Role of Tumor Necrosis Factor in Protein Metabolism

Kevin J. Tracey

The relentless catabolism of body protein during chronic disease may kill. Early investigators presumed that the origins of cachexia lay in the underlying neoplasia or organism usurping the host's energy stores. More recent evidence indicates that immunological and neuroendocrinological mediators produced in response to invasion amplify the catabolism of lean body tissue, diverting substrate from peripheral tissues to liver as part of an acute-phase response. Anorexia and decreased food intake invariably accompany catabolic illnesses, but unlike the metabolic adaptation that occurs during unstressed fasting, the cachectic host fails to downregulate muscle protein catabolism and urinary nitrogen losses, and persistently excretes up to 15 g of nitrogen per day. Since mammals lack a protein storage depot, loss of body protein is associated with loss of function, resulting in immunosuppression, weakness, delayed wound healing, decreased tolerance for surgical and chemotherapeutic regimens, and death.

Cytokines, an expansive family of immunological mediators, have been implicated in mediation of the catabolic response to illness. The number of cytokines that have been identified and isolated continues to increase; at least 30 have been purified, sequenced, and cloned. These protein or glycoprotein mediators, produced in response to invasive stimulae, possess potent activities capable of modulating biochemical and molecular changes in nearly every known cell type. Cytokine-induced responses augment host defense, immune responsiveness, and the mobilization of energy stores during invasion. High doses of some cytokines are also toxic, however, so that the biological effects of an individual cytokine may be either beneficial or injurious to the host, depending on the amount produced. For instance, an acute overproduction of one cytokine, tumor necrosis factor-α (TNF), triggers lethal shock and tissue injury during septicemia (reviewed in 1). Antibodies that prevent TNF toxicity in sepsis are currently being evaluated in clinical trials. Moreover, early toxicity studies revealed that TNF induces the release of catabolic hormones (2) and mediates the development of a protein catabolic state with anorexia and anemia (3).
Subsequently, a great deal of research has been devoted to identifying a role for TNF and other cytokines in the biochemical basis of cachexia.

This chapter is a discussion of the role of TNF in the mediation of whole body protein catabolism. Since TNF mediates catabolic responses through a cascade of secondary cytokines, hormones, and metabolic responses, this brief review presents one model for potential interaction between cytokines and the neuroendocrinological system in mediating the systemic manifestations to injury, infection, or invasion.

**TNF PRODUCTION AND BIOCHEMISTRY**

Human TNF is synthesized by a variety of cells from hematopoietic and non-hematopoietic lineage, including macrophages, T cells, B cells, natural killer (NK) cells, mast cells, eosinophils, astrocytes, and Kupffer cells. The TNF gene, which lies on chromosome 6 in humans, encodes a cell-membrane-associated prohormone which is subsequently cleaved to yield the mature 17-kDa protein. The regulation of TNF expression has been most extensively studied in macrophage systems after stimulation with endotoxin (LPS), enterotoxin, or a variety of antigens derived from parasites, viruses, or tumors. Within minutes after exposure to LPS, macrophages upregulate the transcription and translation of TNF, leading to a 10,000-fold increase in the release of mature protein. Glucocorticoids inhibit and interferon-γ increases LPS-induced TNF expression (4, 5). Serum TNF levels peak within 90 min after experimental endotoxemia in both human volunteers and laboratory animals, then rapidly return to baseline (undetectable) levels (6). This fall of serum TNF levels occurs because cellular biosynthesis ceases shortly after induction (5), and the serum half-life is brief (6–15 min). Mature h-TNF circulates as a non-covalently bound trimer that interacts with two types of TNF receptor present on most cells. These receptors are cleaved in vivo by an unidentified process that yields TNF-binding peptides (7–9). These TNF-binding fragments are present in serum, where they function at low doses to enhance TNF effects (10), but at high doses to inhibit TNF effects (11, 12).

**METABOLIC EFFECTS OF CHRONIC TNF EXPOSURE**

Animals chronically exposed to TNF develop cachexia characterized by anorexia, weight loss, protein and lipid catabolism, insulin resistance, and anemia (Table 1). Some of these metabolic effects are directly attributable to the interaction of TNF with its cellular receptor, but others are attributable to secondary mediators the appearance of which is upregulated by TNF.

