

Cachectin/Tumor Necrosis Factor

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Abdus Salam Khan Gandapur (Department of Basic Medical Sciences, Gomal University, Dera Ismail Khan.)
Salman Malik (Department of Biology, Quaid-e-Azam University, Islamabad.)

Cachectin/tumor necrosis factor (a 17-KD polypeptide hormone produced mainly by the cells of monocyte/macrophage lineage), has been implicated in the pathogenesis of both local and systemic inflammatory reactions. It is a primary mediator in the pathogenesis of infection, injury and inflammation and in the beneficial processes of host defence and tissue homeostasis. Depending on its concentration, duration of cell exposure and presence of other mediators in the cellular environment, the net biological effects of this peptide regulatory factor may be ultimately beneficial or injurious to the host. Thus, acute systemic release of cachectin/TNF causes septic shock and tissue injury. Persisting cachectin/TNF production provokes Cachexia and these sequelae are synergistically influenced by other mediators like interleukin-1 (IL-1) and interferon- γ (IFN- γ). When lesser amounts are released in tissues, the beneficial effects may predominate to mediate enhanced host defence, against pathogens and to coordinate normal tissue remodelling. In this article we shall review the history, chemistry, synthesis, mechanism of action, beneficial and injurious effects, as well as diagnostic and therapeutic potentials of the molecule.

History

Cachectin was first isolated during a research directed at the identification of basic mechanisms of cachexia in chronic disease. When rabbits are infected with *Typanosoma brucei*, they lose up to 50% of their body weight. The rabbits develop striking lipaemia during the final stage of the disease^{1,2}. The hyperlipemia (hypertriglyceridemia) is due to complete suppression of the enzyme lipoprotein lipase (LPL); hence preventing the uptake of exogenous triglycerides by fat cells and causing paradoxical lipaemia frequently associated with infection^{3,4} or neoplastic disease⁵⁻⁷. Subsequent observations suggested that a serum factor produced by macrophages in response to invasive stimuli resulted in LPL suppression and contributed to the development of cachexia⁸.

Beutler and coworkers first isolated cachectin from the supernatant of murine macrophages activated by endotoxin/lipopolysaccharide⁹. Later, when the gene encoding cachectin was identified and cloned¹⁰ it was found to be identical to tumor necrosis factor - (TNF α), a molecule that had been studied for its role as macrophage mediator of haemorrhagic necrosis of certain transplantable tumors¹¹. The availability of recombinant human cachectin/TNF (rh-TNF)¹² and the realization that single mediator participates in the development of both cell cytotoxicity and cachexia prompted investigation into the biologic properties of this newly identified cytokine.

Biochemistry and Biosynthesis

The gene for human TNF is located on the short arm of chromosome 6 and encodes, a polypeptide of 233 amino acids, that is processed to a 157 residue mature protein (MW 17KD) by cleavage of a 76 residue single peptide.

Mature cachectin/TNF polypeptide shares a 28% amino acid sequence homology with another cytokine, lymphotoxin (TNFB), with which it shares some biological properties and these molecules may compete for a common receptor. Lymphotoxin is encoded by a separate gene and is also located on the short arm of chromosome 6 in man possibly indicating ancestral tandem duplication. The biological function of cachectin/TNF also overlaps with that of another 17/KD cytokine IL-1, but the molecules are structurally different and do not compete for a common receptor and several other inflammatory mediators contain 33 nucleotide receptor. The mRNA transcripts of these 3-untranslated sequence is

composed of repeated and overlapping copies of the consensus octamer UUAUUUAU¹⁰. This sequence apparently shortens mRNA life, thereby, reducing the chances for incidental or inappropriate production of large quantities of these potent polypeptides¹³

Synthesis

Cachectin/TNF is synthesized by various activated phagocytic and non-phagocytic cells, including macrophages, monocytes, lymphocytes, natural killer cells, astmytes and microglial cells of the brain and kupifer cells of the liver. A pivotal role of TNF in inflammation is suggested by a wide variety of infectious or inflammatory stimuli capable of triggering cachectin/TNF biosynthesis e.g., bacterial endotoxin, lipopolysaccharide (LPS), enterotoxin, toxic shock syndrome, toxin-I mycobacterial card factor, viruses, c5a, fungal or parasitic antigens, IL-I and in an autocrine manner cachectin/TNF itself. In response to lipopolysaccharide (LPS), both transcription and translation of the cachectin/TNF precursor are increased and large amounts of the mature protein are released within minutes.

Dexamethasone inhibits cachectin/TNF biosynthesis, but this effect is not observed if the steroid is given after the cells have been exposed to LPS. By contrast interferon (IFN γ) exerts a permissive influence that enhances cachectin/TNF biosynthesis. The up or down regulation of cachectin/TNF biosynthesis by IFN γ or Dexamethasone, respectively, probably contributes to the pro-inflammatory or anti-inflammatory effect of these mediators¹⁴. Two different forms of cachectin/TNF are produced; the 17-KD secreted form and a 26KD membrane associated form¹⁵.

