Mini-Review

Cachectin/Tumor Necrosis Factor Exerts Endocrine, Paracrine, and Autocrine Control of Inflammatory Responses

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PARASITIC, bacterial, and viral infections and neoplastic disease profoundly affect host metabolism, disrupting normal homeostatic mechanisms both locally and systemically. A large body of research has documented that many of the observed biological responses to invasive stimuli are mediated by host-secreted cytokines, in particular, the secretory products of activated macrophages. One such cytokine, cachectin/tumor necrosis factor (TNF),[1] has emerged as a particularly important mediator of inflammatory responses. Among its pleiotropic effects, cachectin/TNF has been shown to play a major endocrine role in the pathogenesis of gram-negative endotoxic shock (14, 118) and to induce catabolic responses which could contribute to the profound wasting (cachexia) associated with many chronic diseases (24, 83, 104, 121). It has been implicated in a variety of disease states including meningococcal septicemia (126), cerebral malaria (43), graft vs. host disease (93), and cancer cachexia (2, 8). Systemic exposure to this potent mediator might elicit the pathologic and catabolic derangements associated with such disease states.

Cachectin/TNF has also been shown to exert local, tissue-specific effects. Its wide range of bioactivities includes stimulation of collagenase activity and prostaglandin E_2 production by synovial cells (33), stimulation of osteoclast activity and bone resorption (9), promotion of angiogenesis (41, 60), and stimulation of procoagulant and platelet-activating factor activity in endothelial tissue (19, 75). It has also been shown to stimulate proliferation of normal fibroblasts (40, 114, 125), and to induce the release of certain growth factors including interleukin 1 (IL-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), β_2-interferon, and platelet-derived growth factor (46, 55, 58, 73). The ability of cachectin/TNF to modulate such activities raises the intriguing possibility that it may play a paracrine role in the regulation of normal tissue homeostasis.

Cachectin/TNF also exerts profound effects on its own principal cell of origin, the macrophage. It serves as an autocrine immunomodulator, activating macrophages and enhancing their cytotoxic potential in vitro (92). It has been shown to be chemotactic for monocytes, suggesting that its production at a site of injury might function both to recruit additional macrophages and to activate those macrophages already present.

The capacity of this potent cytokine to mediate the inflammatory response, to modulate the metabolic activities of diverse tissues, and to augment the function of other cytokines necessitates that its synthesis and release be closely controlled in vivo. Local production of cachectin/TNF at a site of injury might act to limit tissue damage and to promote wound healing and tissue remodeling. Moderate systemic levels of the hormone might confer a survival advantage with respect to bacterial or viral infection by providing a useful mobilization of energy reserves for the acute metabolic demands of inflammatory responses. In sharp contrast to these beneficial effects, prolonged exposure to even low levels of cachectin/TNF might contribute to the cachexia associated with many chronic disease states. Rapid uncontrolled production of cachectin/TNF, like that observed in response to endotoxemia or overwhelming gram-negative sepsis, could act systemically to induce the metabolic derangements of septic shock leading to cardiovascular collapse, acute organ failure, and death.

History of Cachectin/TNF

A number of parasitic diseases including trypanosomiasis and leishmaniasis can profoundly alter host physiology leading to severe wasting of the body (cachexia), and ultimately death of the host. Early studies by Rouzer and Cerami elucidated the underlying mechanisms involved in the cachexia associated with parasitic infection (98). Rabbits chronically infected with *Trypanosoma brucei brucei* were observed to become anorectic and to undergo a life-threatening wasting of >50% of their original lean body mass (98). Surprisingly, this wasting syndrome was observed in animals with only a low parasitic burden, and was associated with a marked lipemia (45, 98). The elevated plasma triglyceride levels observed in infected animals were shown to result from systemic suppression of lipoprotein lipase, an enzyme which catalyzes the hydrolysis of lipoprotein-derived triacylglycerol, providing free fatty acids to the circulation for storage or catabolism (79, 108). A similar suppression of lipoprotein lipase (LPL) activity has also been observed in mice after treatment with endotoxin (100). Such observations led to the hypothesis that suppression of LPL activity and the onset of a lipolytic state were regulated by host factor(s) which were released in response to either parasitic or bacterial challenge. Taking advantage of the availability of two genetically similar strains of mice, one sensitive to lipopolysaccharide (LPS) (C3H/HeN) and the other resistant to LPS (C3H/HeJ),

1. *Abbreviations used in this paper*: IL-1, interleukin 1; LPL, lipoprotein lipase; LPS, lipopolysaccharide; TNF, tumor necrosis factor.

Kawakami and Cerami showed that although LPL activity remained normal in LPS-resistant mice in response to LPS administration, serum from LPS-treated LPS-sensitive mice, when transfused into LPS-resistant mice, acted to suppress LPL activity in those mice (53). Macrophages isolated from LPS-sensitive mice were shown to secrete a factor in response to LPS stimulation in vitro which acted to suppress LPL activity in LPS-resistant mice. RAW 264.7, a mouse macrophage cell line, secreted a factor with identical properties when stimulated by LPS (11). Using the suppression of LPL activity in 3T3-L1 adipocytes as a bioassay, the factor responsible for the down-regulation of LPL activity in vivo in endotoxin-treated mice and in vitro in cultured adipocytes was isolated from LPS-stimulated RAW 264.7 cell supernatants, purified to homogeneity, and sequenced (11). The purified protein, referred to as cachectin, had a subunit molecular mass of 17 kD as determined by SDS-PAGE analysis. Its NH_2-terminal amino acid sequence revealed a strong homology to that of human TNF, a macrophage-derived polypeptide hormone previously shown to cause hemorrhagic necrosis of certain tumors (10). With the observation of this striking sequence homology between cachectin and TNF, two disparate lines of research converged: the study of underlying mechanisms of cachexia and the study of mechanisms of endotoxin-induced necrosis of tumors.

The historical background of TNF has been extensively reviewed elsewhere and will only be discussed briefly here (81, 82, 84). As early as the late 1800's, the physician William Coley had observed hemorrhagic necrosis of tumors in selected patients, those with concurrent bacterial infections of streptococcal or serratia origin. Subsequent work established that endotoxin, a component isolated from the cell walls of gram-negative bacteria, was extremely effective at inducing tumor necrosis (106). In 1985, Carswell et al. documented that a factor was present in the serum of BCG-primed, endotoxin-treated mice that was able to induce hemorrhagic necrosis of a mouse sarcoma and complete regression of tumors in a proportion of treated animals (23). Macrophages were determined to be the principal source of this factor (63, 66–68), which was subsequently isolated from supernatants of HL-60 promyelocytic leukemia cells stimulated with PMA (4). The factor was purified to homogeneity and partially sequenced by Aggarwal et al. (4). Purified material induced necrosis of tumors in vivo and exerted cytostatic or cytocidal effects on certain tumor cell lines in vitro. A sensitive bioassay for TNF was developed based on its in vitro cytolytic action on actinomycin-D–treated murine fibroblast L929 cells.

A great deal of enthusiasm was generated with the hope that purification of TNF would enable clinicians to separate the beneficial antitumor properties of endotoxin treatment from its harmful effects, that of circulatory collapse, shock, and death. When sequence analysis by Beutler et al. confirmed the identity of cachectin, the mediator of cachexia and shock, with TNF, the mediator of tumor necrosis, it became apparent that scientists were dealing with a single pleiotropic mediator capable of eliciting a number of complex biological phenomena.