**Protein Loss**

Body composition analysis of laboratory rats subjected to twice daily injection of rh-TNF for 7–10 days shows that whole body protein is depleted (3). This net protein
TABLE 1. Biological responses to TNF that have been implicated in the development of catabolic illness

<table>
<thead>
<tr>
<th>Tissue or cell type</th>
<th>Biological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Anorexia</td>
</tr>
<tr>
<td></td>
<td>Increase adrenocorticotropicin, growth hormone, prolactin</td>
</tr>
<tr>
<td></td>
<td>Decrease of thyroid stimulating hormone</td>
</tr>
<tr>
<td>Muscle</td>
<td>Net whole body protein catabolism</td>
</tr>
<tr>
<td></td>
<td>Increased efflux of amino acids from isolated extremity</td>
</tr>
<tr>
<td></td>
<td>Reduction of resting transmembrane potential</td>
</tr>
<tr>
<td></td>
<td>Suppression of GLUT-4</td>
</tr>
<tr>
<td></td>
<td>Glycogenolysis</td>
</tr>
<tr>
<td></td>
<td>Lactate efflux</td>
</tr>
<tr>
<td>Adipose</td>
<td>Suppression of lipoprotein lipase</td>
</tr>
<tr>
<td></td>
<td>Increased free fatty acid efflux</td>
</tr>
<tr>
<td></td>
<td>Increased lipolysis</td>
</tr>
<tr>
<td></td>
<td>Decreased lipogenesis</td>
</tr>
<tr>
<td>Liver</td>
<td>Acute-phase protein biosynthesis</td>
</tr>
<tr>
<td></td>
<td>Decreased albumin biosynthesis</td>
</tr>
<tr>
<td></td>
<td>Increased lipogenesis</td>
</tr>
<tr>
<td></td>
<td>Enhanced glucagon-mediated amino acid transport</td>
</tr>
<tr>
<td></td>
<td>Increased gluconeogenesis</td>
</tr>
</tbody>
</table>

catabolism was not attributable to decreases in food intake in the TNF-treated animals, since pair-fed controls retained protein despite similar body weight losses (3). Although skeletal muscle mass and protein content were depleted after TNF, there was a simultaneous increase in hepatic mass and protein content (3, 13). Skeletal muscle protein synthesis is suppressed, as indicated by quantitative analysis of expressed mRNA for myofibrillar proteins (13). Net losses of skeletal muscle protein were also observed when TNF was infused continuously rather than intermittently in freely feeding rats (14). Rats injected with TNF and analyzed by \( ^{14} \text{C} \)leucine tracer methodology had increased dilution of labeled leucine, suggesting that systemic exposure to TNF promoted skeletal muscle proteolysis (15). Provision of parenteral nutrition during continuous TNF infusion failed to prevent urinary nitrogen loss and depletion of whole body protein (15, 16). By contrast, concurrent provision of insulin during 5 days of twice daily TNF administration prevented the net losses of whole body nitrogen (17). TNF-mediated protein loss is enhanced by glucocorticoids, but steroids alone do not account for all of the catabolized protein (18, 19).

### Anorexia

Animals exposed to TNF develop anorexia dependent on the dose given (3, 20, 21). This observation was first made in studies of systemically administered TNF; a direct role for the central nervous system was suggested by studies employing trace quantities of TNF injected intracerebrally (22). The onset of anorexia induced by
intracerebral TNF was associated with suppressed activity of glucose-sensitive neurons in the lateral hypothalamus, a region that contributes to the regulation of feeding (22). Exposure of the brain to even higher TNF levels by implantation of a genetically engineered TNF-secreting tumor in the forebrain caused lethal anorexia (23). The development of anorexia in this study was mildly attenuated by insulin, but not by depletion of brain serotonin (23). These studies suggest that TNF is capable of mediating anorexia by a direct effect on the hypothalamus, but leave open the possibility that the mechanism may involve the induction of secondary anorexigenic mediators (e.g., interleukin-1) in the hypothalamus.

Other Cytokines

The recognition that a catabolic state develops after exposure to TNF was followed by the realization that the mechanisms underlying net protein catabolism involved other catabolic cytokines as well. An abbreviated list of other factors that are produced in response to TNF is given in Table 2. TNF occupies a pivotal position in triggering the appearance of other cytokines during inflammation, injury, and invasion. IL-1, IL-2, IL-3, IL-6, and interferon-γ all appear in the circulation after the administration of TNF, and these cytokines have in turn been implicated in the development of catabolic illness. The pivotal role of TNF in mediating this cascade is suggested by the observation that inhibiting TNF during overwhelming septicemia prevents the appearance of the other cytokines (24). Once the humoral cytokine cascade is induced, it is self-propagating, so that the subsequent catabolic responses may be dissociated in time from the initial triggering event (e.g., TNF or LPS).

