Revisiting the Role of Tumor Necrosis Factor α and the Response to Surgical Injury and Inflammation

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Tumor necrosis factor α (TNF-α) is a pleiotropic cytokine with diverse biological actions. Studies originally identified TNF-α as a systemic mediator of endotoxemic shock, cachexia, and tumor regression. We now recognize that TNF-α is a member of a large family of proteins, including Fas ligand, whose actions are primarily paracrine in nature, and serve to regulate both cell proliferation and apoptotic death. Although clinical trials with TNF-α inhibitors in sepsis syndrome have been disappointing to date, and TNF-α administration has not proven widely successful as an antineoplastic agent, preliminary successes with TNF-α inhibition have been recently reported in more chronic inflammatory diseases, including rheumatoid arthritis and ulcerative colitis. The recent description of the TNF-α converting enzyme responsible for the processing of cell-associated to secreted TNF-α has opened a new therapeutic avenue to address inflammatory diseases dependent on the release of 17-kd secreted TNF-α. Similarly, inhibitors of nuclear factor Kappa B activation can increase TNF-α–mediated apoptosis and have rejuvenated efforts to explore TNF-α's antineoplastic potential. The multiple and often conflicting TNF-α signaling pathways reveal a diversity to TNF-α's actions not fully appreciated in the past. Such investigations have opened a number of novel therapeutic interventions to either inhibit or potentiate the actions of TNF-α during surgical injury or acute inflammation. *Arch Surg. 1998;133:558-567*

In 1986, Beutler and Cerami[1] described cachectin/tumor necrosis factor α (TNF-α) as "two sides of the same biological coin." At that time, the authors were referring primarily to the ability of TNF-α to produce both shock and cachexia.[1] Although it is now recognized that several proinflammatory cytokines share overlapping biological actions with TNF-α (for review see van der Poll and Lowry[2]), TNF-α remains a proximal mediator of the host response to acute infection and inflammation. As knowledge of TNF-α has increased, so has the apparent complexity and diversity of its actions. While interest originally focused on the pathological sequelae produced by exaggerated systemic production of TNF-α, particularly as it applied to septic shock, evidence has recently emerged that endogenous TNF-α is essential in mediating a successful response to bacterial infections and to acute inflammation.[3] Disease induced by TNF-α is secondary to either an inappropriate or exaggerated local production, as in the case of rheumatoid arthritis, ulcerative colitis, and Crohn disease, and multiple sclerosis.[4-6]

There is also a growing recognition that TNF-α not only is involved in tissue inflammation and injury, but also appears to be a prominent ligand for the activation of programmed cell death through apoptosis.[7,8] This latter function occurs during normal growth and development, but may also result from pathological conditions in which local and systemic production of TNF-α is increased. Exogenous administration of TNF-α has antineoplastic activity secondary to its ability to induce apoptosis in selected tumor cell populations[9] and to disrupt neovascularization of solid cancers.[10]

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ROLE OF TNF-α IN INFLAMMATION

From 1986 through 1988, Tracey and colleagues[11,12]at Rockefeller University, New York, NY, and Beutler and associates[13] demonstrated that overproduction of TNF-α preceded shock and death induced by endotoxin or gram-negative bacteria. These initial studies, conducted in rodents and primates, established that the variety of inflammatory host responses seen in lethal endotoxemia or gram-negative bacteremia could be reproduced in healthy animals by administering recombinant TNF-α.[11-13] Subsequent studies in mice and *Papio* (baboons) demonstrated that an exaggerated endogenous TNF-α response contributed to the mortality associated with gram-negative bacteremia, and that survival could be improved by inhibiting TNF-α activity with polyclonal or monoclonal antibodies.[14-16]

The benefits of a TNF-α blockade could not, however, be reproduced in other models of sepsis or acute inflammation, particularly in those where infection arose from a nidus. Echtenachter et al[3] demonstrated that blocking an endogenous TNF-α response rendered lethal a nonlethal peritonitis model,[3] while others observed that TNF-α blockade had no impact on the outcome to peritonitis.[17-19] These results suggested that the TNF-α response to infection was not simply a question of exaggerated production. Under certain conditions, an endogenous TNF-α response could be essential, and efforts to block TNF-α could have unfavorable effects.

The preclinical experience, based primarily on rodent and primate models of endotoxemia or bacteremia, nevertheless led to clinical trials with anti–TNF-α therapies for sepsis syndromes. Current data suggests that anti–TNF-α therapies have not lived up to the expectations raised by preclinical studies and have only shown modest benefit in selected patient populations.[20,21] Studies with monoclonal antibodies directed against TNF-α or chimeric TNF receptor constructs (immunoadhesins) in patients with sepsis syndrome have shown no significant beneficial effect.[22-25] In some of the trials, retrospective analysis of prospectively defined subgroups, including patients with shock, have shown some benefit from anti-TNF therapies, but these findings have not been confirmed prospectively.[22,24]

The reasons these clinical trials have failed to show efficacy are multiple, and result from both theoretical and methodological limitations in their study design. One such limitation is the difficulty in prospectively identifying the patient population likely to benefit from anti–TNF-α therapies. Clinical studies have used nonspecific physiological entry criteria, such as the presence of organ system dysfunction and a systemic inflammatory response syndrome, rather than specific disease states, often resulting in a heterologous patient population with diverse underlying disease processes.[26] In the majority of patients receiving such therapies, there was no convincing evidence of an increase in plasma cytokine levels at the time of drug administration. In fact, studies from our laboratory demonstrated that only in a small fraction of such patients (usually less than 5%) could bioactive TNF-α be detected in the circulation of patients with sepsis syndrome.[27,28] In contrast, the plasma concentrations of the major TNF-α inhibitors (p55 and p75 TNF receptors) and interleukin (IL)-1 inhibitors (IL-1RA and p68 IL-1RII) were increased in the blood of septic patients,[28,29] suggesting that these patients had an excess of TNF-α and IL-1 inhibitory activity in the plasma compartment rather than an excess of TNF-α or IL-1.