Biosynthesis and Structure

Cachectin/TNF has a subunit molecular mass of 17 kD and a pI of 3.9 (11). The physical properties of this molecule are quite similar irrespective of the species from which it is isolated (11, 47, 99). The amino acid sequence of murine, human, and rabbit cachectin/TNF have now been determined from cDNA clones (39, 51, 64, 127). The mature mouse protein is 156 residues long (39). The mature human protein is comprised of 157 residues due to the insertion of a histidine residue at position 73 (90), while the mature rabbit protein is only 154 residues in length due to the absence of two NH_2-terminal residues (52).

In all three mammalian species characterized to date, cachectin/TNF is synthesized as a prohormone, which is subsequently cleaved at several discrete sites during processing to yield the mature 17-kD hormone (20, 39, 90). The uncleaved precursor sequence is composed of 79 amino acids in the mouse, 76 in the human, and 80 in the rabbit (39, 51, 90). The amino acid sequences of both the propeptide and the mature protein are highly conserved between mouse and human, with 79% of the residues in the mature hormone, and 86% of the residues in the propeptide sequence being conserved between the two species (20, 39, 89). The fact that the propeptide sequence is so highly conserved suggests that it may possess a distinct biological function. It will be of interest to determine if the higher molecular mass prohormone species exhibit preferential tissue specificity or unique biological functions, or if the clipped propeptides themselves are active in eliciting any biological activities distinct from those of either the uncleaved prohormone or the mature protein. Isolated human monocytes do not secrete the same array of precursor species after stimulation by γ-interferon and LPS, but they do secrete three distinct proteins (20, 23, and 25 kD), all three of which are recognized by polyclonal antisera raised against human recombinant cachectin/TNF (85).

Neither rabbit nor human cachectin/TNF contain potential glycosylation sites (20, 52, 89). In contrast, the amino acid sequence of native murine cachectin/TNF contains one N-linked glycosylation site, an asparagine located at position 7 (39). Glycosylation of murine cachectin/TNF has been shown to include sialic acid and galactosamine (44). What role, if any, glycosylation plays in the mechanism of hormone action is still unclear as recombinant murine cachectin/TNF expressed in *Escherichia coli*, although not glycosylated, retains potent biological activity.

All three species of cachectin/TNF sequenced to date contain two cysteine residues per subunit located at positions 69 and 101 (4). Circular dichroism studies and secondary structure prediction indicate that these two cysteine residues form an intrachain disulfide bridge on the surface of the molecule (4, 32). Reduction of the disulfide bridge has no detectable effect on either the secondary or the tertiary structure of the molecule (32). Studies involving cachectin/TNF analogues with substitutions at the two cysteine residues indicate that the disulfide bridge is not required for maintenance of either conformation or bioactivity of the molecule (74).

Amino acid sequence analysis indicates that cachectin/TNF is moderately hydrophobic (64). Hydrophobicity plots of rabbit, mouse, and human cachectin/TNF indicate characteristic hydrophobic regions in both the prohormone and the mature protein, which are conserved among all three species (51, 64). Sequence data indicate that cachectin/TNF bears an unusual secretory protein signal sequence. It con-

tains a centrally located hydrophobic region, but it is significantly longer than the typical signal sequence, and it contains a variety of basic and acidic residues in the first 30 amino acids (78, 90). Recently, a transmembrane form of cachectin/TNF has been characterized (56). This transmembrane form is rapidly inducible in response to LPS and PMA, and has been shown to be cytotoxic for L929 cells. It has been postulated that such a membrane-bound form of cachectin/TNF might allow for localization of the action of this potentially lethal hormone.

It has been reported that cachectin/TNF associates into dimeric, trimeric, or pentameric forms depending on the species from which it is isolated and the method of purification (4, 11, 44, 64, 107). Recombinant human cachectin/TNF has been shown to associate into noncovalently linked trimers under conditions of both low and high salt (5). Recent cross-linking studies indicate that the trimer form is approximately eightfold more active than the monomer with respect to receptor binding (109).

Gene Expression and Regulation

The cachectin/TNF gene is located on chromosome 6 in man (78, 112) and chromosome 17 in the mouse (72, 77). In both species it appears to be linked to the major histocompatibility complex (72, 112). The human cachectin/TNF gene has been linked to HLA-B and HLA-C on the one side and to the class III complement/steroid 21-hydroxylase gene cluster on the other using pulsed-field gel electrophoresis (22). Upstream from the cachectin/TNF gene lies the gene which codes for lymphotoxin, a related hormone which is produced by mitogen-stimulated lymphocytes and is thought to play a role in lymphocyte-mediated killing (31). Cachectin/TNF and lymphotoxin, sometimes referred to as TNFα and TNFβ, respectively, share a 28% sequence homology (78), and have been shown to exhibit a number of shared biological activities including cytotoxicity for L929 cells and the ability to cause hemorrhagic necrosis of tumors in vivo (3). In addition, these two molecules share a common receptor. It has been postulated that both genes were derived from a common ancestral gene by means of a tandem duplication event. It is of interest to determine the cellular and tissue sources of both molecules and to determine whether they are secreted in response to the same or distinct triggering mechanisms.

The biosynthesis of cachectin/TNF appears to be tightly regulated both at the transcriptional level and the posttranscriptional level. Nuclear transcription assays indicate that cachectin/TNF gene transcription is enhanced some threefold in macrophages after stimulation by endotoxin (12). Dexamethasone was found to suppress this endotoxin-induced increase in cachectin/TNF mRNA content (12). The transcription of the cachectin/TNF gene may also be under the control of short-lived repressor molecules which normally serve to suppress its release (26). A comprehensive study of the upstream region of the cachectin/TNF gene with respect to promoter and enhancer sequences has not yet been reported. Such a study would be revealing, especially with regard to any homology that might exist between the regulatory sequences of cachectin/TNF and the regulatory sequences of other mediators of the host inflammatory response.

Posttranscriptional regulation of cachectin/TNF production involves in part the presence of a highly conserved AU-rich sequence located in the 3′-untranslated region of cachectin/TNF mRNA (20). This sequence is observed not only in cachectin/TNF mRNA, but also in the mRNA of a variety of inflammatory mediators including lymphotoxin, interferon, and interleukin-1α and β, and more recently in the mRNA of certain oncogenes including c-*myc* and c-*fos* (20). It is thought to be a critical regulatory element controlling both the half-life of mRNA molecules and the efficiency with which they are translated (10, 20). This is supported by the fact that insertion of the AU-rich sequence of GM-CSF into the 3′-untranslated region of the globin gene seems to confer upon the resultant mRNA a significantly shortened half-life in fibroblasts (105). Beutler et al. have now identified a nuclease present in the lysates of certain mammalian tissues, including macrophages, that selectively degrades mRNA molecules containing this 3′-untranslated AU sequence (15). From model studies using synthetic mRNAs they conclude that the degree of message instability is dependent upon the number of copies of inserted AU sequence present in the target mRNA molecule (15). Tagging cachectin/TNF mRNA for rapid degradation may be one mechanism of controlling the effects of this potent hormone.

