New Mediators in Cancer Cachexia

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Approximately two thirds of patients who die with advanced cancer suffer from cancer cachexia. Indeed, the degree of cachexia is inversely correlated with the survival time of the patient and it always implies a poor prognosis. This syndrome is characterized by marked weight loss, anorexia, asthenia and anemia [1]. Perhaps one of the most paradigmatic characteristics of cachexia is that of asthenia, which seems to be the most prevalent symptom of patients with advanced cancer and very well reflects one of the most interesting factors involved in cachexia, that of muscle waste [2]. Asthenia is characterized by a generalized weakness as well as physical and mental fatigue. Indeed lean body mass depletion is one of the main trends of cachexia, and it involves not only skeletal muscle but also affects cardiac protein, resulting in important alterations in heart performance.

Cancer-induced cachexia is invariably associated with the presence and growth of the tumor and leads to a malnutrition status due to the induction of anorexia or decreased food intake. In addition, the competition for nutrients between the tumor and the host leads to an “accelerated” starvation state which promotes severe metabolic disturbances in the host including hypermetabolism, which leads to an increased energetic inefficiency. Although the search for the “cachectic” factor(s) started a long time ago, and although many efforts have been devoted to its discovery, we are still a long way from knowing the whole truth. However, a lot of progress has been made, and the suggested mediators (associated with both depletion of fat stores and muscular tissue) can be divided into two categories: tumor origin and humoral factors (mainly cytokines). Indeed cells of the immune system release cytokines that act on multiple target cells such as bone
Fig. 1. Cytokines and cachexia. The balance between pro-inflammatory (pro-cachectic), their soluble receptors and the anti-inflammatory (anti-cachectic) cytokines plays a key role in the development of the cachectic syndrome.

Malnutrition

Malnutrition is one of the hallmarks of cancer cachexia and it is basically associated with anorexia. But, are cytokines involved in the induction of anorexia? Feeding is a complex function resulting from the integration of peripheral and
central nervous impulses in the ventral hypothalamus. Stimulation of the medial hypothalamic nucleus inhibits feeding, while stimulation of the lateral nucleus promotes food intake. Oral stimulation by pleasant tastes elicits feeding, while gastrointestinal distention prevents it. Concerning the possible involvement of cytokines in tumor-induced anorexia, although not precisely conclusive, several points have to be taken into consideration. Cytokines such as IL-1 [3] and TNF [4] have been proposed as being involved in cancer-related anorexia, possibly by increasing the levels of corticotropin-releasing hormone, a central nervous system neurotransmitter that suppresses food intake and the firing of glucose-sensitive neurons, which would also decrease food intake. Anorexia seems to be the effect rather than the cause of weight loss during cancer; cachexia perpetuates and worsens itself through a mechanism involving anorexia in a sort of positive feedback which usually leads to death. There is some evidence in favor of this argument. Firstly, total parenteral nutrition in patients with a high degree of cachexia has led to controversial results, some being beneficial and some resulting in no improvement in survival time. However, even in cases in whom it promoted an increase in body weight, this was not associated with an increase in lean body mass but it seemed rather to increase water retention, which manifested as peripheral edema together with decreased hematocrit. Secondly, in different experimental models, pair-feeding does not lead to the same extent to either weight loss or metabolic abnormalities as found in tumor-bearing animals. In fact, tumor burden induces metabolic changes which do not resemble those of caloric restriction or starvation, but rather those found in infection or injury. Thirdly, sometimes tumors involve the alimentary tract and result in diminished food intake as a consequence of mechanical obstruction, pain or early satiety. In addition, anticancer treatment (such as chemotherapy, radiotherapy or immunotherapy) may be associated with varying degrees of nausea, vomiting and, consequently, anorexia. Furthermore, alterations in food perception (taste and smell) together with psychological causes (such as depression) may contribute to the reduced food intake.