Current treatment strategies consist of infusing TNF-α inhibitors (monoclonal antibodies or receptor constructs) intravenously. However, systemic administration of these inhibitors may not be an optimal means to block the paracrine actions of TNF-α. The paracrine nature of TNF-α's action has been recognized only recently. The failure to regularly detect TNF-α in the circulation may not adequately reflect local TNF-α production or levels at sites of inflammation. Tumor necrosis factor α has been recovered from the bronchoalveolar fluids of patients with adult respiratory distress syndrome at levels as high as 15 ng/mL, whereas concentrations in the plasma rarely exceeded 100 pg/mL.[30] Levels of TNF-α in the peritoneal fluid of patients with peritonitis often exceeded 10 ng/mL, while plasma concentrations were undetectable by immunoassays or bioassays.[31] Thus, intravenously administered TNF-α inhibitors must be given at levels sufficient to block the concentrations encountered in the inflamed tissues. It is unclear whether the current systemic approach achieves an adequate drug concentration in tissues, since anti–TNF-α monoclonal antibodies and soluble receptor fusion proteins are predominantly sequestered in the plasma compartment.[32-34] Additionally, such therapies often were administered after the onset of symptoms and, presumably, the initial release of the proinflammatory cytokines. Finally, the primary outcome variable in these studies (28-day all-cause mortality) was not consistent with the pharmacokinetics of the administered drugs. The half-life of the TNF-α inhibitors is frequently less than 1 week and, in 1 recent clinical trial, improved outcome was seen only at 7 but not 28 days.[24]

Despite the mitigated results of clinical trials with sepsis syndrome, anti–TNF-α therapies have shown initial success in patients with such chronic inflammatory processes as rheumatoid arthritis. The recent studies from Moreland and colleagues[35] have demonstrated that 75% of patients with refractory rheumatoid arthritis treated with a p75 TNF receptor–Fc fusion protein had at least 20% improvement in symptoms after 3 months. The study was too brief to determine whether the treatment actually interrupted disease progression in this population with a long-standing history of intractable disease, and it was unresolved whether such patients were made more susceptible to opportunistic infections by suppression of TNF-α activity. However, the use of this TNF-α inhibitor in an elderly and potentially immunosuppressed patient population without any significant adverse effect strongly suggests that chronic suppression of TNF-α can be achieved and correlated with a clinically important outcome. Further exploration of the

usefulness of this therapeutic approach in other TNF-α–dependent chronic inflammatory diseases is feasible and warranted.

REGULATION OF TNF-α BIOSYNTHESIS

These therapeutic advances have been achieved in large part due to a better understanding of TNF-α biology. Tumor necrosis factor α biosynthesis and processing are tightly controlled. The synthesis and release of TNF-α are regulated at several levels, including gene transcription, message translation, and protein processing.[13,36] Tumor necrosis factor α is expressed on the cell membrane as a bioactive 26-kd protein, and is cleaved into its 17-kd soluble form by a specific TNF-α–converting enzyme (TACE), an adamolysin and member of the matrix metalloproteinase family.[37,38] Shedding of the extracellular domains of the 2 TNF receptors (by matrix metalloproteinases as well) can further alter the biological activity of TNF-α by decreasing the number of cell signaling sites on target tissues and by increasing the amount of circulating inhibitors.[28,39] Proinflammatory signals including TNF-α itself and IL-1 regulate the shedding of TNF receptors and the increased appearance of shed TNF receptors in the circulation.[39-43] Thus, TNF-α down-regulates its own bioactivity in an autocrine manner by altering the number of cell receptors and by releasing self-acting inhibitors.

Moreover, TNF-α directly induces the expression and release of several humoral factors, including IL-10,[44,45] corticosteroids,[46] and prostanoids,[47] which act in a negative feedback loop to suppress TNF-α production and processing. Tumor necrosis factor α is the principal inducer of IL-10 biosynthesis,[48] and IL-10 effectively suppresses TNF-α release in response to endotoxin.[49] When considered in toto, this feedback system works effectively to permit an initial proinflammatory and innate immune response to inflammation mediated by TNF-α, and then subsequently to limit its magnitude and duration.

Using this model, TNF-α–mediated disease is associated with either excessive initial production of TNF-α or an inadequate production of its inhibitors. Restoration of a normal balance between TNF-α and its inhibitors or antagonists represents the theoretical basis for therapies now considered in several acute and chronic inflammatory diseases including sepsis, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.

This model of TNF-α being a proximal mediator of inflammation must be expanded, however. In the past several years, data have accumulated to suggest that TNF-α and Fas ligand (FasL) are also potent inducers of apoptosis in several cell populations. This process is thought to explain the programmed loss of immune cell populations during acute inflammation.[50] Removal of T-cell populations during sepsis,[51] and neutrophils from inflammatory foci,[52] may result from TNF-α–mediated apoptosis. Thymic involution and loss of leukocyte populations in bone marrow during sepsis can be explained in part by TNF-α–mediated apoptosis.[53] More recently, there has been suggestion that hepatic, renal, and vascular endothelial injury in models of sepsis are due at least in part to increased apoptosis.[54-57] However, it is still unclear how important this process is compared with inflammation-induced necrotic injury.

