the studies of Kriegler et al. that TNFα can exist in both cell-associated and secreted forms, and both forms are bioactive,[83] the role these two species play in organ injury and apoptosis is unclear. We and others have previously shown that it is the secreted 17-kDa TNFα, circulating in a trimeric form, that is primarily responsible for shock and organ injury (for review see Ksontini et al.[62]); but the concept that TNFα signaling of inflammation and apoptosis in vivo

TABLE 16.6. Role of Various Humoral Mediators and Cell Signaling Pathways in Increased Apoptosis after CLP or Thermal Injury.

Mediator/pathway	Role
TNFα	May be associated with hepatocyte apoptosis in models of T cell-mediated liver injury, such as in hepatitis B or C or adenoviral gene therapy, or in macrophage-mediated hepatocyte apoptosis in the presence of transcriptional inhibitors
	Does not appear to play a significant role in lymphoid cell apoptosis in sepsis syndromes or thermal injury
FasL	Does not appear to play a significant role in the mortality due to endotoxoin shock or in liver apoptosis in either macrophage or T cell models of hepatocyte apoptotic injury
	Does not appear to play a significant role in the apoptosis of lymphoid tissues after CLP, with the exception of some gut-associated B cell populations
Granzymes/perforin	Role in mediating apoptosis in sepsis or burn injury unclear
TRAIL	Probably plays little role in organ apoptosis after acute inflammation
Lymphotoxin, CD40L/CD30L, other members of the TNF superfamily	Role in mediating apoptosis in sepsis or burn injury unclear
Glucocorticoids	Major player in the early apoptotic responses by lymphoid tissues to CLP Induces apoptosis in several T and B cell classes Activates apoptosis through caspase-3-dependent pathways

occurs *solely* through p55 has been challenged by Grell and associates.[84] Grell et al. compellingly demonstrated that the diversity of TNFα actions arises from a differential responsiveness of the two TNFα receptors for the secreted and cell-associated forms of TNFα (Fig. 16.2). We observed that the principal form of TNFα recovered from livers of burned and septic rats was a 26- to 29-kDa protein.[85] TNFα is synthesized as a 26-kDa membrane-associated precursor that is cleaved to the 17-kDa form by TNFα converting enzyme *TACE*, a novel matrix metalloproteinase recently cloned and described.[86] Grell et al. argued that the principal ligand for the p55 receptor is the 17-kDa secreted form of TNFα. The on–off kinetics of the 17-kDa TNFα with p75 receptor are fast. In conditions of low TNFα concentrations, p75 may serve as a ligand passer for the p55 receptor and increase TNFα binding to p55.[87,88] Conversely, close juxtaposition of the 26-kDa cell-associated TNFα to the p75 receptor, as occurs during cell-to-cell contact, allows formation of complexes with increased stability and signaling potential (Fig. 16.2). Grell et al. further proposed that cell-associated TNFα is the prime physiologic activator of the p75 receptor, implying that p75 controls the local TNFα response in tissues.[84]

Data published to date suggest that the 17-kDa secreted TNFα (not the 26-kDa cell-associated form) is primarily responsible for mortality due to endotoxin- or bacteremia-induced shock. Studies conducted in the baboon further suggest that these 17-kDa TNFα actions occur principally through p55 signaling.[13,18] In two recent reports we demonstrated that blocking the secreted form of TNFα with a matrix metalloproteinase inhibitor improves survival after lipopolysaccharide (LPS)/D-galactosamine-induced shock in the mouse but does not protect against the accompanying liver injury.[63,89] In concanavalin A-induced hepatitis, matrix metalloproteinase inhibitors exacerbated hepatocellular necrosis and apoptosis despite more than 90% reduction in plasma TNFα concentrations. Interestingly, treatment with the matrix metalloproteinase inhibitor had minimal effect on the concentration of membrane-associated TNFα in the livers of animals with hepatitis. In contrast, a TNFα-binding protein[90] that neutralized both membrane-associated and soluble TNFα attenuated both LPS/D-galactosamine- and concanavalin A-induced hepatitis in the presence or absence of a matrix metalloproteinase