Adding to this already complex picture, cytokines produced during this cascade are capable of increasing or decreasing the biological activities of other cytokines,

<table>
<thead>
<tr>
<th>TABLE 2. Abridged list of factors implicated in catabolic illness that are produced in response to tumor necrosis factor (TNF)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokines</strong></td>
</tr>
<tr>
<td>TNF</td>
</tr>
<tr>
<td>Interferon-γ</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
including TNF. For instance, IL-1, interferon-γ, and LPS each have protein catabolic activities when administered chronically to animals. Moreover, each of these factors synergistically enhances the toxicity and catabolic activities of TNF itself, such that sublethal doses of these factors lower the LD50 of TNF (25–27). Thus, when assessing the catabolic mechanisms underlying the biological response to TNF, consideration must be given to the activities of other cytokines in the cellular milieu.

Catabolic Hormones

TNF administration is followed by the release of glucose counterregulatory hormones that may participate in the mediation of net protein loss. Early studies of dogs infused with increasing doses of h-TNF showed rising serum levels of cortisol, epinephrine, norepinephrine, and glucagon (2); more recently, these observations have been extended to humans (28, 29). TNF interacts with the hypothalamic-pituitary axis to alter hormone production with resultant increases of ACTH, and decreases in growth hormone, prolactin, and TSH (reviewed in 30). A direct glucocorticoid stimulating effect of TNF on the adrenal cortex has been demonstrated (31). In addition to altering the hormonal profile, TNF is also capable of altering the magnitude of cellular responsiveness to these hormones. For example, TNF enhances glucagon-mediated amino acid transport in hepatocytes, alters muscle cell responsiveness to insulin by downregulating glucose transporters, and is synergistic with glucocorticoids in enhancing muscle protein breakdown (32–34). Since glucocorticoids are capable of suppressing TNF biosynthesis, the emerging picture is that protein metabolic interactions between TNF and the neuroendocrinological system occur at the level of cytokine production, hormone release, and cellular responsiveness.

Insulin Resistance

TNF has been implicated in the development of insulin resistance in laboratory animals and human subjects. Serum glucose increases after infusion of increasing doses of TNF, with a TNF dose-dependent influence on rising glucose levels (2, 35). A similar observation has been made in animals receiving total parenteral nutrition during a continuous infusion of TNF (16). The physiological basis for this hyperglycemia lies in both reduced peripheral clearance and reduced suppression of hepatic glucose output (36). The cellular basis for reduced glucose uptake has been studied in adipocytes and myocytes co-cultured with increasing concentrations of TNF (33). These results indicate that the expression of the glucose transporter GLUT-4 is reduced in TNF-treated cells, suggesting that decreased transport underlies the reductions of insulin-mediated glucose uptake (33). Relative insulin resistance in the adipocyte also occurs because TNF suppresses the expression of lipogenic enzymes, effectively preventing the incorporation of glucose into lipid even in the presence of high insulin levels (37). Insulin resistance is known to develop in both cachexia and
obesity, and a recent study implicated TNF in the mediation of insulin resistance in genetically obese animals (38). The mechanisms by which insulin resistance influences body weight are unknown, but it is plausible that TNF-mediated insulin resistance may participate in both cachexia and obesity, depending on the concurrent food intake (decreased or increased).

**Tolerance**

Chronic exposure to TNF increases the tolerance of the animal to subsequent TNF administration, so that progressively higher TNF doses must be administered to maintain the same level of biological response (3). The molecular basis of this tolerance is unknown, but antibody production against exogenous TNF has been excluded as the explanation (39). Other cytokines, including IL-1, IL-6, interferon-γ, and D-factor, induce tolerance that protects experimental animals against the lethal effects of endotoxemia. Less is known, however, about catabolic responses in tolerant animals. The results presently available suggest that several factors may be involved, including (a) suppressed production of the cytokine cascade, (b) suppressed cellular responsiveness to cytokine effects, (c) altered activity or clearance of released cytokines, (d) altered expression of cytokine receptors, or (e) altered expression of cytokine inhibitors.