Interestingly, recent studies suggest that TNF receptor signaling of NF-κB actually antagonizes apoptosis, suggesting a delicate balance within and between TNF-α signaling pathways.[58-61] Evocative studies from several groups have shown that inhibition of TNF-α–mediated nuclear factor kappa B (NK-κB) activation exaggerates apoptotic pathways initiated by TNF-α and FasL. Further exploration of this balance between inflammation and apoptosis, and efforts to manipulate these distinct TNF-α functions may herald a new understanding of the antineoplastic, immune, and inflammatory properties of TNF-α.[58,62]

TNF-α SIGNALING OCCURS THROUGH 2 DISTINCT RECEPTORS

Tumor necrosis factor α signaling occurs through 2 distinct receptors, TNFR1 (p55) and TNFR2 (p75).[63] These receptors share extensive homology in their extracellular domains but have unrelated cytoplasmic domains. Expression of the 2 TNF receptors appears to be differentially regulated and exhibits some tissue specificity. Thus, although both receptors are coexpressed on many cells, one is typically quantitatively dominant. For instance, lymphoid cells predominantly express p75, whereas epithelial cells usually express p55.[64] Experiments on a rhabdomyosarcoma cell line using antibodies specific for each receptor type suggested that each receptor activates a distinct signaling pathway.[65]

Unfortunately, data from in vivo and in vitro studies are often conflicting in identifying the quantitative importance of the individual receptors to TNF-mediated signaling. For instance, in vitro studies have revealed that both p55 and p75 transduce a signal for apoptosis and NF-κB activation on binding of the ligand,[66] while in vivo studies have suggested that p55 is the receptor primarily responsible for TNF-α's proinflammatory properties. Studies from our laboratory showed that in primates, TNF-α muteins with specificity for the p55 receptor were proinflammatory,[67] whereas p75 receptor agonists were not.[68] As shown in **Figure 1**, administration of p55 TNF-α receptor agonists to the healthy baboon produced hypotension, tachycardia, and leukocytic changes comparable to that seen with wild-type TNF-α. In contrast, administration of p75 TNF-α receptor agonists produced none of the physiological responses seen with wild-type TNF-α, with the exception of fever and shedding of the p55 and p75 receptors.

These data are consistent with earlier studies performed using transgenic mice or receptor-specific antagonists. Antibodies that prevented TNF-α binding to the p55, but not p75 receptor, protected mice from lethal endotoxic shock but blocked development of a protective response against infection with *Listeria monocytogenes*.[69] Similarly, transgenic mice lacking a functional p55 receptor were more resistant to TNF-α, but more susceptible to infection with *L monocytogenes*.[70] p75-Deficient mice exhibited a nor-

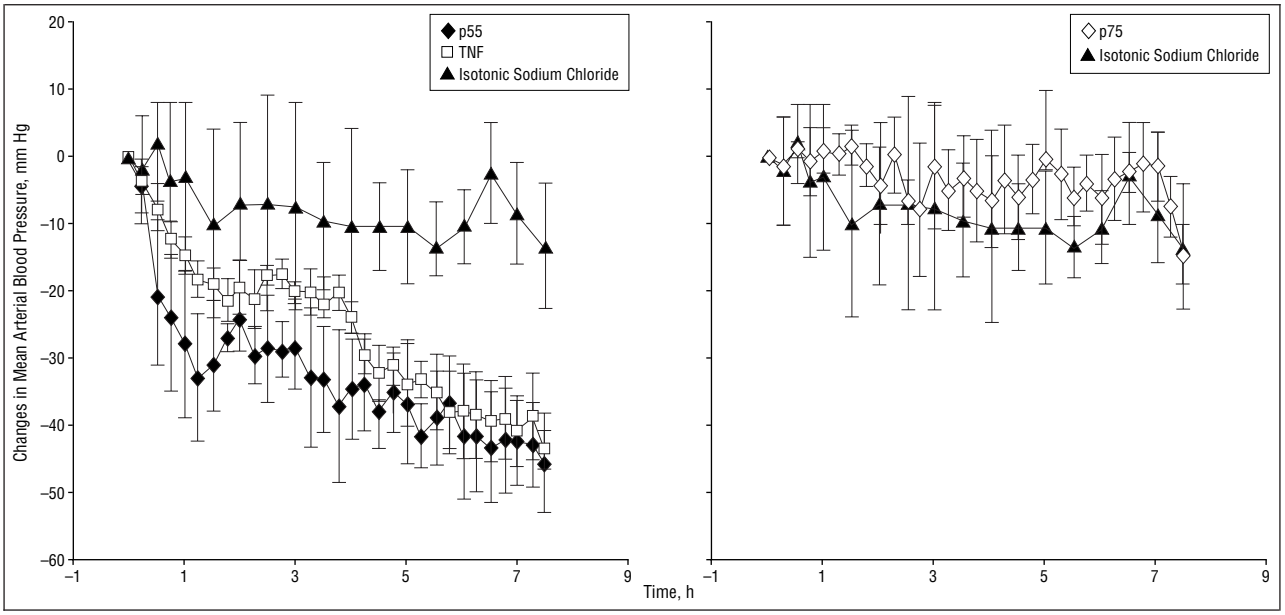

Figure 1. *Changes in mean arterial blood pressure in baboons treated with (left) wild-type tumor necrosis factor α (TNF-α) p55 or (right) p75 TNF receptor agonists (vertical bars represent SEM). Healthy baboons were anesthetized and received an intravenous administration of either 100 µg/kg body weight of recombinant TNF-α, 100 µg/kg body weight of a p55 agonist, or 1000 µg/kg body weight of a p75 agonist. Hemodynamic variables were obtained from anesthetized animals at regular intervals for 8 hours. Wild-type TNF-α and the p55 agonist produced similar changes in hemodynamics and proinflammatory cytokine response. Administration of the p75 agonist had no hemodynamic or proinflammatory effects. (From Welborn et al.[68])*

mal T-cell development and activity. These animals, however, were also more resistant to TNF-α–induced death,[71] suggesting that p75 may have no intrinsic proinflammatory properties of its own, but can potentiate p55 actions.