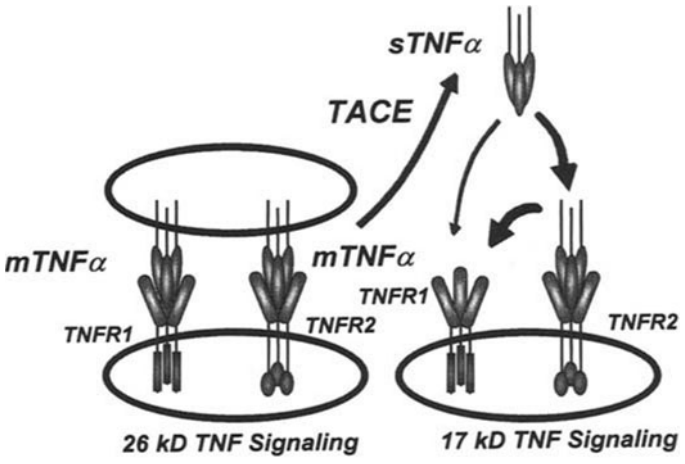

FIGURE 16.2. Proposed TNFα signaling pathways through the p55 and p75 receptor. The primary receptor for the solid 17-kDa TNFα is likely to be p55, whereas low concentrations of TNFα are effectively "passed" from the p75 to the p55 receptor. In contrast, because of steric hindrance, cell-associated 26-kDa TNFα is not easily passed from the p75 receptor to the p55 receptor, and signal transduction through both receptors may occur.

inhibitor. These results suggest that 26-kDa cell-associated TNFα, not the 17-kDa secreted form, plays a critical role in the hepatocellular necrosis and apoptosis that accompany LPS/D-galactosamine- or concanavalin A-induced hepatitis. Therefore the sole blockade of soluble TNFα may be ineffective in preventing this type of injury. Similarly, Georgopolous et al.[91] and Kollias's group[65] demonstrated (using a novel transgenic mouse) that expression of the transmembrane form of TNFα was adequate to produce experimentally induced arthritis.

Although there is now a general consensus that the cell-associated form of TNFα is bioactive and contributes to its juxtacrine effects, confirmation of preferential p75 signaling by cell-associated TNFα remains controversial. Challenging their own hypothesis, Grell and associates demonstrated that endothelial cell apoptosis following irradiation and endotoxemia involved the transmembrane form of TNFα, but that it could be blocked by inhibiting antibodies against the p55, but not the p75, receptor.[92] Similarly, Leist et al. observed that D-galactosamine-sensitized mice expressing a null form of the p55 receptor were resistant to TNFα-induced hepatic injury,[93] suggesting that in experimental hepatitis cell-associated TNFα also signals predominantly through the p55 receptor.

Conclusions

The role of cytokines in the sepsis response has become considerably clearer since the original descriptions of TNFα and IL-1. Although there is agreement that these two cytokines play critical roles in the pathogenesis of sepsis syndrome, interest in them as a therapeutic target in sepsis is waning. A better understanding of the cytokine response to sepsis reveals that several classes of cytokines are induced simultaneously, including proinflammatory and T-helper lymphocyte (Th1)-type cytokines such as TNFα, IL-1, INFγ, and IL-12, as well as antiinflammatory cytokines such as IL-10, and cytokine antagonists such as IL-1ra, p55, p75, and p68. These cytokines not only serve to initiate the innate immune response, but especially IL-10, results in a sustained immunosuppressive response characterized by a Th2-type response and increased lymphoid apoptosis. There is also increasing interest in other members of the TNFα family and the role they play in apoptotic injury. Although FasL expression is increased in animal models of acute inflammation and its expression seems to parallel that of TNFα, its role in the host response to sepsis is unclear. Increased FasL expression does not seem to play a significant role in the mortality or the increased apoptosis in most lymphoid organs. Although progress has been made in understanding the role played by individual cytokines, the lack of a successful therapy for the patient with sepsis suggests that our knowledge of the role cytokines play in the host response to sepsis is still incomplete.