It has been suggested that the membrane-bound form may act primarily as a mediator of local (paracrine) tissue effects and is processed to the 17-KD circulating form. High affinity membrane receptors for TNF are present in a variety of tissues (most notably liver, kidney, spleen, lung, muscle, endothelium and intestine) and mediate maximal cellular responses even with low receptor occupancy. Two different TNF receptors (P55 and P75) have recently been identified and sequenced and their genes cloned^{16,17}.

Mechanism of action and metabolic effects of cachectin

After binding to its receptor, TNF induces transcriptional changes that lead to a reduction of the mRNA, for several key lipogenic enzymes and LPL, without altering the biosynthesis of normal house keeping genes. The metabolic effects of these cellular responses is lipolysis, with net losses of free fatty acids and a depletion of the triglyceride storage pool. Muscle cells also become catabolic when incubated with TNF, they are rapidly depleted of glycogen stores, release lactate and develop a defect in the ability to maintain a normal resting transmembrane potential¹⁸. In contrast to the monocyte and adipocyte effects, hepatocytes display relatively anabolic responses to TNF and manifest increased rates of lipogenesis and glucagon-mediated amino acid uptake accelerated biosynthesis of acute phase proteins and decreased albumin biosynthesis¹⁹.

Taken together, the data suggests that the net effects of TNF induces mobilization of peripheral tissue energy stores from adipose and muscle, that can then be used by the liver in order to meet the increased energy and synthetic demands associated with infection or cancer. However, as with other cases immunologically mediated tissue injury or death (e.g., anaphylaxis), the inappropriate or prolonged production of TNF is capable of mediating progressive catabolism and injurious wasting that may ultimately kill the host.

Septic shock

After intravenous administration of endotoxin/LPS in man Cachectin/TNF achieve peak levels within two hours, coinciding with the onset of fever, rigors, myalgia, headache and nausea²⁰.

Larger quantities of LPS stimulate much higher serum cachectin/TNF concentrations, that trigger lethal shock and tissue injury. Administration of human recombinant cachectin/TNF, that is virtually endotoxin free, produces metabolic effects and lethal tissue injury that is almost identical to septic/toxic shock syndrome²¹.

It has recently become clear that many of the systemic effects of TNF are mediated by secondary factors (Table)

Table. Factors induced by cachectin (TNF).

Cytokines	IL-1
	IL-6
	GM-CSF
	PDGF
	Cachectin/TNF
Hormones	Corticotropin/cortisol
	Epinephrine
	Nor epinephrine
	Glucagon
Eicosanoids	Platelet activating factor
	Prostaglandins
	Leukotriens
Acute-phase Proteins	C reactive protein
	α_1 acid glyco-protein
	α_2 macroglobulin
	Complement proteins factor
	β and C_3
Other	Collagenase
	Elastase
	Superoxide radicals
	Increased endothelial
	expression of ICAM-1, ELAM-1 and VCAM-1

* Modified from Tracey and others^{24,25}.

IL-1: interleukin-1

GM-CSF: Granulocyte-macrophage colony stimulating factor

PDGF: Platelet derived growth factor.

TGFB: Transforming growth factor B.

ICAM: intercellular leukocyte adhesion molecule.

ELAM: Extracellular leukocyte adhesion molecule.

VCAM: Vascular cell adhesion molecule.

but the importance of cachectin/TNF in lethal/toxic and septic shock has been established by studies showing a protective effect of anticachectin/TNF antibodies in vivo. In primates disassociated from the presence of bacteria or LPS. Septic shock syndrome seems to be the result of immunological over-

responsiveness (similar to anaphylactic shock), when the death of the host may be caused by an excessive immune response to an invasive stimulus.

Cachexia

Initial experiments with partly purified material from murine macrophages indicated that persistent exposure to cachectin/TNF may induce cachexia, these observations were confirmed with recombinant cachectin/TNF²⁵. Recent workers have used antibodies against TNF in animal models of tumor-associated cachexia to identify the role of endogenously produced TNF. The antibody-treated animals were partially protected against the development of anorexia and wasting, as evidenced by improved food intake and reduced losses of whole body protein and lipid²⁶. Interestingly, the antibody treatment did not protect against the development of anaemia or acute phase protein biosynthesis, suggesting either that the antibodies did not completely neutralize the cachexia-inducing effects of TNF produced locally in tissues or that other factors directly mediated these effects. Stovroff and others (in animal studies) demonstrated that endogenous production of TNF during tumor invasion contributed to the development of malnutrition and wasting²⁷.

Studies in patients and healthy volunteers given rh-TNF offer additional evidence of the role of TNF in cachexia and of the involvement of a complex cascade of secondary mediators.