The proposal that TNF-α signaling of inflammation and apoptosis in vivo occurs principally through p55 has recently been challenged by Grell and associates.[72] Grell et al have compellingly argued that the diversity of TNF-α actions arises from a differential responsiveness of the 2 TNF-α receptors for the secreted and cell-associated forms of TNF-α. In 1988, Kriegler et al[73] reported that biologically active TNF-α existed both as a 17-kd secreted and a 26-kd cell-associated form. We observed that the principal form of TNF-α recovered from livers of burned and septic rats was a 26- to 29-kd protein.[74] Tumor necrosis factor α is synthesized as a 26-kd membrane-associated precursor that is cleaved to the 17-kd form by TNF-α–converting enzyme, a novel matrix metalloproteinase that has been recently cloned and described.[37,38] Grell et al[72] have argued that the principal ligand for the p55 receptor is the 17-kd secreted form of TNF-α. The on-off kinetics of the 17-kd TNF-α with the p75 receptor are very fast.[75] In conditions of low TNF-α concentrations, p75 may serve as a ligand passer for the p55 receptor and increase TNF-α binding to p55.[76,77] Conversely, close juxtaposition of the 26-kd cell-associated TNF-α to the p75 receptor, as occurs during cell-to-cell contact, allows formation of complexes with increased stability and signaling potential (**Figure 2**). Grell et al further propose that cell-associated TNF-α is the prime physiological activator of the p75 receptor, implying that p75 controls local TNF-α response in tissues.

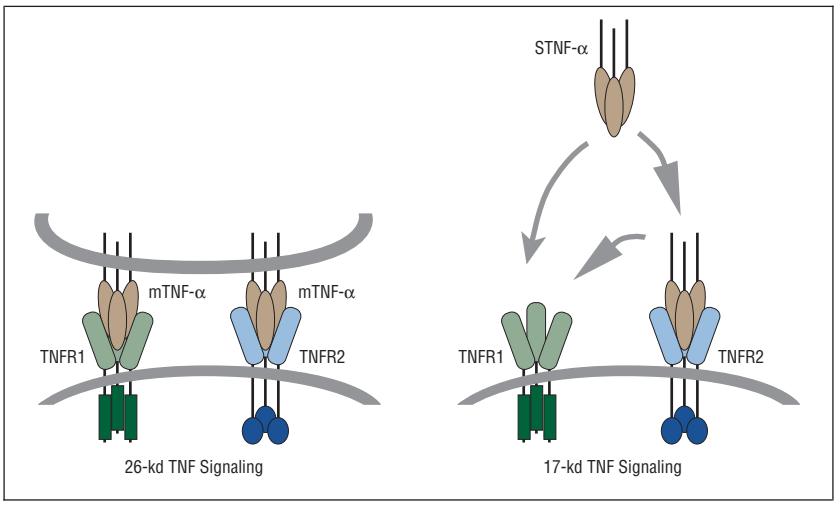

Figure 2. *Secreted 17-kd tumor necrosis factor α (STNF-α) and membrane-associated 26-kd tumor necrosis factor α (mTNF-α) signaling though the p55 and p75 receptors. The STNF-α presumably signals primarily through the p55 TNF receptor (TNFR). Ligand passing from the p75 to p55 receptor may occur, due in part to the fast on-off kinetics of the p75 receptor compared with the p55 receptor. In contrast, membrane-associated TNF-α may signal through both the p55 and p75 receptor because of stearic hindrance stabilizing receptor-ligand contact and preventing ligand passing. TNFR1 and TNFR2 indicate TNFR types 1 and 2. (From Grell et al.[72])*

Data published to date suggest that the 17-kd secreted TNF-α (and not the 26-kd cell-associated form) is primarily responsible for mortality in endotoxin- or bacteremia-induced shock. Studies conducted in the baboon further suggest that these 17-kd TNF-α actions occur principally

Effect of a TACE and TNF-α Inhibitor on Concanavalin A–Induced Hepatitis*

	Plasma AST, U/mL	Plasma TNF-α, pg/mL
Isotonic sodium chloride	3009 ± 444	680 ± 70
TACE inhibitor	9145 ± 1465†	40 ± 20†
TNF-α inhibitor	95 ± 19†	<10†
TACE + TNF-α inhibitors	341 ± 103†	<10†

**Data are given as mean ± SEM. TACE indicates tumor necrosis factor α (TNF-α)–converting enzyme; AST, aspartate aminotransferase.*

†P<.05 vs isotonic sodium chloride.