Cachectin/TNF Receptor

Most cell types including liver, kidney, muscle, and adipose tissue have been shown to possess specific high affinity receptors for cachectin/TNF (11), and the consequences of ligand binding appear to be tissue specific. Cachectin/TNF exhibits a high affinity for its receptor with a K_a estimated at 10^9 M^{-1} (7, 11, 48, 123). Polyclonal antisera raised in rabbits against peptides representing the NH_2 terminus of human recombinant cachectin/TNF have been shown to block both receptor binding and bioactivity (111). Studies involving NH_2-terminal deletion mutants of recombinant human cachectin/TNF support the requirement for an intact NH_2 terminus in receptor binding (21, 28). Depending on cell type, several hundred to several thousand cell surface receptors for cachectin/TNF are available. Preincubation of cells with γ-interferon significantly enhances the number of cell surface receptors for cachectin/TNF (3, 50). This phenomenon may be a contributing factor to the synergy observed between these two cytokines (25, 40, 129). The receptor has not yet been isolated, and the nature of the signal transduced upon binding remains to be determined. A number of cross-linking studies with radiolabeled cachectin/TNF ligand have indicated the existence of at least two polypeptide chains of 75 and 90 kD that participate in binding (57, 103, 110, 122), and one recent study suggests that as many as four proteins may be involved (29). In vitro and in vivo studies indicate that the biological effects of cachectin/TNF are maximally expressed at receptor occupancies as low as 5–10%. Binding of cachectin/TNF to its receptor is followed by rapid internalization (7) and degradation (123).

Cachectin/TNF: Role in Endotoxic Shock

During lethal endotoxemia a complex pattern of responses including metabolic acidosis, fever, hypotension, peripheral tissue lactate release, elevation of plasma catecholamines, disseminated intravascular coagulation, and renal, hepatic,

and lung injury eventually leads to shock and death of the host. Although originally attributed directly to endotoxin, it is now accepted that most, if not all, of these metabolic derangements are mediated by endogenous cytokines, and cachectin/TNF has been shown to play a major role. Significant quantities of cachectin/TNF are secreted by macrophages after stimulation by LPS both in vivo and in vitro (1, 13, 102). In vivo studies have demonstrated that nanomolar levels of cachectin/TNF are present in the sera of mammals after administration of lethal doses of endotoxin (13). Passive immunization of mice with polyclonal antibody to murine cachectin/TNF 6 h before the administration of otherwise lethal doses of endotoxin conferred on them a significant survival advantage (14). In a rabbit model of endotoxic shock, prior infusion of polyclonal antibody raised against human recombinant cachectin/TNF resulted in neutralization of endotoxin-induced serum cachectin/TNF activity and provided significant protection from the development of hypotension, histopathologic changes, and lethality (65). In vivo studies in mice, rats, dogs, and humans have documented that many of the pathophysiologic changes observed after challenge with endotoxin can be reproduced by administration of purified cachectin/TNF (13, 117, 120). In mice, the injection of recombinant cachectin/TNF induces piloerection, diarrhea, fever, and eventual death (14). Infusion of human recombinant cachectin/TNF into rats causes hypotension, tachypnea, and death after respiratory arrest (117). As with endotoxin administration, metabolic acidosis, hemoconcentration and biphasic changes in blood glucose are observed (117). There is a significant accumulation of neutrophils in the lungs, hemorrhagic necrosis of the adrenals and pancreas, and tubular necrosis in the kidney. All of the pathologic responses noted above were prevented by pretreatment of the rats with neutralizing murine monoclonal antibody against recombinant human cachectin/TNF.

Tracey et al. recently reported that passive immunization of baboons with murine monoclonal antibody against recombinant human cachectin/TNF protected them against the cardiovascular collapse and acute organ failure resulting from infusion of otherwise lethal quantities of live *E. coli* (118). It is important to note that throughout the course of this study animals passively immunized against cachectin/TNF exhibited a significant bacteremia indicating that the presence of bacteria alone, in the absence of circulating cachectin/TNF, is not sufficient to induce the full scope of metabolic derangements associated with sepsis (118). These findings document that in addition to being a principal mediator of endotoxic shock, cachectin/TNF is a critical mediator of lethal bacterial sepsis.

In the baboon model described above, peak serum cachectin/TNF levels (20,500 ± 9890 pg/ml) were observed 90 min after the infusion of a lethal dose of live *E. coli*. A similar temporal pattern of cachectin/TNF release, an early burst followed by a rapid decline to undetectable levels, was observed when a bolus injection of endotoxin was given to human volunteers (49). In the latter study, peak serum cachectin/TNF levels (358 ± 166 pg/ml) were observed within 90 min of endotoxin challenge. These results indicate that cachectin/TNF is an early mediator of endotoxemia and bacteremia, and that although the magnitude is significantly different, the time course of cachectin/TNF appearance is similar in nonlethal and lethal models of acute sepsis.

Cachectin/TNF: Role in Cachexia

One of the original motives for studying cachectin/TNF stemmed from the belief that it was involved in the severe wasting syndrome associated with chronic parasitic infection (11). Since then, the availability of recombinant material has allowed further characterization of the role cachectin/TNF plays in cachexia. Cachectin/TNF has been shown to induce a catabolic state in cultured 3T3-L1 adipocytes by suppressing key lipogenic enzymes including lipoprotein lipase, acetyl CoA carboxylase, and fatty acid synthetase (11, 87, 130); stimulating hormone sensitive lipase (86, 88); and increasing lactate production (86). Torti et al. demonstrated that cachectin/TNF inhibits adipocyte gene expression in TA 1 adipocytes inducing an in vitro "cachectic state" (116). Cachectin/TNF acts to prevent accumulation of lipid within differentiating preadipocytes and to cause mature lipid-laden adipocytes to lose stored triglycerides and to decrease adipose specific mRNAs by >90% (116). By analogy one can envision an in vivo situation in which cachectin/TNF, while functioning to provide a necessary mobilization of peripheral energy reserves for the acute metabolic demands of inflammatory responses, may at the same time be contributing to the cachexia associated with many chronic disease states.

Differentiated L6 myotubes respond to cachectin/TNF in a similar catabolic manner, with a marked stimulation of glycogenolysis characterized by a rapid increase in fructose 2,6-bisphosphate, depletion of glycogen reserves, and increased production of lactate (59). These rapid changes are followed more slowly by increased synthesis of hexose transporters and concomitant stimulation of glucose uptake. In vitro, isolated rat muscle fibers exhibit decreased membrane potential when incubated with low doses of cachectin/TNF (119). These decreases can be completely blocked by monoclonal antibody specific for this hormone. In vivo, dogs infused with cachectin/TNF exhibit reduced resting hindlimb muscle transmembrane potentials and increased lactate release (120). The latter study raises the possibility that the reduced resting skeletal muscle membrane polarization observed in many critically ill patients (30) may result at least in part from the action of this mediator. Recombinant cachectin/TNF does not appear to affect skeletal protein balance, suggesting the involvement of other mediators as well (71).

Recent in vivo studies using a rat model further support the role of cachectin/TNF in cachexia. Metabolic derangements typically associated with the cachexia of chronic disease (including anorexia, weight loss, depletion of whole body protein and lipid stores, and anemia) were reproduced in rats given twice-daily sublethal injections of recombinant human cachectin/TNF for 8 d (121). In addition, daily injections of anti-cachectin antibodies protected cachectin/TNF-treated animals against subsequent weight loss and anorexia (121). This study suggests that continual in vivo exposure to cachectin/TNF is sufficient to elicit many of the clinical manifestations of cachexia.

Additional support for the causative role of cachectin/TNF in cachexia was obtained in experiments in which the cachectin/TNF gene was inserted into a mammalian expression vector and transfected into Chinese hamster ovary cells (83). When these cells, which continuously secrete cachectin/TNF both in vitro and in vivo, were implanted into the hind

limbs of nude mice they produced measurable levels of cachectin/TNF in the circulation. Mice treated in this manner developed a wasting syndrome reminiscent of that seen in cancer cachexia. In sharp contrast, mice implanted with cells transfected with the expression vector alone continued to gain weight during the course of the experiment.