**Tumors**

Tumorigenic cell lines have been genetically engineered to constitutively secrete TNF, IL-6, or interferon-γ. When implanted into nude mice, these cells form tumors that continuously produce a single cytokine, providing models for the effects of chronic cytokine overexposure. These studies provide conclusive evidence that chronic overproduction of a single gene product (either TNF, IL-6, or interferon-γ) is capable of inducing net protein catabolism, as determined by analysis of body composition (23, 40–43). In each case animals developed increased serum cytokine levels relative to the transfected cytokine gene, developed some degree of anorexia, and had carcass composition analyses that verified net protein catabolism. A similar observation has been made in rats bearing a sarcoma that induced local macrophage activation and TNF production; the severity of cachexia correlated with the quantity of TNF produced (44). Administration of anti-TNF antibodies to animals bearing either this sarcoma (44), another transplantable tumor (45), or a genetically engineered TNF-secreting tumor (46) partially ameliorated the development of anorexia, weight loss, and lipid and protein depletion, suggesting that TNF was involved in triggering the catabolic state, but that it acts via indirect mechanisms.

**CELL BIOLOGY OF CYTOKINE-INDUCED PROTEIN CATABOLISM**

Despite the weighty evidence from *in vivo* studies implicating TNF in the mediation of net protein catabolism, the molecular basis of TNF-induced protein catabolism is
TNF IN PROTEIN METABOLISM

enigmatic. TNF, IL-1, interferon-γ, and LPS fail to induce protein breakdown when incubated with skeletal muscle in vitro, suggesting that in vivo protein loss occurs via some unknown indirect mechanism (47). Myocytes in vitro respond directly to TNF with a variety of other biological effects, including reduction of resting transmembrane potential (48, 49), enhanced glycogenolysis (50), increased lactate release (50), and decreased expression of glucose transporters (33). Hepatocytes co-cultured with TNF are triggered to upregulate acute-phase protein biosynthesis (51), downregulate albumin production (41), increase lipogenesis (52), and increase glucagon-mediated amino acid uptake (32). Extrapolating from these in vitro data, it appears that the net result of these TNF-mediated cellular responses in vivo would contribute to a redistribution of amino acids from peripheral tissue to the hepatic compartment for incorporation into acute phase proteins, carbohydrates, and urea.

METABOLIC EFFECTS OF TNF IN HUMANS

TNF Administration

Recombinant human TNF has been administered to cancer patients as an experimental antineoplastic drug. Although these studies have not yet identified clinical efficacy for TNF in the treatment of cancer, they have provided a great deal of information about the biological effects of this cytokine in humans.

TNF infusion is followed by the development of fever, myalgia, tachycardia, headache, hypotension, and a capillary leakage syndrome that is dose-dependent (53-55). Forearm efflux of amino acids is increased, primarily because of increased rates of alanine and glutamine efflux (28). Muscle amino acid efflux occurred in association with increases in circulating levels of cortisol and glucagon, and a fall in serum insulin (28). Resting energy expenditure increased because of increases in oxygen consumption and carbon dioxide production, and lactate levels rose by 50% (29). Whole body protein turnover, assessed by the [15N]glycine method of Picou and Taylor-Roberts, was increased, and synthesis was decreased. Whole body protein catabolism was increased, but the magnitude of the increase did not reach statistical significance (29). A similar trend toward negative nitrogen balance was observed with the higher doses of TNF used in this study (29). Taken together, these data indicate that exposure to a single dose of TNF is capable of triggering metabolic responses that are typical of the catabolic response to invasive illness. What remains unclear is whether these metabolic effects persist during prolonged TNF administration, or whether tolerance develops.

TNF Production in Disease

Increased TNF levels have been detected in body fluids obtained from patients with a variety of catabolic illnesses, including parasite infection, sepsis, malnutrition, cancer, and AIDS (reviewed in 56). Attempts to correlate circulating TNF levels with
the magnitude of nutritional depletion have been thwarted, however, by a number of factors that confound the analysis. These factors include (a) trace amounts of other cytokines synergistically increasing or decreasing the effects of TNF; (b) the fact that fragments of the TNF receptor may increase or decrease the biological response to TNF; (c) TNF acting locally in vital organs (e.g., brain or liver) to exert metabolic responses to the whole body without attaining detectable serum levels; and (d) the fact that TNF is produced sporadically and appears only transiently in the serum during chronic catabolic illness [e.g., burn injury (57)], so that periodic serum sampling may miss the TNF peak. These difficulties of interpreting serum levels do not exclude a role for TNF in protein catabolism. Rather, this approach points out the complexity of the catabolic response induced by TNF, and propagated by other humoral factors, that ultimately leads to protein loss. Perhaps assigning a role for TNF in cachexia by measuring serum TNF is analogous to assigning a role for insulin in diabetes by assaying serum insulin levels (which may be increased, decreased, or normal). Unlike hemoglobin-A1c, which is useful for monitoring the chronic effect of insufficient insulin action in diabetics, there are no available tests to assess the impact of chronic TNF excess in cachexia.