**Body Weight Loss and Circulating Cytokines**

Weight loss, the most paradigmatic trend of cancer cachexia, can be mimicked by cytokines under some experimental conditions. Nevertheless, episodic TNF administration has proved unsuccessful at inducing cachexia in experimental animals [5]. Indeed, repetitive TNF administrations initially induce a cachectic effect, although tolerance to the cytokine soon develops and food intake and body weight return to normal. Other studies have shown that escalating doses of TNF are necessary to maintain the cachectic effects. However, a very elegant approach involving the implantation of Chinese hamster ovary cells which were transfected with the human TNF gene in nude mice seems to indicate that TNF may play an important role in the induction of cachexia [6].
Raised concentrations of TNF have been detected in the serum of about 70% of patients with parasitic infections and septicemia, pathological states in which a high degree of cachexia is achieved. The increase in plasma TNF in septicemia is likely to be due to increased concentrations of endotoxin or lipopolysaccharide (LPS), which can elicit a transitory rise in plasma TNF when administered to healthy control subjects. In contrast, evidence for increased TNF in the plasma of cancer patients is controversial. Balkwill et al. [7] found that 50% of serum samples from cancer patients had a positive response for TNF with an enzyme-linked immunosorbent assay, and more recently the presence of this cytokine has been observed in the serum of children with acute lymphoblastic leukemia; by contrast, other studies have reported no increase. Similarly, no TNF could be detected in the plasma of tumor-bearing mice but, in contrast, other studies have found considerable amounts of TNF in the blood of tumor-bearing rats. These divergent findings may be due to the different sensitivities of the assay methods, stability of TNF on storage, short half-life of TNF in vivo or localized paracrine production of TNF.

Strassmann et al. [8], using a murine colon adenocarcinoma, have shown that treatment with an anti-mouse IL-6 antibody was successful in reversing the key parameters of cachexia in tumor-bearing mice. These results seem to indicate that, at least in certain types of tumors, IL-6 could have a more direct involvement than TNF in the cachectic state. Conversely, other studies have revealed that IL-6 is not involved in cachexia in a very similar mouse tumor model. In addition, studies using incubated rat skeletal muscle have clearly shown that IL-6 had no direct effect on muscle proteolysis.

Another interesting candidate for cachexia is IFN-γ, which is produced by activated T and natural killer cells and possesses biological activities that overlap those of TNF. Matthys et al. [9], using a monoclonal antibody against IFN-γ, were able to reverse the wasting syndrome associated with the growth of the Lewis lung carcinoma in mice, thus indicating that endogenous production of IFN-γ occurs in tumor-bearing mice and is instrumental in bringing about some of the metabolic changes characteristic of cancer cachexia. The same group also demonstrated that severe cachexia develops rapidly in nude mice inoculated with Chinese hamster ovary cells constitutively producing IFN-γ, as a result of the transfection of the corresponding gene.

Other cytokines, such as the leukemia-inhibitory factor (LIF), transforming growth factor-α or IL-1 have also been suggested as mediators of cachexia. Thus, mice engrafted with tumors secreting LIF develop severe cachexia. Concerning IL-1, although its anorectic and pyrogenic effects are well known, administration of the IL-1 receptor antagonist (IL-1ra) to tumor-bearing rats did not result in any improvement in the degree of cachexia, thus suggesting that its role in cancer cachexia may be secondary to the actions of other mediators. Interestingly the levels of both IL-6 and LIF have been shown to be increased in patients with different types of malignancies.
Ciliary neurotropic factor (CNTF) is a member of the family of cytokines which includes IL-6 and LIF and is produced predominantly by glial cells of the peripheral nervous system; however, this cytokine also seems to be expressed in skeletal muscle. Henderson et al. [10] have demonstrated that CNTF induced potent cachectic effects and acute-phase proteins (independent of the induction of other cytokine family members) in mice implanted with C6 glioma cells, genetically modified to secrete this cytokine.

Bearing all this in mind, it may be concluded that, although TNF plays a very important role in the induction of cachexia, the metabolic derangement leading to this pathological state can also be influenced by other cytokines produced by immune cells in response to invasive stimuli.