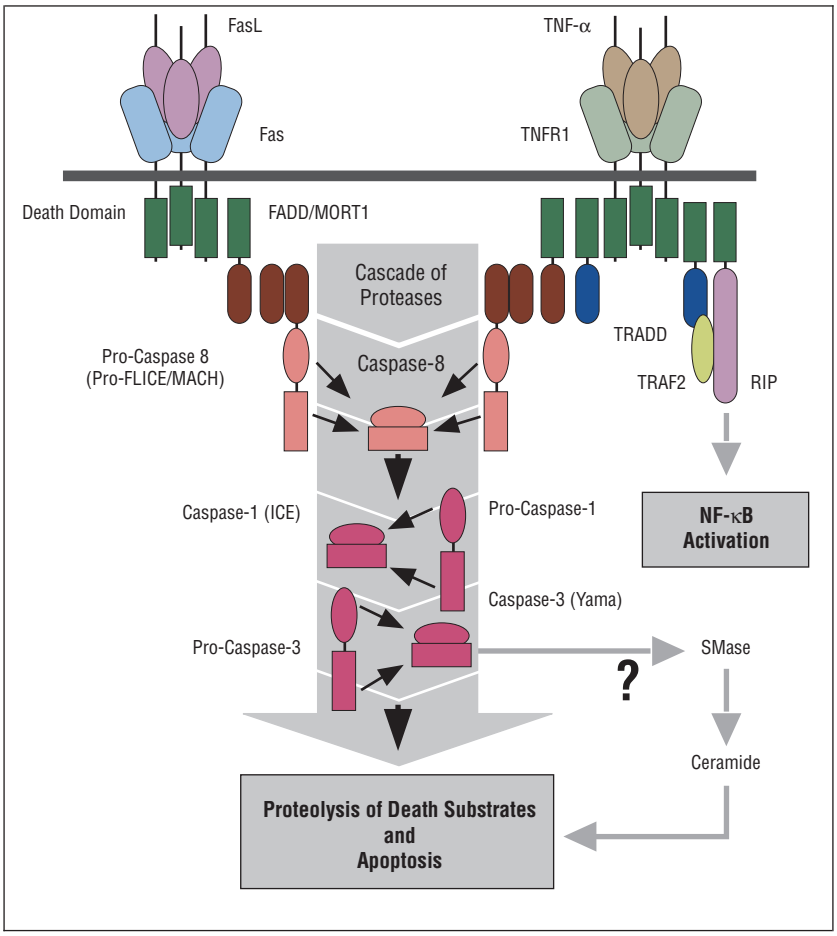

Figure 3. *p55 and CD95/APO-1/Fas signaling of apoptosis. Proposed model by which tumor necrosis factor α (TNF-α) and Fas ligand (FasL) signal apoptosis through caspase-dependent pathway. p55 and Fas signaling converge at caspase-9 (FLICE/MACH). FasL signaling appears more direct since Fas interacts directly with Fas-associated death domain protein (FADD). In contrast, p55 signaling involves TNF receptor accessory factor's (TRAF) interaction with TNF-associated death domain protein (TRADD), which then recruits caspase-9. TNFR1 indicates TNF receptor type 1; RIP, receptor interacting protein; ICE, interleukin-1β–converting enzyme; SMase, sphingomyelinase; and FLICE, FADD-like 1L-1β converting enzyme.*

through p55 signaling.[67] However, in 2 recent reports,[78,79] data from our laboratory demonstrated that blocking the secreted form of TNF-α with a matrix metalloproteinase inhibitor improves survival to lipopolysaccharide plus D-galactosamine–induced shock in the mouse, but does not protect against the accompanying liver injury. In concanavalin A (conA)-induced hepatitis, matrix metalloproteinase inhibitors actually exacerbate hepatocellular necrosis and apoptosis despite greater than 90% reduction in plasma TNF-α concentrations (**Table**). Interestingly, treatment with the matrix metalloproteinase inhibitor had a minimal effect on the concentration of membrane-associated TNF-α in the livers of animals with hepatitis. In contrast, a TNF-α binding protein,[80] which neutralized both membrane-associated and soluble TNF-α, attenuated both lipopolysaccharide plus D-galactosamine and conA-induced hepatitis in the presence or absence of a matrix metalloproteinase inhibitor. These results suggest that 26-kd cell-associated TNF-α, and not the 17-kd secreted form, plays a critical role in the hepatocellular necrosis and apoptosis that accompany lipopolysaccharide plus D-galactosamine or conA-induced hepatitis. Therefore, the sole blockade of soluble TNF-α may be ineffective in preventing this type of injury. Similarly, Georgopoulous et al[81] recently demonstrated, using a novel transgenic mouse, that expression of the transmembrane form of TNF-α was adequate to produce experimentally induced arthritis.

Although there is now a general consensus that the cell-associated form of TNF-α is bioactive and contributes to its juxtacrine effects, confirmation of a preferential p75 signaling by cell-associated TNF-α remains controversial. Challenging their own hypothesis, Grell and associates[82] recently demonstrated that endothelial cell apoptosis following irradiation and endotoxemia involved the transmembrane form of TNF-α, but could be blocked by inhibiting antibodies against the p55, but not the p75, receptor.[82] Similarly, Leist et al[83] observed that D-galactosamine–sensitized mice expressing a null form of the p55 receptor were resistant to TNF-α–induced hepatic injury, suggesting that in experimental hepatitis, cell-associated TNF-α also signals predominantly through the p55 receptor.

TNF-α AND FasL SIGNALING PATHWAYS TO APOPTOSIS AND INFLAMMATION

Tumor necrosis factor α is a member of a growing family comprising at least 10 cytokines,[84] including TNF-β (lymphotoxin), FasL, TNF-related apoptosis–inducing ligand, nerve growth factor, and CD40 ligand. With the exception of lymphotoxin, members of this family are all homotrimers and exist primarily in a membrane-associated form. Their receptors are characterized by a cysteine-rich motif that repeats 3 to 6 times in the extracellular domain. As a rule, members of the

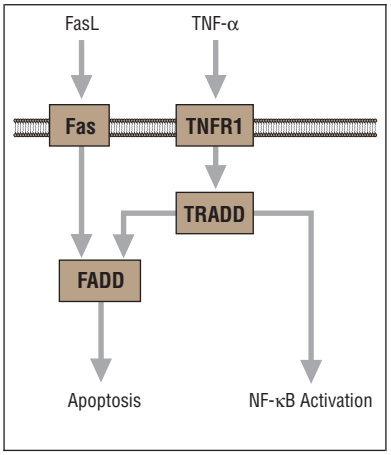

Figure 4. *Convergence and divergence of Fas ligand (FasL) and tumor necrosis factor α (TNF-α) signaling pathways. (NF-κB) indicates nuclear factor kappa B. See legend to Figure 3 for explanation of other terms.*