SUMMARY AND FUTURE

The search for a humoral factor that mediates protein catabolism has been under way since the demonstration that normal animals develop cachexia when parabiotically joined to a tumor-bearing animal (58). TNF has been implicated as a mediator that is capable of participating in this response because (a) it is produced during protein catabolic illness in humans; (b) administration of highly purified recombinant human TNF induces anorexia and a protein catabolic state in humans and animals; (c) the cellular biology of TNF suggests a role in promoting a transfer of amino acids from peripheral tissue to liver for use in acute-phase protein synthesis and gluconeogenesis; and (d) blocking TNF with antibodies attenuates the development of protein catabolism and insulin resistance. Much less is known about the precise molecular mechanisms underlying TNF-induced protein catabolism. It is hoped that the identification of the mechanisms by which TNF triggers protein losses in cachexia will foster the development of novel therapies to prevent protein catabolism and its complications.

REFERENCES


DISCUSSION FOLLOWING THE PRESENTATION OF DR. TRACEY

**Dr. Uauy:** Were your experiments conducted in growing animals; in other words, were you demonstrating stunting, or were you demonstrating cachexia in fully grown animals?

**Dr. Tracey:** My experiments were done in animals with a starting body weight of about 200–220 g, so they were still growing but they were adolescent. Other studies have looked at older animals and the results appear to be fairly reproducible. In a more general sense your question is asking whether cytokines participate in stunting. I don’t think anybody knows the answer to this. It is an important area that has to be investigated. A few studies have looked at the effects of toxic amounts of cytokines on embryos and neonates, but I don’t think the specific question of stunting has been addressed. There is a transgenic mouse that overproduces tumor necrosis factor, and those animals become stunted or runted and seem to have abnormal thymus function as well.

**Dr. Guesry:** You show that the end result of these TNF and interleukin studies is cachexia, and we know that the origin of this is partly anorexia. But is the effect on muscle mass due primarily to excessive muscle breakdown or is it due to impaired protein synthesis?

**Dr. Tracey:** The problem with answering your question is that in the animal model the data suggest that there is an effect on both synthesis and breakdown. This has not been confirmed in tissue culture, so it is fair to say that since we don’t understand the molecular basis of the net loss, we don’t know the answer. But we do know that there seems to be both decreased synthesis and increased breakdown *in vivo*.

**Dr. Garlick:** It is a complex issue as to whether or not protein synthesis is altered by these agents *in vivo*. Kevins has showed that when either TNF or IL-1 was injected over periods of days there was no reduction in muscle protein synthesis, although I think there was an increase in liver protein synthesis (1). Grimble, in Southampton, gave injections of TNF but measured the effects at much shorter time intervals, within hours after the injection, when the animals showed fever. At that stage muscle protein synthesis did fall quite substantially after 9 h and liver protein synthesis increased (2). This is very similar to the effect of any acute-phase response.

In our laboratory, Peter Ballmer injected rats with IL-1β and got exactly the same effect as you get with TNF. In other words, at about 9 h there was a big fall in muscle protein synthesis and a large rise in liver protein synthesis. In acute experiments we were unable to measure protein breakdown.

The question I would like to ask is this. If you inject these cytokines in large amounts, there appears to be an acute-phase response. Can the final response be mimicked by other agents that induce acute-phase response: for example, stress hormones, trauma, and so on?

**Dr. Tracey:** You can reproduce some of the aspects of the acute-phase response, as you suggest, by giving infusions or injections of classical hormones, but these hormones don’t induce the entire spectrum of cachexia. They don’t induce the anemia and they don’t induce the severity of the muscle protein loss that are found after administration of cytokines. Cytokines by themselves seem able to induce the whole spectrum of cachexia, particularly TNF and IL-1.