Mice bearing a transplantable methylcholanthrene-induced sarcoma become cachectic, exhibiting significant weight loss, hypoalbuminemia, and increased serum amyloid P concentrations (62). Tumors recovered from these animals have been shown to spontaneously secrete cachectin/TNF in vitro (62). Studies are in progress to document to what extent in vivo production of cachectin/TNF in this model might contribute to the ensuing cachectic state.

Tissue-specific Effects of Cachectin/TNF

Endothelial Cells

Cachectin/TNF dramatically alters the properties of endothelial cells. In vitro, cachectin/TNF has been shown both to increase tissue factor procoagulant activity and to suppress cofactor activity for the anticoagulant protein C pathway which normally acts to block clot formation within the vascular bed (75, 113). The expression of thrombomodulin is also down-regulated (113). These changes are dependent upon protein synthesis and favor procoagulant activity on the endothelial cell surface. In addition, cachectin/TNF stimulates endothelial cells to secrete IL-1 which has also been shown to enhance tissue factor–like procoagulant activity (16, 18, 61, 76). The effects of cachectin/TNF and IL-1 in this regard are additive (16, 17). The altered surface properties of endothelial cells observed in vitro in response to cachectin/TNF could play an important role in certain phenomena observed in vivo after infection and injury. In uncompromised tissue the endothelial cell surface acts to control the coagulation of circulating blood and to restrict the penetration of fluid, cells, and macromolecules into the tissues. One of the hallmarks of endotoxemia is disseminated intravascular coagulation with increased capillary permeability and leakage of macromolecules into the tissue space. This could result from the interaction of cachectin/TNF and the vascular endothelium. The fact that activated protein C anticoagulant activity has been shown to protect against coagulopathy and death in bacteremic shock in a baboon model (115) suggests that the ability of cachectin/TNF to suppress this activity is an important component of the toxicity observed after bacterial challenge.

Cachectin/TNF also stimulates the expression of endothelial cell surface antigens including ICAM 1 (95) and the surface antigens recognized by monoclonal antibodies H4/18 and 60.1, which function to modulate adherence of inflammatory leucocytes to cultured endothelial cells (42, 94–96). In addition to these effects on the endothelial cell surface, cachectin/TNF induces the synthesis of an endothelial cell surface factor(s) that promotes neutrophil adherence to the luminal surface of the vascular endothelium by a mechanism involving the neutrophil membrane adhesion complex CDw18 (LFA-1/Mac-1/p150,95) (97). The expression of HLA-A and HLA-B on the surface of endothelial cells is also increased after exposure to cachectin/TNF (27). The adherence of blood cells to the endothelium is a critical factor in the development of an inflammatory state, and the ability of cachectin/TNF to modulate the expression of surface antigens that are involved in this process suggests that it may play a critical role.

Bone, Cartilage, and Fibroblasts

Inflammatory processes often lead to the destruction of host tissues. Cachectin/TNF has been implicated in a number of these processes including abscess formation and the resorption of cartilage and bone observed in rheumatoid arthritis (9). Cultured human synovial cells show an increased production of collagenase and prostaglandin E_2 in response to cachectin/TNF (9). Collagenase can function to disrupt the extracellular collagen matrix in inflammed tissues, and PGE_2 is thought to stimulate the production of intracellular proteases involved in tissue destruction and remodeling. Human recombinant cachectin/TNF causes chondrocytes to both degrade proteoglycan through limited proteolysis and to inhibit the synthesis of new proteoglycan (101). The destruction of proteoglycan, an essential component of the matrix of cartilage enabling tissue to resist compression during load bearing, results in severe impairment of the function of cartilage. Cachectin/TNF also acts indirectly, stimulating fibroblasts to produce IL-1, another mediator which is postulated to play an important role in the induction of proteolysis observed in inflammatory joint diseases (35). The two cytokines may act in concert to bring about the tissue destruction observed in rheumatoid arthritis, osteoarthritis, and other joint diseases.

Cachectin/TNF modulates the function of a broad range of normal fibroblasts. Dermal fibroblasts respond to cachectin/TNF with an increased production of collagenase and PGE_2 (33). Normal lung fibroblasts show an enhanced production of prostaglandin in response to cachectin/TNF, and this response is augmented by IL-1 (37). Fibroblasts from several tissues have been shown to be growth stimulated by cachectin/TNF (40, 114, 125). This proliferative effect can be explained at least in part by the ability of the mediator to stimulate fibroblasts to produce β_2-interferon, a cytokine shown to function in an autocrine manner enhancing cell proliferation (55). The ability to stimulate fibroblast proliferation may play a role in the repopulation and tissue remodeling which occurs after inflammation. This mediator has also been shown to induce an antiviral state in certain fibroblast cell lines (69).

Leucocytes

During sepsis and bacteremia marked changes are observed in both the number of circulating leucocytes, and in their state of activation. Cachectin/TNF has been shown to play an important role in these changes. It induces neutrophil degranulation (54), and stimulates the release of reactive oxygen intermediates including superoxide anion (124) and hydrogen peroxide (54). It also acts to increase the phagocytic capacity of treated neutrophils and to enhance their cytotoxicity to certain pathogens (36). This multifunctional mediator has also been shown to be chemotactic for neutrophils (70).

Cachectin/TNF acts in an autocrine fashion as well, activating macrophages and enhancing their cytotoxic potential in vitro (34, 92). It elicits an increase in the production of

IL-1 and PGE_2 by cultured macrophages (6). Cachectin/TNF also induces the expression of the class II MHC antigen, I-A, on the surface of macrophages. It synergizes strongly with interferon-γ in this regard. In addition, cachectin/TNF is chemotactic for monocytes (70), and its production locally may serve to attract these cells, as well as neutrophils, to a site of injury.

One of the hallmarks of the cellular response to tissue injury and sepsis is the recruitment and activation of both polymorphonuclear leukocytes and macrophages. The ability of cachectin/TNF to activate these different effector mechanisms suggests that it may play a pivotal role in mounting a well-coordinated cellular response to infection and injury.

Hepatic Tissue

Cachectin/TNF has been shown to regulate the expression of acute phase proteins in vitro in human hepatoma cell lines through a transcriptional mechanism (91). What role this may play in vivo is still unclear, although similar changes are observed in vivo in response to inflammation and injury. Recombinant human cachectin/TNF has been shown to stimulate rat liver amino acid uptake fivefold in vivo (128), and to increase responsiveness to plasma glucagon (128): two metabolic alterations observed early in acute tissue injury or sepsis. The significance of these findings is unclear as cachectin/TNF has no direct effect on hepatocyte amino acid uptake in vitro (128) but does elevate hepatic lipogenesis in vivo (38).

Conclusions

Cachectin/TNF has been shown to be a pluripotent mediator influencing the growth, differentiation, and function of a broad range of cells and tissues. It has been shown to function at many levels, exerting endocrine, paracrine, and autocrine control of inflammatory responses. The various bioactivities this mediator exhibits in vitro and in vivo lends support to the hypothesis that it plays an important role in both humoral and cell-mediated immune and inflammatory responses to injury and infection, and raises the possibility that it is involved in the regulation of normal tissue homeostasis. In addition to the direct actions of cachectin/TNF, its interaction with other host-secreted cytokines including IL-1, β_2-interferon, γ-interferon, and lymphotoxin allows it to play an even more powerful role in the regulation of cell growth and function. To try to harness the beneficial effects of cachectin/TNF while controlling its adverse effects is a daunting task, but one which if achieved, even to some degree, would reap substantial rewards.