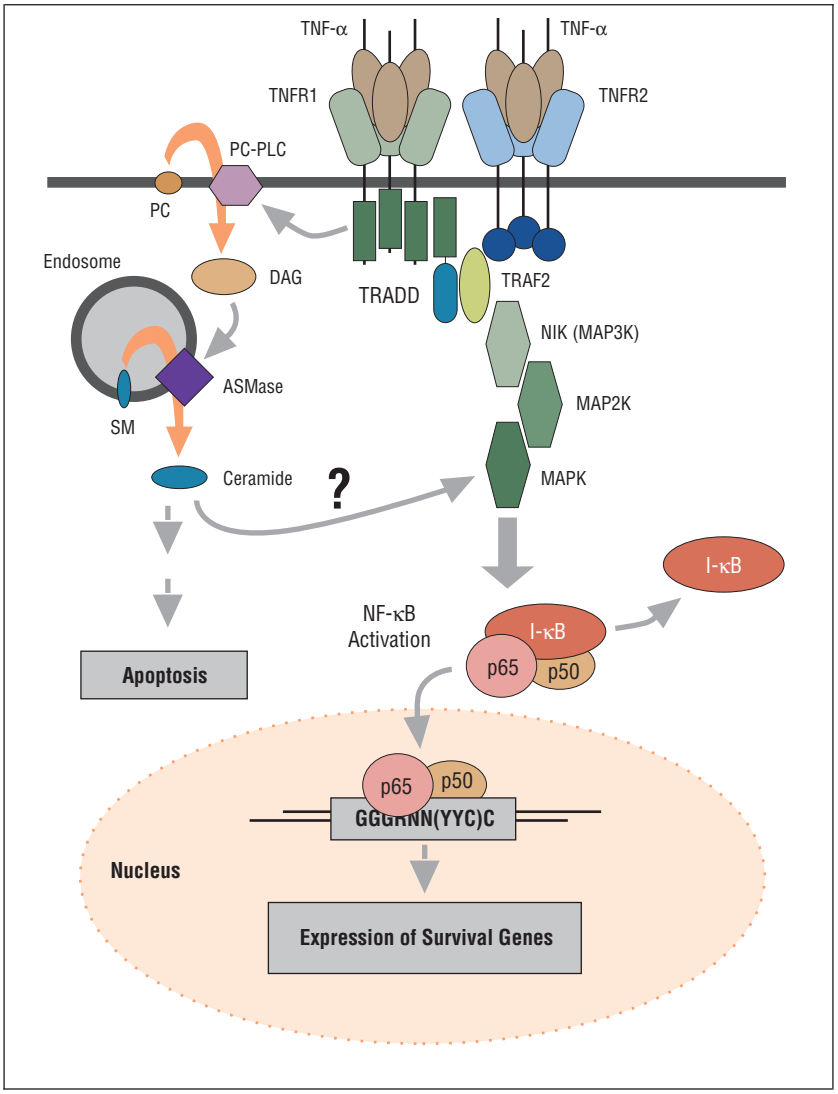

Figure 5. *p55 and p75 signaling of nuclear factor kappa B (NF-κB) activation. p55 and p75 signaling of NF-κB converges at the level of TRADD. TRADD recruits a newly described protein,[66] termed* NIK *(NF-κB–inducing kinase) or* MAP3K*, which initiates the phosphorylation cascade by MAP kinase, leading to NF-κB translocation. Interleukin-1 and Fas ligand activation of NF-κB translocation is presumed to occur through these intermediates. PC-PLC indicates phosphatidylcholine-phospholipase C; PC, phosphatidylcholine; DAG, diacylglycerol; ASMase, acid sphingomyelinase; SM, sphingomyelin; and MAPK, mitogen activated protein kinase. See legend to Figure 3 for explanation of other terms.*

TNF-α family are primarily involved in the regulation of cell proliferation and apoptosis. Tumor necrosis factor α appears to be somewhat unique in this regard, since it has proinflammatory properties in addition to its ability to regulate cell proliferation or apoptosis.

Tumor necrosis factor α and FasL trigger apoptosis on binding to their respective receptors, p55 and CD95/APO-1/Fas. Although primarily produced by macrophages, TNF-α is expressed on many cell types whereas FasL appears to be restricted mainly to the surfaces of cytotoxic T lymphocytes,[85] activated macrophages,[86] and neutrophils.[87]

The p55 TNF-α receptor and Fas share a highly conserved intracellular domain called the "death domain" (DD). This sequence of approximately 70 amino acids plays a pivotal role in triggering apoptosis in the cell.[88] Binding of their respective ligands causes trimerization of the receptors. Their intracellular DDs recruit other DD-containing molecules and initiate the intracellular signaling cascade (**Figure 3**). The Fas DD recruits FADD/MORT1 (Fas-associating protein with DD) and RIP (receptor interacting protein).[89,90] p55 also binds RIP, but requires another DD-containing protein, TRADD (TNFR1-associated DD protein)[90,91] to recruit FADD. FasL may thus provide a more direct route to cell death than TNF-α. TRADD can also interact with 2 other proteins, TRAF1, and TRAF2, from another family of signal-transducing proteins called TNF receptor-associated factors (TRAFS).[92] TRAF2 is an intermediary in the activation of NF-κB by TNF-α. FADD therefore appears to be the converging point of TNF-α and FasL pathways to apoptosis, whereas TRADD lies at the bifurcation of the apoptotic and proinflammatory signaling pathways of TNF-α (**Figure 4**).