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References

1. Pennica D, Nedwin GE, Hayflick JS, et al: Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 1984;312:724–729.
2. Pennica D, Hayflick JS, Bringman TS, Palladino MA, Goeddel DV: Cloning and expression in *Escherichia coli* of the cDNA for murine tumor necrosis factor. Proc Natl Acad Sci USA 1985;82:6060–6064.
3. Lomedico PT, Gubler U, Hellmann CP, et al: Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli.* Nature 1984;312:458–462.
4. Auron PE, Rosenwasser LJ, Matsushima K, et al: Human and murine interleukin 1 possess sequence and structural similarities. J Mol Cell Immunol. 1985;2:169–177.
5. Beutler B, Greenwald D, Hulmes JD, et al: Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature 1985;316:552–554.
6. Tracey KJ, Beutler B, Lowry SF, et al: Shock and tissue injury induced by recombinant human cachectin. Science 1986;234: 470–474.
7. Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC: Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 1990;348:550–552.
8. Fischer E, Marano MA, Van Zee KJ, et al: Interleukin-1 receptor blockade improves survival and hemodynamic performance in *Escherichia coli* septic shock, but fails to alter host responses to sublethal endotoxemia. J Clin Invest 1992;89:1551–1557.
9. Zeni F, Freeman B, Natanson C: Anti-inflammatory therapies to treat sepsis and septic shock: a reassessment [editorial; comment]. Crit Care Med 1997;25:1095–1100.
10. Baue AE: Multiple organ failure, multiple organ dysfunction syndrome, and systemic inflammatory response syndrome: why no magic bullets? Arch Surg 1997;132:703–707.
11. Cain BS, Meldrum DR, Harken AH, Mclntyre RC Jr: The physiologic basis for anticytokine clinical trials in the treatment of sepsis. J Am Coll Surg 1998;186:337–350.
12. Vincent JL: Search for effective immunomodulating strategies against sepsis [comment]. Lancet 1998;351:922–923.
13. Welborn MB III, Van Zee K, Edwards PD, et al: A human tumor necrosis factor p75 receptor agonist stimulates in vitro T cell proliferation but does not produce inflammation or shock in the baboon. J Exp Med 1996;184:165–171.
14. Tracey KJ, Lowry SF, Fahey TJ, et al: Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. Surg Gynecol Obstet 1987;164:415–422.
15. Van der Poll T, Romijn JA, Endert E, Borm JJ, Buller HR, Sauerwein HP: Tumor necrosis factor mimics the metabolic response to acute infection in healthy humans. Am J Physiol 1991;261:E457–E465.
16. Van der Poll T, Jansen J, Levi M, et al: Regulation of interleukin 10 release by tumor necrosis factor in humans and chimpanzees. J Exp Med 1994;180:1985–1988.
17. Van der Poll T, Jansen PM, Van Zee KJ, et al: Tumor necrosis factor-alpha induces activation of coagulation and fibrinolysis in baboons through an exclusive effect on the p55 receptor. Blood 1996;88:922–927.