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References

1. Abe, S., T. Gatanaga, M. Yamazaki, G. Soma, and D. Mizuno. 1985. Purification of rabbit tumor necrosis factor. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 180:203–206.
2. Aderka, D., S. Fisher, Y. Levo, H. Holtman, T. Hahn, and D. Wallach. 1985. Cachectin/tumor necrosis factor production by cancer patients. *Lancet.* 2ii:1190.
3. Aggarwal, B. B., T. E. Eessalu, and P. E. Hass. 1985. Characterization of receptors for human tumor necrosis factor and their regulation by γ-interferon. *Nature (Lond.).* 318:665–667.
4. Aggarwal, B. B., W. J. Kohr, P. E. Hass, B. Moffat, S. A. Spencer, W. J. Henzel, T. S. Bringman, G. E. Nedwin, D. V. Goeddel, and R. N. Harkins. 1985. Human tumor necrosis factor: production, purification, and characterization. *J. Biol. Chem.* 260:2345–2354.
5. Arakawa, T., and D. A. Yphantis. 1987. Molecular weight of recombinant human tumor necosis factor-α. *J. Biol. Chem.* 262:7484–7485.
6. Bachwich, P. R., S. W. Chensue, J. W. Larrick, and S. W. Kunkel. 1986. Tumor necrosis factor stimulates interleukin-1 and prostaglandin E_2 production in resting macrophages. *Biochem. Biophys. Res. Commun.* 136:94–101.
7. Baglioni, C., S. McCandless, J. Tavernier, and W. Fiers. 1985. Binding of human tumor necrosis factor to high affinity receptors on HeLa and lymphoblastoid cells sensitive to growth inhibition. *J. Biol. Chem.* 260: 13395–13397.
8. Balkwill, F., F. Burke, D. Talbot, J. Tavernier, R. Osborne, S. Naylor, H. Durbin, and W. Fiers. 1987. Evidence for tumour necrosis factor/cachectin production in cancer. *Lancet.* 2:1229.
9. Bertolini, D. R., G. E. Nedwin, T. S. Bringman, D. D. Smith, and G. R. Mundy. 1986. Stimulation of bone resorption and inhibition of bone formation *in vitro* by human tumor necrosis factors. *Nature (Lond.).* 319: 516–518.
10. Beutler, B., D. Greenwald, J. D. Hulmes, M. Chang, Y.-C. E. Pan, J. Mathison, R. Ulevitch, and A. Cerami. 1985. Identity of tumor necrosis factor and the macrophage secreted factor cachectin. *Nature (Lond.).* 316:552–554.
11. Beutler, B., J. Mahoney, N. LeTrang, P. Pekala, and A. Cerami. 1985. Purification of cachectin, a lipoprotein lipase suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *J. Exp. Med.* 161: 984–995.
12. Beutler, B., N. Krochnin, I. W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms for endotoxin resistance. *Science (Wash. DC).* 232:977–980.
13. Beutler, B., I. W. Milsark, and A. Cerami. 1985. Cachectin/tumor necrosis factor: Production, distribution, and metabolic fate *in vivo*. *J. Immunol.* 135:3972–3977.
14. Beutler, B., I. W. Milsark, and A. Cerami. 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science (Wash. DC).* 229:869–871.
15. Beutler, B., P. Thompson, J. Keyes, K. Hagerty, and D. Crawford. 1988. Assay of a ribonuclease that preferentially hydrolyzes mRNAs contain g cytokine-derived UA-rich instability sequences. *Biochem. Biophys. Res. Commun.* 152:973–980.
16. Bevilacqua, M. P., J. S. Pober, G. R. Majeau, R. S. Cotran, and M. A. Gimbrone, Jr. 1984. Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. *J. Exp. Med.* 160:618–623.
17. Bevilacqua, M. P., J. S. Pober, G. R. Majeau, W. Fiers, R. S. Cotran, and M. A. Gimbrone, Jr. 1986. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. *Proc. Natl. Acad. Sci. USA.* 83:4533–4537.
18. Bevilacqua, M. P., J. S. Pober, M. E. Wheeler, R. S. Cotran, and M. A. Grimbrone, Jr. 1985. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J. Clin. Invest.* 76:2003–2011.
19. Camussi, G., F. Bussolino, G. Salvidio, and C. Baglioni. 1987. Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. *J. Exp. Med.* 166:1390–1404.
20. Caput, D., B. Beutler, K. Hartog, R. Thayer, S. Brown-Shirmer, and A. Cerami. 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl. Acad. Sci. USA.* 83:1670–1674.
21. Carlino, J. A., L. S. Lin, and A. A. Creasey. 1987. Use of a sensitive receptor binding assay to discriminate between full-length and truncated human recombinant tumor necrosis factor proteins. *J. Biol. Chem.* 262:958–961.
22. Carroll, M. C., P. Katzman, E. M. Alicot, B. H. Koller, D. E. Geraghty, H. T. Orr, J. L. Strominger, and T. Spies. 1987. Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. *Proc. Natl. Acad. Sci. USA.* 84:8535–8539.
23. Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA.* 72:3666–3670.
24. Cerami, A., Y. Ikeda, Le N. Trang, P. J. Hotez, and B. Beutler. 1985. Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: The role of cachectin (tumor necrosis factor). *Immunol. Lett.* 11:173–177.
25. Chang, R. J., and S. H. Lee. 1986. Effects of interferon-γ and tumor necrosis factor-γ on the expression of an Ia antigen on a murine macrophage cell line. *J. Immunol.* 137:2853–2856.
26. Collart, M. A., D. Belin, J.-D. Vassalli, S. deKossodo, and P. Vassalli. 1986. γ-Interferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase genes, which are controlled by short lived repressors. *J. Exp. Med.* 164:2113–2118.
27. Collins, T., L. A. Lapierre, W. Fiers, J. L. Strominger, and J. S. Pober. 1986. Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A,B antigens in vascular endothelial cells and dermal fibroblasts in vitro. *Proc. Natl. Acad. Sci. USA.* 83:446–450.