Following intravenous administration of TNF, whole body energy expenditure, protein turnover and lipogenesis are enhanced²⁸ and amino-acid release from extremity skeletal muscle is increased²⁹. In addition to these catabolic responses, higher doses of TNF are extremely toxic and have been complicated by the development of hypotension, capillary leak syndrome, fever, anorexia and inflammation at the site of injection³⁰. Recently, it has been found that the systemic effects of TNF are mediated by secondary factors. For example, TNF stimulates pituitary biosynthesis of ACTH that in turn stimulates cortisol secretion. The role of ACTH in the regulation of increased protein breakdown is well documented³¹. In addition, TNF stimulates interleukin 1 (IL-1) biosynthesis from endothelial cells and macrophages, which in turn synergistically increases biological responses to TNF³². Thus the available evidence indicates that the net catabolic effect of TNF *in vivo* are the result of the complex interplay between its direct catabolic effects and the influence of other factors that are induced during illness. Tracey and Cerami demonstrated that the whole body nutritional responses to TNF are dependent on the site of tissue production, regardless of the predominant blood level³².

Malignancy

Identification of cachectin/TNF as the agent promoting tumor lysis in the serum of endotoxaemic mice initially stimulated interest in its development, as a potential antineoplastic agent. It is cytotoxic to some human tumor cell lines and causes haemorrhagic necrosis of the centres of some implantable tumors *in vivo*. However, animal studies indicate that the therapeutic efficacy of cachectin/TNF is unimpressive and limited by severe toxicity. Moreover, various human epithelial cell lines are resistant to and actually synthesize the cytokine³³.

Clinical trials of recombinant human Cachectin/TNF are underway. Phase-I studies confirmed many of the observations initially made in animals; profound systemic and organ specific toxicity has been observed, fever, rigors and myalgias develop frequently and dose limiting after-effects include hypotension, increased fluid requirements and hepatotoxicity³⁰. It remains to be established whether the toxicity of cachectin can be decreased without reducing any intrinsic antineoplastic efficacy. Trials of cachectin given in combination with other cytokines are also in progress, but the evidence available suggests that the effects of cachectin/TNF in cancer are not specifically tumouricidal, but resemble the effects of bacterial endotoxin.

Parasitic diseases

In 1986, Scuderi and his co-workers for the first time measured the serum levels of tumor necrosis factor (TNF α) in healthy people and patients with neoplastic or infectious disease. Only patients with

Kala-azar (visceral leishmaniasis) and malaria were found to have strikingly increased frequency of raised TNF levels. They found that 7.9% of samples from both healthy subjects and patients with neoplastic disease contained measurable TNF. The discovery of elevated TNF levels in the sera of patients with parasitic diseases suggests that this cytokine may play a part in host defence against parasitic infections³⁴.

Malaria

Raised TNF levels were detected in patients with malaria in 1986³⁴. Since then, interest has focussed on the possible relation of TNF to severe and cerebral malaria. There is now considerable evidence linking TNF with various aspects of malarial pathology³⁴. Recently more detailed studies have shown that serum TNF levels correlate with disease severity in malaria³⁵. The authors also found very highly significant difference ($P < 0.001$) in TNF levels in severe malaria as compared to mild malaria (unpublished observations).

Several hypotheses have been proposed to explain the development of coma in cerebral malaria. The most important one is called cytokines hypothesis. It is based on the observation that in African children with cerebral malaria, plasma concentrations of TNF, interleukin-1 and other cytokines correlate with disease severity, as judged by parasitaemia, hypoglycemia, case fatality and the incidence of neurological sequelae. Cytokines, particularly tumor necrosis factor released by macrophages under the influence of a malaria toxin Glycosyl phosphatidyl inositol (GPI) released at schizont rupture could be involved in enhancing cytoadherence by increasing the expression of endothelial receptors) such as intercellular adhesion molecule-1 (ICAM-1), CD³⁶ and can induce fever, hypoglycaemia, coagulopathy, dyserythropoiesis and leukocytosis³⁷.

Saissy and his co-workers compared the clinical and biologic aspects of adult severe falciparum malaria, with those found in Children in West Africa (in malaria endemic area) and confirmed the prognostic significance of serum levels of TNF, IL-6 and IL-2SR in severe malaria³⁸. Kumaratilake and co-workers³⁹ synthesized tumor necrosis factor, agonist peptide TNF (70-80) and demonstrated that it enhanced the human PMN-mediated killing of plasmodium falciparum in vitro and reduced the plasmodium chabaudi parasitaemia in mice. They concluded that the host protective effects of TNF can be retained while the toxic effects are eliminated using a selected, characterized subunit of the cytokine. Treatment of malaria with neutralizing antibodies to TNF seems a distinct possibility, but, the role of antibodies, directed against other cytokines, can be of immense clinical importance.

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