18. Van Zee KJ, Stackpole SA, Montegut WJ, et al: A human tumor necrosis factor (TNF) α mutant that binds exclusively to the p55 TNF receptor produces toxicity in the baboon. J Exp Med 1994;179:1185–1191.
19. Fong Y, Tracey KJ, Moldawer LL, et al: Antibodies to cachectin/tumor necrosis factor reduce interleukin 1 beta and interleukin 6 appearance during lethal bacteremia. J Exp Med 1989;170:1627–1633.
20. Van Zee KJ, Moldawer LL, Oldenburg HS, et al: Protection against lethal *Escherichia coli* bacteremia in baboons (*Papio anubis*) by pretreatment with a 55-kDa TNF receptor (CD120a)-Ig fusion protein, Ro 45–2081. J Immunol 1996;156: 2221–2230.
21. Jansen J, van der Poll T, Levi M, et al: Inhibition of the release of soluble tumor necrosis factor receptors in experimental endotoxemia by an anti-tumor necrosis factor-α antibody. J Clin Immunol 1995;15:45–50.
22. Van der Poll T, van Deventer SJ, ten Cate H, Levi M, ten Cate JW: Tumor necrosis factor is involved in the appearance of interleukin-1 receptor antagonist in endotoxemia. J Infect Dis 1994;169: 665–667.
23. Remick D, Manohar P, Bolgos G, Rodriguez J, Moldawer L, Wollenberg G: Blockade of tumor necrosis factor reduces lipopolysaccharide lethality, but not the lethality of [ceccal ligation and puncture]. Shock 1995;4:89–95.
24. Bagby GJ, Plessala KJ, Wilson LA, Thompson JJ, Nelson S: Divergent efficacy of antibody to tumor necrosis factor-alpha in intravascular and peritonitis models of sepsis. J Infect Dis 1991;163:83–88.
25. Echtenacher B, Falk W, Mannel DN, Krammer PH: Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. J Immunol 1990;145:3762–3766.
26. Bone RC: Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med 1996;24:1125–1128.
27. Van Zee KJ, Kohno T, Fischer E, Rock CS, Moldawer LL, Lowry SF: Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha in vitro and in vivo. Proc Natl Acad Sci USA 1992;89:4845–4849.
28. Fisher CJ Jr, Dhainaut J-FA, Opal SM, et al: Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome: results from a randomized, double-blind, placebo-controlled trial. JAMA 1994;271:1836–1843.
29. Pruitt JH, Welborn MB, Edwards PD, et al: Increased soluble interleukin-1 type II receptor concentrations in postoperative patients and in patients with sepsis syndrome. *Blood* 1996;87: 3282–3288.
30. Clark MA, Plank LD, Connolly AB, et al: Effect of a chimeric antibody to tumor necrosis factor-alpha on cytokine and physiologic responses in patients with severe sepsis—a randomized, clinical trial. Crit Care Med 1998;26:1650–1659.
31. Bone RC: Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). Ann Intern Med 1996;125:680–687.
32. Derkx B, Marchant A, Goldman M, Bijlmer R, van Deventer S: High levels of interleukin-10 during the initial phase of fulminant meningococcal septic shock. J Infect Dis 1995; 171:229–232.
33. Marchant A, Deviere J, Byl B, De Groote D, Vincent J-L, Goldman M: Interleukin-10 production during septicaemia. Lancet 1994;343:707–708.
34. Neidhardt R, Keel M, Steckholzer U, et al: Relationship of interleukin-10 plasma levels to severity of injury and clinical outcome in injured patients. J Trauma 1997;42: 863–870.
35. Van Dissel JT, Van Sangevelde P, Westendorp RG, et al: Anti-inflammatory cytokine profile and mortality in febrile patients. Lancet 1998;351:950–953.
36. Cassatella MA, Meda L, Bonora S, Ceska M, Constantin G: Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes: evidence for an autocrine role of tumor necrosis factor and IL-1 beta in mediating the production of IL-8 triggered by lipopolysaccharide. J Exp Med 1993;178:2207–2211.
37. Kasama T, Strieter RM, Lukacs NW, Lincoln PM, Burdick MD, Kunkel SL: Interleukin-10 expression and chemokine regulation during the evolution of murine type II collagen-induced arthritis. J Clin Invest 1995;95:2868–2876.
38. Fong YM, Marano MA, Moldawer LL, et al: The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. J Clin Invest 1990;85:1896–1904.
39. Fischer E, Van Zee KJ, Marano MA, et al: Interleukin-1 receptor antagonist circulates in experimental inflammation and in human disease. Blood 1992;79:2196–2200.
40. Van Zee KJ, Coyle SM, Calvano SE, et al: Influence of IL-1 receptor blockade on the human response to endotoxemia. J Immunol 1995;154:1499–1507.
41. Calvano SE, Thompson WA, Coyle SN, et al: Changes in monocyte and soluble tumor necrosis factor receptors during endotoxemia or sepsis. Surg Forum 1993;44:114–116.
42. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR: Interleukin-10. Annu Rev Immunol 1993;11: 165–190.
43. de Waal Malefyt R, Haanen J, Spits H, et al: Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 1991;174:915–924.
44. Wanidworanun C, Strober W: Predominant role of tumor necrosis factor alpha in human monocyte IL-10 synthesis. J Immunol 1996;151:6853–6861.
45. Cassatella MA, Meda L, Gasperini S, Calzetti F, Bonora S: Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. J Exp Med 1994;179:1695–1699.
46. Wang P, Wu P, Anthes JC, Siegel MI, Egan RW, Billah MM: Interleukin-10 inhibits interleukin-8 production in human neutrophils. Blood 1994;83:2678–2683.
47. Van der Poll T, Jansen PM, Montegut WJ, et al: Effects of IL-10 on systemic inflammatory responses during sublethal primate endotoxemia. J Immunol 1997;158:1971–1975.
48. Howard M, Muchamuel T, Andrade S, Menon S: Interleukin 10 protects mice from lethal endotoxemia. J Exp Med 1993;177:1205–1208.
49. Gerard C, Bruyns C, Marchant A, et al: Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med 1993;177:547–550.
50. Huhn RD, Radwanski E, O'Connell SM, et al: Pharmacokinetics and immunomodulatory properties of intravenously administered recombinant human interleukin-10 in healthy volunteers. Blood 1996;87:699–705.