28. Creasey, A. A., L. V. Doyle, M. T. Reynolds, T. Jung, L. S. Lin, and C. R. Vitt. 1987. Biological effects of recombinant human tumor necrosis factor and its novel muteins on tumor and normal cell lines. *Cancer Res.* 47:145–149.
29. Creasey, A. A., R. Yamamoto, and C. R. Vitt. 1987. A high molecular weight component of the human tumor necrosis factor receptor is associated with toxicity. *Proc. Natl. Acad. Sci. USA.* 84:3293–3297.
30. Cunningham, Jr., S. N., N. W. Carter, F. C. Rector, Jr., and D. W. Seldin. 1971. Resting transmembrane potential in normal subjects and severely ill patients. *J. Clin. Invest.* 50:49–50.
31. Cuturi, M. C., M. Murphy, M. P. Costa-Gione, R. Weinmann, B. Perussia, and G. Trinchieri. 1987. Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. *J. Exp. Med.* 165:1581–1594.
32. Davis, J. M., M. A. Narachi, N. K. Alton, and T. Arakawa. 1987. Structure of human tumor necrosis factor α derived from recombinant DNA. *Biochemistry.* 26:1322–1326.
33. Dayer, J. M., B. Beutler, and A. Cerami. 1985. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. *J. Exp. Med.* 162:2163–2168.
34. De Titto, E. H., J. R. Catterall, and J. S. Remington. 1986. Activity of recombinant tumor necrosis factor on *Toxoplasma gondii* and *Trypanosoma cruzi*. *J. Immunol.* 137:1342–1345.
35. Dinarello, C. A., J. G. Cannon, S. M. Wolff, H. A. Bernheim, B. Beutler, A. Cerami, I. S. Figari, M. A. Palladino, Jr., and J. V. O'Connor. 1986. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J. Exp. Med.* 163:1433–1450.
36. Djeu, J. Y., D. K. Blanchard, D. Halkias, and H. Friedman. 1986. Growth inhibition of *Candida abicans* by human polymorphonuclear neutrophils: activation by interferon-γ and tumor necrosis factor. *J. Immunol.* 137:2980–2984.
37. Elias, J. A., K. Gustilo, W. Baeder, and B. Freunlich. 1987. Synergistic stimulation of fibroblast prostaglandin release by recombinant interleukin 1 and tumor necrosis factor. *J. Immunol.* 138:3812–3816.
38. Feingold, K. R., and C. Grumfeld. 1987. Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat in vivo. *J. Clin. Invest.* 80:184–190.
39. Fransen, L., R. Muller, A. Marmenout, J. Tavernier, J. Van der Heyden, E. Kawashima, A. Chollet, R. Tizard, H. Van Heuverswyn, A. Van Vliet, M. R. Ruysschaert, and W. Fiers. 1985. Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. *Nucleic Acids Res.* 13:4417–4429.
40. Fransen, L., J. Van der Heyden, R. Ruysschaert, and W. Fiers. 1986. Recombinant tumor necrosis factor: Its effects and its synergism with interferon-γ on a variety of normal and transformed cell lines. *Eur. J. Cancer Clin. Oncol.* 22:419–426.
41. Frater-Schroeder, M., W. Risau, R. Hallmann, P. Gautschi, and P. Bohlen. 1987. Tumor necrosis factor typeα, a potent inhibitor of endothelial cell growth *in vitro*, is angiogenic *in vivo*. *Proc. Natl. Acad. Sci. USA.* 84:5277–5281.
42. Gamble, J. R., J. M. Harlan, S. J. Klebanoff, and M. A. Vadas. 1985. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc. Natl. Acad. Sci. USA.* 82:8667–8671.
43. Grau, G. E., L. F. Fajardo, P.-F. Piguet, B. Allet, P.-H. Lambert, and P. Vassalli. 1987. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. *Science (Wash. DC).* 237:1210–1212.
44. Green, S., A. Dobrjansky, E. A. Carswell, R. L. Kassel, L. J. Old, N. Fiore, and M. K. Schwartz. 1976. Partial purification of a serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA.* 73:381–385.
45. Guy, M. W. 1975. Serum and tissue fluid lipids in rabbits experimentally infected with *Trypanosoma brucei*. *Trans. R. Soc. Trop. Med. Hyg.* 69:429.
46. Hajjar, K. A., D. P. Hajjar, R. L. Silverstein, and R. L. Nachman. 1987. Tumor necrosis factor–mediated release of platelet-derived growth factor from cultured endothelial cells. *J. Exp. Med.* 166:235–245.
47. Haranaka, K., E. A. Carswell, B. D. Williamson, J. S. Prendergast, N. Satomi, and L. J. Old. 1986. Purification, characterization, and antitumor activity of nonrecombinant mouse tumor necrosis factor. *Proc. Natl. Acad. Sci. USA.* 83:3949–3953.
48. Hass, P. E., A. Hotchkiss, M. Mohler, and B. B. Aggarwal. 1986. Characterization of specific high affinity receptors for human tumor necrosis factor on mouse fibroblasts. *J. Biol. Chem.* 260:12214–12218.
49. Hesse, D. G., K. J. Tracey, Y. Fong, K. R. Manogue, M. A. Palladino, Jr., A. Cerami, G. T. Shires, and S. F. Lowry. 1988. Cytokine appearance in human endotoxemia and primate bacteremia. *Surg. Gynecol. Obstet.* 166:147–153.
50. Israel, S., T. Hahn, H. Holtmann, and D. Wallach. 1986. Binding of human TNF-α to high-affinity cell surface receptors: effect of IFN. *Immunol. Lett.* 12:217–224.
51. Ito, H., T. Shirai, S. Yamamoto, M. Akira, S. Kawahara, C. W. Todd, and R. B. Wallace. 1986. Molecular cloning of the gene encoding rabbit tumor necrosis factor. *DNA.* 5:157–165.
52. Ito, H., S. Yamamoto, S. Kuroda, H. Sakamoto, J. Kajihara, T. Kiyota, H. Hayashi, M. Kato, and M. Seko. 1986. Molecular cloning and expression in *Escherichia coli* of the cDNA coding of rabbit tumor necrosis factor. *DNA.* 5:149–156.
53. Kawakami, M., and A. Cerami. 1981. Studies of endotoxin-induced decrease in lipoprotein lipase activity. *J. Exp. Med.* 154:631–639.
54. Klebanoff, S. J., M. A. Vadas, J. M. Harlan, L. J. Sparks, J. R. Gamble, J. M. Agosti, and A. M. Waltersdorph. 1986. Stimulation of neutrophils by tumor necrosis factor. *J. Immunol.* 136:4220–4225.
55. Kohase, M., D. Henriksen-DeStefano, L. T. May, J. Vilcek, and P. B. Sehgal. 1986. Induction of β_2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. *Cell.* 45: 659–666.
56. Kriegler, M., C. Perez, K. DeFay, I. Albert, and S. D. Lu. 1988. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell.* 53:45–53.
57. Kull, Jr., F. C., S. Jacobs, and P. Cuatrecasas. 1985. Cellular receptor for ^{125}I-labeled tumor necrosis factor: specific binding, affinity labeling, and relationship to sensitivity. *Proc. Natl. Acad. Sci. USA.* 82:5756–5760.
58. Le, J., D. Weinstein, U. Gubler, and J. Vilcek. 1987. Induction of membrane-associated interleukin 1 by tumor necrosis factor in human fibroblasts. *J. Immunol.* 138:2137–2142.
59. Lee, M. D., A. Zentella, W. Vine, P. H. Pekala, and A. Cerami. 1987. Effect of endotoxin-induced monokines on glucose metabolism in the muscle cell line L6. *Proc. Natl. Acad. Sci. USA.* 84:2590–2594.
60. Leibovich, S. J., P. J. Polverini, H. M. Shepard, D. M. Wiseman, V. Shively, and N. Nuseir. 1987. Macrophage-induced angiogenesis is mediated by tumor necrosis factor-α. *Nature (Lond.).* 329:630–632.
61. Libby, P., J. M. Ordovas, K. R. Auger, A. H. Robbins, L. K. Biriyi, and C. A. Dinarello. 1986. Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. *J. Path.* 124:179–185.
62. Lonnroth, C., L. L. Moldawer, L. Kindblom, B. Sherry, S. B. Mizel, and K. Lundholm. 1988. Spontaneous cachectin/tumor necrosis factor-alpha and interleukin 1-alpha production in cachectic, sarcoma-bearing mice. *Cancer Res.* In press.
63. Mannel, D. N., R. N. Moore, and S. E. Mergenhagen. 1980. Macrophages as a source of tumoricidal activity (tumor-necrotizing factor). *Infec. Imm.* 30:523–530.
64. Marmenout, A., L. Fransen, J. Tavernier, J. Van Der Heyden, R. Tijard, E. Kawashima, A. Shaw, M.-J. Johnson, D. Semon, R. Muller, M.-R. Ruysschaert, A. Van Vliet, and W. Fiers. 1985. Molecular cloning and expression of human tumor necrosis factor and comparison with mouse tumor necrosis factor. *Eur. J. Biochem.* 152:512–519.
65. Mathison, J. C., E. Wolfson, and R. J. Ulevitch. 1988. Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide induced injury in rabbits. *J. Clin. Invest.* 81:1925–1937.
66. Matthews, N. 1978. Tumour-necrosis factor from the rabbit. II. Production by monocytes. *Br. J. Cancer* 38:310–315.
67. Matthews, N. 1981. Production of an anti-tumour cytotoxin by human monocytes. *Immunology.* 44:135–142.
68. Matthews, N. 1981. Tumour-necrosis factor from the rabbit. V. Syntheses in vitro by mononuclear phagocytes from various tissues of normal and BCG-injected rabbits. *Br. J. Cancer.* 44:418–424.
69. Mestan, J., W. Digel, S. Mittnacht, H. Hillen, D. Blohm, A. Moller, H. Jacobsen, and H. Kirchner. 1986. Antiviral effects of recombinant tumour necrosis factor in vitro. *Nature (Lond.)* 323:816–819.
70. Ming, W. J., L. Bersan, and A. Mantovani. 1987. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J. Immunol.* 138:1469–1474.
71. Moldawer, L. L., G. Svaninger, J. Gelin, and K. G. Lundholm. 1987. Interleukin 1 and tumor necrosis factor do not regulate protein balance in skeletal muscle. *Am. J. Physiol.* 253:766–773.
72. Muller, U., C. V. Jongeneel, S. A. Nedospasov, K. F. Lindahl, and M. Steinmetz. 1987. Tumour necrosis factor and lymphotoxin genes map close to H-2D in the mouse major histocompatibility complex. *Nature (Lond.).* 325:265–267.
73. Munker, R., J. Gasson, M. Ogawa, and H. P. Koeffler. 1986. Recombinant human TNF induces production of granulocyte-monocyte colony-stimulating factor. *Nature (Lond.).* 323:79–82.
74. Narachi, M. A., J. M. Davis, Y.-R. Hsu, and T. Arakawa. 1987. Role of single disulfide in recombinant human tumor necrosis factor-α. *J. Biol. Chem.* 262:13107–13110.
75. Nawroth P. P., and D. M. Stern. 1986. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J. Exp. Med.* 163:740–745.
76. Nawroth, P. P., I. Bank, D. Handley, J. Cassimeris, L. Chess, and D. Stern. 1986. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. *J. Exp. Med.* 163: 1363–1375.
77. Nedospasov, S. A., B. Hirt, A. N. Shakhov, V. N. Dobrynin, E. Kawashima, R. S. Accolla, and C. V. Jongeneel. 1986. The genes for tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta) are tandemly arranged on chromosome 17 of the mouse. *Nucleic Acids Res.* 14: 7713–7725.
78. Nedwin, G. E., S. L. Naylor, A. Y. Sakaguchi, D. Smith, J. Jarrett-Nedwin, D. Pennica, D. V. Goeddel, and P. W. Gray. 1985. Human lymphotoxin and tumor necrosis factor genes: structure, homology and