The p75 TNF-α receptor presumably participates in the proinflammatory signal of TNF-α via TRAF2 (**Figure 5**). Some investigators, however, have observed the ability of p75 receptor agonists to induce apoptosis.[65,93,94] Induction of apoptosis by p75 does not share the same pathways as p55, but seems to rely on the induction of acid or neutral sphingomyelinases (SMase). Like p55, p75 has been found to associate with the C-terminus of TRAF2, which mediates activation of NF-κB. Recently a new protein kinase, NIK (NF-κB–inducing kinase), which binds to TRAF2 and stimulates NF-κB activity has been described.[66] This latter signal transduction peptide appears to be involved in TNF-α–, FasL-, and IL-1– induced pathways of NF-κB activation.

The Fas/p55 apoptotic signaling pathway involves a family of cys-

teine proteases structurally and functionally related to the product of the *ced-3* gene, which orchestrates cell death in the nematode worm *Caenorhabditis elegans*. The mammalian prototype for this family of proteases is IL-1β–converting enzyme (ICE).[95] Renamed caspase-1, this protease is best characterized by its ability to cleave the inactive IL-1β precursor into the inflammatory cytokine, IL-1β,[96,97] and more recently, its ability to activate interferon-inducing factor or IL-18.[98] The caspase family currently consists of 10 known members, which are divided into 3 principal subgroups, as shown below.[96]

Caspase Nomenclature
ICE-like
ICE (caspase-1)
TX/ICH-2/ICErel-II (caspase-4)
ICErel-III, TY (caspase-5)
ICH-1-like
ICH-1 (caspase-2)
ICE-LAPG (caspase-9)
CPP32-like
CPP32/Yama/apopain (caspase-3)
Mch2 (caspase-6)
Mch3/ICE-LAP3/CMH-1 (caspase-7)
FLICE, MACH, Mch5 (caspase-8)
ICE-LAPG, Mch6 (caspase-9)
Mch4 (caspase-10)

The link between FADD (and therefore p55 and Fas) and these ICE-like proteases was recently established by the discovery of caspase-8 (MACH, FLICE, Mch5).[99,100] In addition to a DD that binds to Fas, TRADD and RIP,[92,101] FADD carries in its N-terminal region a "death effector domain" that is required for the induction of apoptosis by both Fas and TNFR1.[89,102] Caspase-8 is an ICE-like protease that contains in its N-terminal region 2 death effector domains susceptible to interact with the death effector domain of FADD. In its C-terminal region, a 260–amino acid sequence has a strong homology to caspases and includes all known residues required for protease activity.[99,100] Recently, another adapter molecule, RAIDD (receptor-interacting protein-associated ICH-1/CED-3-homologous protein with a death domain), was described. RAIDD contains a carboxy terminal DD that binds to the homologous region in RIP and an amino terminal that has homology to caspase-2 (ICH-1). RAIDD, which when bound to RIP can form a complex with TRADD, binds caspase-2 and thus like caspase-8, provides a direct link to the "death" proteases.[103]

All caspases or ICE-like proteases are synthesized as proenzymes and are proteolytically cleaved to form an active heterodimer. With the exception of caspase-9, whose active site is QACGG, caspases share an active pentapeptide QACRG site, in which the cysteine residue is catalytic. Caspases are the only mammalian proteases known to cleave substrates following an aspartate residue.[97] Apart from caspase-8, which was identified as a FADD binding protein by yeast 2-hybrid analysis,[89] caspases represent the effector components of the apoptotic machinery mediated by Fas and p55.

Inhibitors of caspases, which include the baculovirus p35 protein, the poxvirus serpin CrmA, and aldehyde or fluoromethyl ketone (fmk) derivatives of synthetic peptides, block TNF-α– and FasL-mediated apoptosis in mammals.[97,104,105] These inhibitors of apoptosis have helped delineate the sequence of events occurring during the caspase cascade. IL-1β-converting enzyme (caspase-1) activation is an early event that precedes caspase-3 activation following Fas ligation or p55 receptor signaling. Administration of agonist anti-Fas antibodies into mice causes massive apoptotic death of hepatocytes, which results in total liver destruction and death within a few hours.[106] This process is concurrent with the sequential activation of caspase-1 and caspase-3 in the liver.[105]

It is unclear how caspases orchestrate the morphologic changes that occur during TNF-α– or FasL–mediated apoptosis, but a number of their targets have been identified. These include the caspases themselves and nuclear components such as U1 ribonucleoprotein, nuclear laminins, the nuclear proteins poly-ADP-ribose polymerase, and DNA-dependent protein kinase. The latter 2 enzymes are involved in sensing and repairing DNA damage. In the cytoplasm, the caspases have been found to act on protein kinase C and various cytoskeletal components, such as actin.[85] Another possible target is the outer mitochondrial membrane where the enzymes Bcl-2 and Bcl-xL are located. The Bcl-2 family of enzymes are homologues of the *ced-9* gene product from the nematode worm *C elegans*. Both Bcl-2 and *ced-9* prevent programmed cell death in *C elegans*.[107] Members of the Bcl-2 family inhibit apoptosis in mammals whereas Bcl-x's activate apoptosis. Both Bcl-2 and Bcl-xL have been found to inhibit apoptosis in vitro and in vivo.[108-110] Specifically, overexpression of Bcl-2 in the liver has been found to protect mice from Fas-induced fulminent liver destruction.[110] Because Fas activation damages mitochondrial function that is inhibited by ICE protease inhibitors, it has been proposed that the mitochondrial damage occurs downstream of the caspase cascade.