It is hard to study cachexia during sepsis, and hard to treat it because it goes on for so
long. The situation is confused by antigenic responses and it is difficult to do studies over a sufficiently long period of time. People therefore try to extrapolate from the acute sepsis syndrome to the chronic situation. During the acute sepsis syndrome, many cytokines can give you changes in blood pressure or catabolic rate, but so far TNF is the only one that can of its own induce the entire spectrum of septic shock syndrome. If you look at the effects of each cytokine, they suggest that these agents play a role in the host responses that may perhaps give rise to cachexia. So it is difficult to assign priorities to these factors. However, by understanding that there is a cascade effect which, once started, becomes self-perpetuating, we shall hopefully be better able to target therapies.

\textbf{Dr. Räihä:} Do you have any information on cytokines during the neonatal period? I ask this because we know that normal breast-fed newborn infants show very little decrease in weight after birth, whereas formula-fed infants usually lose quite a lot and take longer before they regain their birthweight. Do you think that cytokines could have anything to do with this? We are inducing proteins into these infants that are not species specific.

\textbf{Dr. Tracey:} This is a reasonable hypothesis. Malnourished adults have raised levels of TNF in the blood. This has been reported by two different groups. This is suggestive that malnutrition or aberrant nutrition may somehow trigger a cytokine response. Maybe the cytokines are involved in mobilizing energy as a beneficial response. There has been a great deal of interest in cytokine production in the neonatal period in relation to necrotizing enterocolitis. Babies with this condition seem to have a reduced cytokine response, at least initially, but they also develop a different syndrome of septic shock from the adult, so there is much speculation in this area.

\textbf{Dr. Yamashiro:} Do you think that the stunting that occurs with chronic steroid therapy has something to do with cytokines?

\textbf{Dr. Tracey:} That is a very important point. The mechanism by which glucocorticoids produce their anti-inflammatory action has been an enigma since they were first developed. A great deal of work has now been done on the fact that glucocorticoids seem, as a group, specifically to turn off the production of many of these pro-inflammatory cytokines. So you could be describing a sort of rebound effect. This is an important area for further investigation.

\textbf{Dr. Beatty:} In the process of development of cachexia, do you see a rise in immunoglobulins and acute-phase proteins in your mouse model?

\textbf{Dr. Tracey:} Yes, in all studies on cachexia where indices of acute-phase proteins have been sought they have been found to be increased.

\textbf{Dr. Rassin:} TNF and some of the IL cytokines are present in human milk. What function do you think they might have when they enter the baby by the enteral route?

\textbf{Dr. Tracey:} I don't know. But I was struck by the data showing lactoferrin-specific receptors and I wonder what the cytokine receptor profile is. Many of the cytokines are fairly tough biochemically and would probably survive gastric acid. Some would probably also survive proteolysis in the upper gastrointestinal tract and get absorbed.

\textbf{Dr. Rassin:} We have been working very hard on the mechanism of TPN-associated cholestasis and one of the mechanisms we have been looking at is the fact that light-exposed parenteral nutrition mixtures produce toxic products capable of stimulating TNF release. We have not yet been able to pin down exactly what it is that stimulates TNF release. When this substance gets in the liver it appears to affect amino acid transport at the bile formation site. However, it is hard to understand the sequence of events.

\textbf{Dr. Tracey:} There are now at least two books on TNF both of which have excellent chapters on the regulation of TNF synthesis, each with 100–200 references. This is a very large field because of the obvious medical relevance. The list of things that turn on TNF
synthesis keeps growing. I don’t know the answer to your question but any kind of pro-
inflammatory stimulator may turn on TNF.

*Dr. Pettifor:* Do all peripheral tissues have receptors for TNF? And how is it catabolized?

*Dr. Tracey:* The red cell is the only somatic cell type that does not have TNF receptors. All other cells in the body have two TNF receptors, type 1 (55 kDa molecular weight) and type 2 (75 kDa molecular weight). It appears that the type 1 isoform may be the important one for mediating shock and death in the septic shock syndrome. The catabolism of TNF is another area that is not well understood. The half-life of TNF in humans varies between 6 and 20 minutes and a lot of the TNF is secreted through the kidney. Some of it is not accounted for and it is not clear whether it is bound to cell receptors or bound by circulating fragments.

*Dr. Guesry:* Did you say that the cancer cells keep the TNF receptor?