**Hypermetabolism**

If anorexia is not the only factor involved in cancer cachexia, it becomes clear that metabolic abnormalities must play a very important role. Basically, due to the hypermetabolic state, the tumor-bearing host is energetically more inefficient than in the normal non-tumor-bearing state, and this leads to an increased energy expenditure that, together with the decreased food intake, plays a key role in the development of cachexia. Indeed, body weight maintenance requires energy intake to equal energy expenditure. In fact, these two variables are normally interconnected since when energy intake increases, so does expenditure and *vice versa*. For instance, starvation is characterized by an important drop in oxygen consumption while carbohydrate overeating is associated with an increase in thermogenesis. This relation between caloric intake and energy expenditure can be viewed as a mechanism to save calories when intake is low and to prevent obesity when excess food is eaten. In cancer cachexia, however, the often decreased caloric intake is not accompanied by a drop in energy expenditure. Different mechanisms can be involved in the increase in energy expenditure. Thus, the activity of futile cycles, such as the Cori cycle (glucose to lactate to glucose) or lactate recycling that takes place between the tumor and the host, are certainly involved in generating energetic inefficiency. Indeed the gluconeogenic utilization of the tumor-derived lactate is a very inefficient metabolic process consuming six molecules of ATP per cycle, but it is essential for compensating tumor acidosis. IL-6 seems to be clearly involved in stimulating gluconeogenesis in cultured hepatocytes. Yasmineh and Theologides [11], however, have shown that TNF does not seem responsible for the increase in gluconeogenesis observed in the tumor-bearing host. In addition, Christ and Nath [12] have shown that both TNF and IL-1 impair the glucagon-mediated increase in phosphoenolpyruvate carboxykinase (one of the rate-limiting enzymes in gluconeogenesis) in cultured rat hepatocytes. Conversely, Zentella et al. [13] have clearly shown that TNF action in cultured myocytes is linked with an important activation of a futile cycle. Thus, the cyto-
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Fig. 2. Cytokines and futile cycling. Changes in futile substrate cycling have been suggested as participating in body weight regulation by increasing energy expenditure. Cytokines may participate in the activity of some of these cycles.

Cytokines stimulate glucose utilization and lactate formation activating the substrate cycle between phosphofructokinase and fructose 1,6-bisphosphatase, therefore increasing glucose and glycogen utilization with a subsequent increase in lactate production (Fig. 2). Protein synthesis and degradation (protein turnover) constitute another wasteful metabolic process activated by TNF (Fig. 2). Another futile cycle involves an abnormal function of Na⁺-K⁺-ATPase. The enzyme complex works by pumping Na⁺ out of the cells at a certain energetic cost in order to fulfill the demands of the active transport. Alterations in stoichiometry (more ATP per Na⁺ translocated) have been observed in Ehrlich ascites tumor cells.
Nonshivering thermogenesis takes place in brown adipose tissue (BAT). Brown adipocytes contain numerous mitochondria and are characterized by the presence of a 32-kD protein, thermogenin or uncoupling protein-1 (UCP1), which uncouples oxidative phosphorylation in the mitochondrial compartment. Consequently, the energy associated with substrate oxidation is not employed for ATP synthesis and thus is released as heat. Injection of low doses of TNF either peripherally or into the brain of laboratory animals elicits rapid increases in metabolic rate which are not associated with increased metabolic activity but rather with an increase in blood flow and thermogenic activity of BAT. Interestingly, during cachectic states there is an increase in BAT thermogenesis both in humans and experimental animals. In addition, TNF stimulates lipogenesis in BAT. Consequently, nonshivering thermogenesis due to BAT activity may be a very important factor contributing to the decreased energy efficiency found in the cachectic state.

Until very recently, UCP1 (present only in BAT) was considered to be the only mitochondrial protein carrier that stimulated heat production by dissipating the proton gradient generated during respiration across the inner mitochondrial membrane and therefore uncoupling respiration from ATP synthesis. Recently, two new proteins sharing the same function, UCP2 and UCP3, have been described. While UCP2 is expressed ubiquitously, UCP3 is expressed abundantly and specifically in skeletal muscle in humans and also in BAT of rodents. Our research group has demonstrated that both UCP2 and UCP3 mRNAs are elevated in skeletal muscle during tumor growth and that TNF is able to mimic the increase in gene expression [14].