More recently, a newly cloned cytosolic protein, DFF (for DNA fragmentation factor), has been shown to be activated by caspase-3 to directly induce DNA fragmentation.[111]

TNF-α–MEDIATED NF-κB TRANSLOCATION

The observation that TNF-α has both proinflammatory and apoptosis-inducing properties appears perplexing. Tumor necrosis factor α can induce a variety of other proinflammatory cytokines known to antagonize apoptosis, including IL-1, IL-6, and granulocyte-macrophage colony-stimulating factor. In fact, experimental evidence from several laboratories has recently emphasized that TNF-α may not only directly stimulate apoptotic cell death, but in the same cells actually induce pathways that protect the cells from apopototic cell death.[59] These contrasting roles for TNF-α have led some investigators to speculate that TNF-α–mediated cytotoxicity may be enhanced by therapeutic efforts to block its proinflammatory properties. This has been especially tantalizing for chemotherapeutic efforts where TNF-α has historically been shown to have only modest antineoplastic effects.

In a series of recent reports, it was demonstrated that TNF-α–induced activation of NF-κB was responsible for the inhibition of apoptosis in a variety of cell types, including B-lymphocytes, mouse fibroblasts, and human malignant epithelial cell lines.[60] Historically,

NF-κB was thought to play an early regulatory role in apoptosis, but more recent investigation suggests that NF-κB–induced gene transcription actually antagonizes apoptosis. NF-κB is a ubiquitous transcription factor that plays a critical role in the cellular response to TNF-α's proinflammatory signal.[62] It consists of 2 families of p50 and p65 subunits.[112] All members of this family (NF-B) share a conserved region of approximately 300 amino acids (Rel homology domain) important for their dimerization, nuclear translocation, and DNA binding.[112] These NF-κB proteins form homodimers and heterodimers and their activity is modulated by interactions with inhibitory proteins of the I-κB family. Inactive NF-κB/Rel complexes bound to I-κB are located in the cytoplasm and, on cell activation, dissociate from I-κB and translocate to the nucleus where they bind the κB sites and modulate transcription of genes containing the κB sites in their promoters.[112]

As mentioned above, TNF-α signaling pathways leading to NF-κB activation and/or apoptosis appear to be independent and diverge early in the TNF-α signaling cascade. Conversely, current data suggest that NF-κB is not or only weakly activated by FADD,[92] indicating that FasL does not appear to simultaneously stimulate apoptosis and opposing pathways. Therefore, TNF-α and FasL differ in 2 important regards: while FasL directly activates a cell death pathway, TNF-α does so through a more indirect route and concurrently stimulates pathways that inhibit apoptosis. Two lines of evidence suggest in vitro that it is the balance between induction of NF-κB and the induction of apoptosis that ultimately determines the cell's fate. Van Antwerp and colleagues[60] demonstrated that cells overexpressing a dominant negative form of I-κB that prevented NF-κB activation became exquisitely sensitive to the induction of apoptosis by TNF-α. Similarly, Beg and Baltimore[59] reported that cells with defective NF-κB subunits readily underwent apoptosis on TNF-α stimulation, whereas normal cells were more resistant.[59] In fact, the mechanism by which several growth factors, including IL-1, granulocyte-macrophage colony-stimulating factor, and interferon, inhibit apoptosis is through induction of NF-κB.

Based on this hypothesis, TNF-α–mediated activation of NF-κB may protect some cell populations from apoptotic cell death during acute inflammation. However, this response must be closely balanced because overproduction of TNF-α can also lead to apoptotic injury directly. Furthermore, excessive activation of NF-κB can also have pathological effects, instead of protecting against apoptosis. For example, the exaggerated NF-κB activation that occurs in sepsis syndromes may explain some of the pathophysiological changes, including intravascular coagulopathy. In a recent report, Böhrer and colleagues[113] observed that NF-κB translocation was exaggerated in peripheral blood mononuclear cells from patients dying of sepsis syndrome, when compared with surviving patients. The degree of NF-κB activation was comparable to the Acute Physiology and Chronic Health Evaluation (APACHE) II injury score as a predictor of outcome. Furthermore, the same investigators demonstrated that in a mouse model of endotoxemia, overactivation of NF-κB led to increased renal tissue factor expression, activation of the coagulation system, and renal fibrin and fibrinogen deposition. Such findings suggest that although NF-κB may play a beneficial role in reducing TNF-α–mediated apoptosis, overactivation may be one of the endogenous causes of septic shock.

CONCLUSIONS

Since the late 1980s, considerable knowledge has been gained regarding how TNF-α mediates the host response to inflammation and regulates cell growth and proliferation. With both beneficial and adverse properties, TNF-α activity is essential to a normal inflammatory response, but can also cause the pathological manifestations of acute and chronic inflammation. In general, clinical trials aimed at blocking an endogenous TNF-α response in sepsis syndrome have been disappointing, as have been clinical trials with recombinant TNF-α as an antineoplastic agent. However, early reports from clinical trials with anti–TNF-α therapies in rheumatoid arthritis have been more promising.

The failures with anti–TNF-α therapies in sepsis and with TNF-α administration in cancer can be attributed to an earlier oversimplistic view of sepsis and TNF-α–mediated cytotoxicity. More recent investigations have revealed a surprising level of complexity in the way TNF-α and other members of its superfamily (FasL) signal inflammation and apoptosis. Unfortunately, much of the data exploring TNF-α and FasL signal transduction pathways have been obtained in vitro, and the results have depended to a large extent on the cell lines investigated. Results have often been conflicting and have provided little insight into mechanisms and pathways that lead to inflammation and apoptosis in vivo. Detailed in vivo investigations are now required to explore the physiological significance of these pathways in experimental models of acute inflammation. Only through a detailed understanding of how these systems operate under in vivo conditions can appropriate therapeutic approaches be designed.

Supported in part by grants GM-40586 and GM-53252, awarded by the National Institute of General Medical Sciences, US Public Health Service, Washington, DC.

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