*Dr. Tracey:* Many cancer cells have a TNF receptor, and many cancer cells make TNF. The problem with understanding the biology of TNF in cancer cells is that, depending on type, some tumor cells respond to TNF by dying, while others respond by growing. So there does not appear to be a 1:1 correlation between biological effect and tumor or tumor receptor.

*Dr. Guesry:* At one time people were thinking that they could use TNF as an anti-cancer drug. Of course, if the cancer cells are using the receptor, there is no reason to continue this type of investigation because we know that TNF may have deleterious side effects.

*Dr. Tracey:* Loss of the receptor does not explain cancer resistance or lack of efficacy of TNF. The lack of efficacy in cancer appears to be closely correlated to the development of toxicity, particularly toward endothelial cells. The problem is that the endothelial cells around the tumor are not more sensitive to TNF than endothelial cells in the kidney, or the heart, or the liver, so systemic toxicity is the limiting problem.

*Dr. Uauy:* Considering that this is a nutrition meeting, do you know of any specific nutritional manipulations that modulate TNF responsiveness—zinc, vitamin A, other specific nutrients?

*Dr. Tracey:* There is evidence that the intake of ω-3 fatty acids may alter cytokine production. I believe that the largest effect was on IL-1, while the effect on TNF was to attenuate it without reducing it to zero. It would be reasonable to consider these approaches for nutritional modulation of cytokines.

*Dr. Wharton:* All the conditions we have discussed where there is high TNF in association with wasting are also associated with quite severe curtailment of intake. I have been trying to think of conditions where you get wasting combined with a high intake and wonder what happens to TNF in this situation. Has thyrotoxicosis been studied, or Cushing’s syndrome, where you have a high intake and deposition of fat but there is wasting of muscles?

*Dr. Tracey:* I have not seen any data on TNF in thyrotoxicosis or Cushing’s syndrome. I think you are raising an important point. In the animal model—the mouse with prolonged weight loss—there is not a profound degree of anorexia. It would be within the bounds of the typical patients with cancer or AIDS, where food intake is reduced. If you look at Jeejeeboy’s data, the animals he studied were getting well in excess of their requirements, but they continued to have negative muscle protein balance. We have worked with other tumor models of cachexia characterized by protein and lipid loss but with normal or increased food intake and we have not found TNF to be important in those models.

*Dr. Wharton:* Take another clinical example, such as Crohn’s disease. We know that some children with Crohn’s disease will start to grow if they are fed adequately, whether or not hydrolyzed protein is given. There are also some patients in whom this does not happen, despite what seems to be a very adequate intake. Can you relate this kind of clinical course to TNF levels?
Dr. Tracey: Several companies and several research centers have spent a great deal of time and money investigating inhibitors of TNF and IL-1, particularly the IL-1 receptor antagonist, in Crohn's disease and ulcerative colitis. There is marked upregulation of pro-inflammatory cytokine production in these conditions, but the effects of this over a long period of time in a developing child are largely unknown. It is certainly possible that an overproduction of these pro-inflammatory factors could have deleterious effects on nutritional development.

Dr. Beatty: To follow on what Dr. Wharton was saying, the diencephalic syndrome is characterized by excessive intake and poor weight gain. Is anything known about cytokines in this condition?

Dr. Tracey: In babies with diencephalic syndrome no TNF was found in the blood or in the spinal fluid. Energy expenditure was measured and was found to be in excess of predicted for both age and weight.

Dr. Yamashiro: One established example of cytotoxicity of TNFα in the pediatric field is Kawasaki disease. In patients with Kawasaki disease, the serum level of TNFα is significantly higher than in controls in the initial acute phase of the illness, and those with high levels of TNFα are more likely to have cardiac involvement. TNFα attacks the endothelium of vessels and damages the coronary vasculature.

Dr. Kirsten: Does TNF cross the placenta, and do you know any disease in the mother where TNF is secreted and could have an influence on the fetus?

Dr. Tracey: TNF levels are very high in the amniotic fluid of mothers delivering at the time of chorioamnionitis. It is difficult to diagnose chorioamnionitis and attempts are being made to establish whether there is a possible diagnostic correlation with cytokines locally. I don't know if they cross the placenta. They don't cross the blood-brain barrier very well. There has been renewed interest in the last couple of years on the role of local cytokine production in the pathogenesis of infertility.

REFERENCES