51. Chernoff AE, Granowitz EV, Shapiro L, et al: A randomized, controlled trial of IL-10 in humans: inhibition of inflammatory cytokine production and immune responses. J Immunol 1995;154:5492–5499.
52. Standiford TJ, Strieter RM, Lukacs NW, Kunkel SL: Neutralization of IL-10 increases lethality in endotoxemia: cooperative effects of macrophage inflammatory protein-2 and tumor necrosis factor. J Immunol 1995;155:2222–2229.
53. Van der Poll T, Marchant A, Buurman WA, et al: Endogenous IL-10 protects mice from death during septic peritonitis. J Immunol 1995;155:5397–5401.
54. Hess PJ, Seeger JM, Huber TS, et al: Exogenously administered interleukin-10 decreases pulmonary neutrophil infiltration in a tumor necrosis factor dependent model of acute visceral ischemia. J Vasc Surg 1997;26:113–118.
55. Engles RE, Huber TS, Zander DS, et al: Exogenous human recombinant interleukin-10 attenuates hindlimb ischemi-reperfusion injury. J Surg Res 1997;69:425–428.
56. Kelly JL, Lyons A, Soberg CC, Mannick JA, Lederer JA: Antiinterleukin-10 antibody restores burn-induced defects in T-cell function. Surgery 1997;122:146–152.
57. Song GY, Chung CS, Schwacha MG, Jarrar D, Chaudry IH, Ayala A: Splenic immune suppression in sepsis: A role for IL-10-induced changes in P38 MAPK signaling. J Surg Res 1999;83: 36–43.
58. Steinhauser ML, Hogaboam CM, Kunkel SL, Lukacs NW, Strieter RM, Standiford TJ: IL-10 is a major mediator of sepsis-induced impairment in lung antibacterial host defense. J Immunol 1999;162:392–399.
59. Van der Poll T, Marchant A, Keogh CV, Goldman M, Lowry SF: Interleukin-10 impairs host defense in murine pneumococcal pneumonia. J Infect Dis 1996;174:994–1000.
60. Keystone E, Wherry J, Grint P: IL-10 as a therapeutic strategy in the treatment of rheumatoid arthritis. Rheum Dis Clin North Am 1998;24:629–639.
61. Van Montfrans C, Camoglio L, van Deventer SJ: Immunotherapy of Crohn's disease. Mediators Inflamm 1998;7:149–152.
62. Ksontini R, MacKay SL, Moldawer LL: Revisiting the role of tumor necrosis factor alpha and the response to surgical injury and inflammation. Arch Surg 1998;133:558–567.
63. Solorzano CC, Ksontini R, Pruitt JH, et al: Involvement of 26-kDa cell-associated TNF-alpha in experimental hepatitis and exacerbation of liver injury with a matrix metalloproteinase inhibitor. J Immunol 1997;158:414–419.
64. Kusters S, Tiegs G, Alexopoulou L, et al: In vivo evidence for a functional role of both tumor necrosis factor (TNF) receptors and transmembrane TNF in experimental hepatitis. Eur J Immunol 1997;27:2870–2875.
65. Alexopoulou L, Pasparakis M, Kollias G: A murine transmembrane tumor necrosis factor (TNF) transgene induces arthritis by cooperative p55/p75 TNF receptor signaling. Eur J Immunol 1997;27:2588–2592.
66. Mariani SM, Matiba B, Baumler C, Krammer PH: Regulation of cell surface APO-1/Fas (CD95) ligand expression by metalloproteases. Eur J Immunol 1995;25:2303–2307.
67. Schneider P, Holler N, Bodmer JL, et al: Conversion of membrane-bound Fas (CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. J Exp Med 1998;187:1205–1213.
68. Ksontini R, Colagiovanni DB, Josephs MD, et al: Disparate roles for TNF-alpha and Fas ligand in concanavalin A-induced hepatitis. J Immunol 1998;160:4082–4089.
69. Tannahill CL, Fukuzuka K, Marum T, et al: Discordant TNF-alpha supefamily expression in bacterial peritonitis and endotoxemic shock. Surgery 1999;126:349–357.
70. Griffith TS, Lynch DH: TRAIL: a molecule with multiple receptors and control mechanisms. Curr Opin Immunol 1998;10:559–563.
71. Hiramatsu M, Hotchkiss RS, Karl IE, Buchman TG: Cecal ligation and puncture (CLP) induces apoptosis in thymus, spleen, lung, and gut by an endotoxin and TNF-independent pathway. Shock 1997;7:247–253.
72. Ayala A, Herdon CD, Lehman DL, Ayala CA, Chaudry IH: Differential induction of apoptosis in lymphoid tissues during sepsis: variation in onset, frequency, and the nature of the mediators. Blood 1996;87:4261–4275.
73. Kondo T, Suda T, Fukuyama H, Adachi M, Nagata S: Essential roles of the Fas ligand in the development of hepatitis. Nat Med 1997;3:409–413.
74. Fukuzuka K, Rosenberg JJ Gaines GC, et al: Caspase-3 dependent organ apoptosis early after burn injury [abstract]. Ann Surg 1999;229:851–858.
75. Hotchkiss RS, Swanson PE, Cobb JP, Jacobson A, Buchman TG, Karl IE: Apoptosis in lymphoid and parenchymal cells during sepsis: a findings in normal and T- and B-cell-deficient mice. Crit Care Med 1997;25:1298–1307.
76. Ayala A, Urbanich MA, Herdon CD, Chaudry IH: Is sepsis-induced apoptosis associated with macrophage dysfunction? J Trauma 1996;40:568–573.
77. Ayala A, Xin XY, Ayala CA, et al: Increased mucosal B-lymphocyte apoptosis during polymicrobial sepsis is a Fas ligand but not an endotoxin-mediated process. Blood 1998;91:1362–1372.
78. Hotchkiss RS, Swanson PE, Knudson CM, et al: Overexpression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis. J Immunol 1999;162:4148–4156.
79. Hotchkiss RS, Swanson PE, Freeman BD, et al: Apoptotic cell death in patients with sepsis, shock and multiple organ dysfunction [abstract]. Crit Care Med 1999;27:1230–1251.
80. Nakamura M, Yagi H, Ishii T, et al: DNA fragmentation is not the primary event in glucocorticoid-induced thymocyte death in vivo. Eur J Immunol 1997;27:999–1004.
81. Alam A, Braun MY, Hartgers F, et al: Specific activation of the cysteine protease CPP32 during the negative selection of T cells in the thymus. J Exp Med 1997;186:1503–1512.
82. Clayton LK, Ghendler Y, Mizoguchi E, et al: T-cell receptor ligation by peptide/MHC induces activation of a caspase in immature thymocytes: the molecular basis of negative selection. EMBO J 1997;16:2282–2293.
83. Kriegler M, Perez C, DeFay K, Albert I, Lu SD: A novel form of TNF/cachetin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 1988;53: 45–53.
84. Grell M, Douni E, Wajant H, et al: The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell 1995;83:793–802.
85. Keogh C, Fong Y, Marano MA, et al: Identification of a novel tumor necrosis factor alpha/cachectin from the livers of burned and infected rats. Arch Surg 1990;125:79–84.