**Lipid Metabolism**

During cachexia there is a dramatic loss of white adipose tissue, basically due to a fall in lipoprotein lipase (LPL) activity and an increase in hormone-sensitive lipase activity. In addition to these metabolic events, there is an inhibition of glucose transport and de novo lipogenesis in the tissue. TNF has been shown to decrease LPL activity in 3T3-L1 cells, associated with a decrease in LPL mRNA. Fried and Zechner [15] reported that TNF produced a dose-dependent marked suppression of LPL activity in human adipose tissue maintained in organ culture. In *vivo* administration of TNF results in a decrease in adipose tissue LPL activity in the rat, mouse and guinea pig. This decreased activity has been shown to depress the uptake of exogenous lipid by adipose tissue and to increase circulating triacylglycerols in the rat. Such an elevation may, in part, be the result of stimulation of lipolysis in adipose tissue with subsequent increased secretion of very low-density lipoproteins from the liver. In contrast, in human primary cultures of isolated adipocytes the cytokine was unable to decrease LPL. The addition of TNF to 3T3-L1 cells increased lipolysis, which has been confirmed by using fully differentiated adipocytes. TNF and IL-1 have both been shown to inhibit glucose...
transport in adipocytes and consequently decrease the availability of substrates for lipogenesis. Conversely, no direct action of TNF has been shown on de novo lipogenesis in adipose tissue of starved rats. However, TNF decreased acetyl-CoA carboxylase during preadipocyte differentiation by a decrease in its mRNA; this did not occur in fully differentiated adipocytes.

IFN-γ, like TNF, can inhibit LPL activity in 3T3-L1 adipocytes and can diminish the rate of synthesis of long-chain lipids from smaller chain fatty acids. This effect is similar to that of the inhibition of lipogenesis and LPL seen with TNF. With this ability of IFN-γ to mimic the effects of TNF on fat metabolism, and with its apparent synergy with TNF, IFN-γ may play a prominent role in cancer cachexia. In cultured adipocytes, IL-1, TNF-β (lymphotoxin), IFN-γ and LIF were all shown to decrease LPL activity. Similarly, IL-1 and IFN-α, β and γ increased lipolysis in adipocytes in culture.

Elevation of circulating lipid seems to be a hallmark of cancer-bearing states to the extent that some authors have suggested that plasma levels may be used to screen patients for cancer. Hyperlipemia in cancer-bearing states seems to be the result of an elevation in both triacylglycerols and cholesterol. Hypertriglyceridemia is the consequence of the decreased LPL activity which results in a decrease in the plasma clearance of both endogenous and exogenous triacylglycerols. Muscaritoli et al. [16] have demonstrated that both the fractional removal rate and the maximum clearing capacity are significantly decreased after the administration of an exogenous triacylglycerol load to cancer patients. In tumor-bearing animals with a high degree of cachexia there is also an important association between decreased LPL activity and hypertriglyceridemia. Another factor that could contribute to the elevation of circulating triacylglycerols is an increase in liver lipogenesis. As described above, TNF can affect lipid metabolism in different sites which can produce the serum elevations in lipid levels: adipose tissue LPL and lipolysis. Another important site that can account for hyperlipemia is the de novo fatty acid synthesis that takes place in the liver. Indeed, TNF has been shown to increase hepatic lipogenesis in vivo and subsequent very-low-density lipoprotein production. In vivo administration of IL-1, IL-6 and IFN-α to mice also produces a rapid increase in hepatic lipogenesis. IL-4 is a cytokine that has marked inhibitory properties in regulating the immune response. By itself, IL-4 has no effect on hepatic fatty acid synthesis, but it inhibits the stimulation of hepatic lipogenesis induced by TNF, IL-1 and IL-6. Using a polyclonal rat anti-TNF antibody, we have demonstrated that TNF is involved in the abnormalities in lipid metabolism found in tumor-bearing rodents [17]. In addition, studies involving TNF p55 receptor-deficient mice have shown that via p55 receptor the cytokine is involved in the alterations in lipid metabolism associated with the implantation of a cachexia-induced tumor. In conclusion, it may be suggested that TNF, together with perturbations in hormonal homeostasis, is likely to play an important role in forcing the metabolic balance of the adipocyte towards the catabolic side (Table 1).
### Table 1. Metabolic alterations present in the host that can be mimicked by cytokines