chromosomal localization. *Nucleic Acids Res.* 13:6361–6367.
79. Nillsson-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* 46: 667–693.
80. Okusawa, S., J. A. Gelfand, T. Ikejima, R. J. Connolly, and C. A. Dinarello. 1988. Interleukin 1 induces a shock-like state in rabbits: synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. *J. Clin. Invest.* 81:1162–1172.
81. Old, L. J. 1985. Tumor necrosis factor (TNF). *Science (Wash. DC).* 230: 630–632.
82. Old, L. J. 1985. Tumor necrosis factor. *Sci. Am.* 258:59–75.
83. Oliff, A., D. Defeo-Jones, M. Boyer, D. Martinez, D. Kiefer, G. Vuocolo, A. Wolfe, and S. H. Socher. 1987. Tumors secreting human TNF/cachectin induce cachexia in mice. *Cell.* 50:555–563.
84. Palladino, Jr., M. A., M. R. Shalaby, S. M. Kramer, B. L. Ferraiolo, R. A. Baughman, A. B. Deleo, D. Crase, B. Marafino, B. B. Aggarwal, I. S. Figari, D. Liggitt, and J. S. Patton. 1987. Characterization of the antitumor activities of human tumor necrosis factor-α and the comparison with other cytokines: induction of tumor-specific immunity. *J. Immunol.* 138:4023–4032.
85. Panuska, J. R., K. Fukui, and C. W. Parker. 1988. Secreted proteins of human monocytes. *Biochem. J.* 249:501–511.
86. Patton, J. S., H. M. Shepard, H. Wilking, G. Lewis, B. B. Aggarwal, T. E. Eessalu, L. A. Gavin, and G. Grunfeld. 1986. Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. *Proc. Natl. Acad. Sci. USA.* 83:8313–8317.
87. Pekala, P. H., M. Kawakami, C. W. Angus, M. D. Lane, and A. Cerami. 1983. Selective inhibition of synthesis of enzymes for de novo fatty acid biosynthesis by an endotoxin-induced mediator from exudate cells. *Proc. Natl. Acad. Sci. USA.* 80:2743–2747.
88. Pekala, P. H., S. R. Price, C. A. Horn, B. E. Hom, J. Moss, and A. Cerami. 1984. Model for cachexia in chronic disease: secretory products of endotoxin-stimulated macrophages induce a catabolic state in 3T3-L1 adipocytes. *Trans. Assoc. Am. Physicians.* 97:251.
89. Pennica, D., J. S. Hayflick, T. S. Bringman, M. A. Palladino, and D. V. Goeddel. 1985. Cloning and expression in *Escherichia coli* of the cDNA for murine tumor necrosis factor. *Proc. Natl. Acad. Sci. USA.* 82:6060–6064.
90. Pennica, D., G. E. Nedwin, J. S. Hayflick, P. H. Seeburg, R. Derynck, M. A. Palladino, W. J. Kohr, B. B. Aggarwal, and D. V. Goeddel. 1984. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature (Lond.).* 312:724–729.
91. Perlmutter, D. H., C. A. Dinarello, P. I. Punsa, and H. R. Colten. 1986. Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. *J. Clin. Invest.* 78:1349–1354.
92. Philip, R., and L. B. Epstein. 1986. Tumour necrosis factor as immunomodulator and mediator of monocyte cytoxicity induced by itself, γ-interferon and interleukin-1. *Nature (Lond.).* 323:86–89.
93. Piguet, P.-F., G. E. Grau, B. Allet, and P. Vassalli. 1987. Tumor necrosis factor/cachectin is an effector of skin and gut lesions of the acute phase of graft-vs.-host disease. *J. Exp. Med.* 166:1280–1289.
94. Pober, J. S., M. P. Bevilacqua, D. L. Mendrick, L. A. Lapierre, W. Fiers, and M. A. Gimbrone, Jr. 1986. Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. *J. Immunol.* 136:1680–1687.
95. Pober, J. S., M. A. Gimbrone, Jr., L. A. Lapierre, D. L. Mendrick, W. Fiers, R. Rothlein, and T. A. Springer. 1986. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J. Immunol.* 137:1893–1896.
96. Pober, J. S., L. A. Lapierre, A. H. Stophen, T. A. Brock, T. A. Springer, W. Fiers, M. P. Bevilacqua, D. L. Mendrick, and M. A. Gimbrone, Jr. 1987. Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin 1 species. *J. Immunol.* 138:3319–3324.
97. Pohlman, T. H., K. A. Stanness, P. G. Beatty, H. D. Ochs, and J. M. Harlan. 1986. An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin 1, and tumor necrosis factor-α increases neutrophil adherence by a CDw18-dependent mechanism. *J. Immunol.* 136:4548–4553.
98. Rouzer, C., and A. Cerami. 1980. Hypertriglyceridemia associated with *Trypanosoma brucei brucei* infection in rabbits: Role of defective triglyceride removal. *Mol. Biochem. Parasitol.* 2:31–38.
99. Ruff, M. R., and G. E. Gifford. 1980. Purification and physico-chemical characterization of rabbit tumor necrosis factor. *J. Immunol.* 125:1671–1677.
100. Sakaguchi, O., and S. Sakaguchi. 1979. Alterations in lipid metabolism in mice injected with endotoxin. *Microbiol. Immunol.* 23:71–74.
101. Saklatvala, J. 1986. Tumour necrosis factor-α stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature (Lond.)* 322:547–549.
102. Sayers, T. J., I. Macher, J. Chung, and E. Kugler. 1987. The production of tumor necrosis factor by mouse bone marrow-derived macrophages in response to bacterial lipopolysaccharide and a chemically synthesized monosaccharide precursor. *J. Immunol.* 136:2935–2940.
103. Scheurich, P., U. Ucer, M. Kronke, and K. Pfizenmaier. 1986. Quantification and characterization of high-affinity membrane receptors for tumor necrosis factor on human leukemic cell lines. *Int. J. Cancer.* 38: 127–133.
104. Scuderi, P., K. S. Lam, K. J. Ryan, E. Petersen, K. E. Sterling, P. R. Finley, G. C. Ray, D. J. Slymen, and S. E. Salmon. 1986. Raised serum levels of tumour necrosis factor in parasitic infections. *Lancet.* 2ii:1364–1365.