86. Moss ML, Catherine-Jin SL, Milla ME, et al: Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-α Nature 1997;385:733–736.
87. Tartaglia LA, Pennica D, Goeddel DV: Ligand passing: the 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem 1993;268:18542–18548.
88. Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D: Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med 1992;175:323–329.
89. Solorzano CC, Ksontini R, Pruitt JH, et al: A matrix metalloproteinase inhibitor prevents processing of TNF-alpha and abrogates endotoxin induced lethality. Shock 1997;7:427–431.
90. Solorzano CC, Kaibara A, Hess PJ, et al: Pharmacokinetics, immunogenicity, and efficacy of dimeric TNFR binding proteins in healthy and bacteremic baboon. J Appl Physiol 1998;84:1119–1130.
91. Georgopolous S, Plows D, Kollias G: Transmembrane TNF is sufficient to induce localized tissue toxicity and chronic inflammatory arthritis in transgenic mice. J Inflamm 1996;46:86–97.
92. Eissner G, Kohlhuber F, Grell M, et al: Critical involvement of transmembrane tumor necrosis factor-alpha in endothelial programmed cell death mediated by ionizing radiation and bacterial endotoxin. Blood 1995;86:4184–4193.
93. Leist M, Gantner F, Jilg S, Wendel A: Activation of the 55 kDa TNF receptor is necessary and sufficient for TNF-induced liver failure, hepatocyte apoptosis, and nitrite release. J Immunol 1995;154:1307–1316.