<table>
<thead>
<tr>
<th>Metabolic alteration</th>
<th>Cytokines involved</th>
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<tr>
<td><strong>Carbohydrate metabolism</strong></td>
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<tr>
<td>Increased hepatic gluconeogenesis</td>
<td>IL-6</td>
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<tr>
<td>Increased Cori cycle activity</td>
<td>TNF</td>
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<tr>
<td>Increased glucose turnover</td>
<td>TNF</td>
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<tr>
<td>Decreased muscle insulin-stimulated glucose uptake</td>
<td>TNF</td>
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<tr>
<td><strong>Lipid metabolism</strong></td>
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<tr>
<td>Hyperlipidemia</td>
<td>TNF, IL-1, LIF, IFN-γ</td>
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<tr>
<td>Decreased WAT LPL activity</td>
<td>TNF, IL-1, IFN-γ, LIF</td>
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<tr>
<td>Increased WAT lipolysis</td>
<td>TNF, IL-1, IFN-α, β, γ</td>
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<tr>
<td>Increased BAT thermogenesis</td>
<td>TNF</td>
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<tr>
<td><strong>Protein metabolism</strong></td>
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<tr>
<td>Increased whole body protein turnover</td>
<td>TNF</td>
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<tr>
<td>Increased hepatic protein synthesis</td>
<td>TNF, IL-1, LIF, IL-6</td>
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<tr>
<td>Changes in circulating amino acid pattern</td>
<td>TNF</td>
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<tr>
<td>Increased muscle protein degradation</td>
<td>TNF</td>
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<tr>
<td>Decreased muscle amino acid uptake</td>
<td>TNF</td>
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<tr>
<td>Increased hepatic protein synthesis</td>
<td>TNF, IL-1, LIF, IFN-γ</td>
</tr>
<tr>
<td>Increased BCAA turnover</td>
<td>TNF, IL-1</td>
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<tr>
<td><strong>Hormonal changes</strong></td>
<td></td>
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<tr>
<td>Insulin resistance</td>
<td>TNF</td>
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<tr>
<td>Increased counter-regulatory hormones</td>
<td>TNF, IL-1</td>
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WAT = White adipose tissue; BCAA = branched chain amino acid.

### Muscle Wasting

Asthenia or lack of strength is one of the main characteristics of cancer cachexia and it is directly related to the muscle waste observed in cachectic states. During fasting, muscle proteins are degraded to provide amino acids which are used for gluconeogenesis; however, during longer starvation periods, protein breakdown is decreased in order to conserve nitrogen and maintain lean body mass. This ability, which is essential for conserving nitrogen when the intake is reduced, seems to be absent in cancer-bearing states, leading to a depletion of vital host protein. The skeletal muscle, which accounts for almost half of the whole body protein mass, is severely affected in cancer cachexia and evidence has been provided for muscle protein waste as being associated with enhanced turnover rates. Since cachexia tends to develop at a rather advanced stage of the neoplastic growth, preventing muscle waste in cancer patients is of great potential clinical interest. Whether the negative protein balance results from altered rates of synthe-
sis or breakdown, or from changes on both sides of muscle protein turnover is still debated. It has been suggested that, during cancer cachexia, the muscle mass is decreased as a result of a lower rate of protein synthesis, while changes in protein degradation are secondary. Conversely, studies involving the release of 3-methylhistidine (a marker of myofibrillar protein degradation) from peripheral muscle in cancer patients suggest that protein degradation is increased. Our group has demonstrated that protein synthesis is hardly altered in skeletal muscle during tumor growth and that there is a great increase in protein degradation studied both in vivo and in vitro. In addition, we have identified the proteolytic mechanism which is involved in skeletal muscle during cancer cachexia [18].