105. Shaw, G., and R. Kamen. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell.* 46:659–667.
106. Shear, M. J., and A. Perrault. 1944. Chemical treatment of tumors. IX. Reactions of mice with primary subcutaneous tumors to injection of a hemorrhage-producing bacterial polysaccharide. *J. Natl. Cancer Inst.* 4: 461–475.
107. Shirai, T., H. Yamaguchi, Ito, H., C. W. Todd, and R. B. Wallace. 1985. Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor. *Nature (Lond.).* 313:803–806.
108. Smith, L. C., and P. H. Pownall. 1984. Lipoprotein lipase. *In* Lipases. B. Borgstrom and H. L. Brockman, editors. Elsevier/North Holland, Amsterdam. 263.
109. Smith, R. A., and C. Baglioni. 1987. The active form of tumor necrosis factor is a trimer. *J. Biol. Chem.* 262:6951–6954.
110. Smith, R. A., M. Kirstein, W. Fiers, and C. Baglioni. 1986. Species specificity of human and murine tumor necrosis factor. *J. Biol. Chem.* 261:14871–17874.
111. Socher, S. H., M. W. Riemen, D. Martinez, A. Friedman, J. Tai, J. C. Quintero, V. Garsky, and A. Oliff. 1987. Antibodies against amino acids 1–15 of tumor necrosis factor block its binding to cell-surface receptor. *Proc. Natl. Acad. Sci. USA.* 84:8829–8833.
112. Spies, T., C. C. Morton, S. A. Nedospasov, W. Fiers, D. Pious, and J. L. Strominger. 1986. Genes for the tumor necrosis factors α and β are linked to the human major histocompatibility complex. *Proc. Natl. Acad. Sci. USA.* 83:8699–8702.
113. Stern, D. M., and P. P. Nawroth. 1986. Modulation of endothelial hemostatic properties by tumor necrosis factor. *J. Exp. Med.* 163:740–745.
114. Sugarman, B. J., B. B. Aggarwal, P. E. Hass, I. S. Figar, M. A. Palladino, Jr., and H. M. Shepard. 1985. Recombinant human tumor necross factor-α: effects on proliferation of normal and transformed cells in vitro. *Science (Wash. DC).* 230:943–945.
115. Taylor, Jr., F. B., A. Chang, C. T. Esmon, D'Angelo, A., S. Vigano-D'Angelo, and K. E. Blick. 1987. Protein C prevents coagulopathic and lethal effects of *Escherichia coli* infusion in the baboon. *J. Clin. Invest.* 79:918–925.
116. Torti, F. M., B. Dieckmann, B. Beutler, A. Cerami, and G. M. Ringold. 1985. A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia. *Science (Wash. DC).* 229:867–869.
117. Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R. J. Harir, T. J. Fahey III, A. Zentella, J. D. Albert, G. T. Shires, and A. Cerami. 1986. Shock and tissue injury induced by recombinant human cachectin. *Science (Wash. DC).* 234:470–474.
118. Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and A. Cerami. 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature (Lond.).* 330:662–664.
119. Tracey, K. J., S. F. Lowry, B. Beutler, A. Cerami, J. D. Albert, and G. T. Shires. 1986. Cachectin/tumor necrosis factor mediates changes of skeletal muscle plasma membrane potential. *J. Exp. Med.* 164:1368–1373.
120. Tracey, K. J., S. F. Lowry, T. J. Fahey III, J. D. Albert, Y. Fong, D. Hesse, B. Beutler, K. R. Manogue, S. Calvano, H. Wei, A. Cerami, and G. T. Shires. 1987. Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. *Surg. Gynecol. Obstet.* 164: 415–422.
121. Tracey, K. J., H. Wei, K. R. Manogue, Y. Fong, D. G. Hesse, H. T. Nguyen, G. C. Kuo, B. Beutler, R. S. Cotran, A. Cerami, and S. F. Lowry. 1988. Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. *J. Exp. Med.* 167:1211–1227.
122. Tsujimoto, M., R. Feinman, M. Kohase, and J. Vilcek. 1986. Characterization and affinity crosslinking of receptors for tumor necrosis factor on human cells. *Arch. Biochem. Biophys.* 249:563–568.
123. Tsujimoto, M., Y. K. Yip, and J. Vilcek. 1985. Tumor necrosis factor: Specific binding and internalization in sensitive and resistant cells. *Proc. Natl. Acad. Sci. USA.* 82:7626–7630.
124. Tsujimoto, M., S. Yokota, J. Vilcek, and C. Weissman. 1986. Tumor necrosis factor provokes superoxide anion generation from neutrophils. *Biochem. Biophys. Res. Commun.* 137:1094–1100.
125. Vilcek, J., V. J. Palombella, D. Henriksen-DeStefano, C. Swenson, R. Feinman, M. Hirai, and M. Tsujimoto. 1986. Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. *J. Exp. Med.* 163:632–643.
126. Waage, A., A. Halstensen, and T. Espevik. 1987. Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet.* 1i:355–357.
127. Wang, A. M., A. A. Creasey, M. B. Ladner, L. S. Lin, J. Strickler, J. N.

Van Arsdell, R. Yamamoto, and D. F. Mark. 1985. Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science (Wash. DC)*. 228:149–154.
128. Warren, R. S., D. B. Donner, H. F. Starnes, Jr., and M. F. Brennan. 1987. Modulation of endogenous hormone action by recombinant human tumor necrosis factor. *Proc. Natl. Acad. Sci. USA*. 84:8619–8622.
129. Wong, G. H. W., and D. V. Goeddel. 1986. Tumour necrosis factors α and β inhibit virus replication and synergize with interferons. *Nature (Lond.)*. 323:819–822.
130. Zechner, R., T. C. Newman, B. Sherry, A. Cerami, and J. L. Breslow. 1988. Recombinant human cachectin/tumor necrosis factor but not interleukin-1α downregulates lipoprotein lipase gene expression at the transcriptional level in mouse 3T3-L1 adipocytes. *Mol. Cell. Biol.* 8: 2394–2401.