A large body of evidence suggests that TNF participates in protein wasting and loss of nitrogen associated with cachectic situations. Chronic treatment of rats with TNF resulted in a depletion of body protein compared with pair-fed control animals. Indeed, chronic treatment with either TNF or IL-1β resulted in a body protein redistribution and a significant decrease in muscle protein content, associated with coordinate decreases in muscle mRNA levels for myofibrillar proteins. Studies involving administration of TNF in vivo have shown an increase in nitrogen efflux from skeletal muscle of non-weight-losing humans with disseminated cancer. Flores et al. [19], by infusing 14C-leucine to rats, showed that chronic TNF administration significantly enhanced muscle protein breakdown. Goodman [20], measuring tyrosine and 3-methylhistidine release by incubated muscles of rats acutely treated with the cytokine, concluded that TNF was involved in activating muscle proteolysis. Our research group has also demonstrated that TNF treatment enhances protein degradation measured in vivo in rat skeletal muscle. In addition, we have described that, at least during tumor growth, muscle wasting is associated with the activation of nonlysosomal ubiquitin-dependent proteases [18] and that this activation seems to be mediated via TNF [21]. Ubiquitin can be found free or conjugated in an isopeptide linkage to other cellular proteins, and proteins with multiple ubiquitins are the ones targeted for degradation by an ATP-dependent protease. However, it has been suggested that the activity of this system, which is integrated in a supramolecular structure called the proteasome, can also be related to the turnover of long-lived proteins, such as those found in skeletal muscle. We have also reported that in vivo administration of TNF to rats results in an increased skeletal muscle proteolysis associated with an increase in both gene expression and higher levels of free and conjugated ubiquitin. In addition, the in vivo action of TNF during cancer cachexia does not seem to be mediated by IL-1 or glucocorticoids. Concerning a possible direct action of TNF on muscle proteolysis, the presence of both p55 and p75 TNF receptors has been described and we have demonstrated that the action of the cytokine on the induction of ubiquitin-dependent proteolysis can be direct. Other cytokines such as IL-1 or IFN-γ are also able to activate ubiquitin gene expression. Therefore, TNF (alone or in combination with other cytokines) seems to mediate most of the changes concerning nitrogen metabolism associated with cachectic
Fig. 3. Muscle waste and acute-phase response. Perhaps the most paradigmatic metabolic derangement induced by the tumor is the activation of proteolysis in skeletal muscle and the redistribution of protein synthesis in the liver. These alterations can be mimicked by different cytokines.

states (Table 1). In conclusion, muscle protein degradation is perhaps the most important metabolic feature of the cachectic cancer-bearing host and future studies will no doubt concentrate on the discovery of compounds which are able to block the activation of the proteolytic systems responsible for the enhanced degradation.

**Acute-Phase Response**

The result of enhanced muscle proteolysis is a large release of amino acids from skeletal muscle which takes place especially as alanine and glutamine (Fig. 3). The release of amino acids is also potentiated by an inhibition of amino acid transport into skeletal muscle. While glutamine is basically taken up by the tumor to sustain both its energy and nitrogen demands, alanine is mainly channelled to the liver for both gluconeogenesis and protein synthesis. Interestingly, liver fractional rates of protein synthesis are increased in tumor-bearing animals, accounting for the production of the so-called acute-phase proteins, while there is a decrease in albumin synthesis.

The acute-phase response is a systemic reaction to tissue injury, typically observed during infection or trauma, characterized by a series of hepatocyte-
derived plasma proteins known as acute-phase reactants (including C-reactive protein, serum amyloid A, α1-antitrypsin, fibrinogen, and complement factors B and C3) and by reduced synthesis of albumin and transferrin. In cancer patients, an acute-phase response is observed [22]. For many years investigators have been searching for mediators involved in the regulation of acute-phase protein synthesis. Interestingly the cytokines IL-6, IL-1 and TNF are now regarded as the major mediators of acute-phase protein induction in the liver [23]. In fact, acute-phase proteins can be divided into two groups: type I and type II acute phase proteins. Type I proteins include serum amyloid A, C-reactive protein, C3, haptoglobin (rat) and α1-acid glycoprotein, and are induced by IL-1 and TNF. Type II proteins include fibrinogen, haptoglobin (human), α1-antichymotrypsin, and α2-macroglobulin (rat), and are induced by IL-6, LIF, oncostatin M, CNTF and cardiotropin-1. Unfortunately, the role of acute-phase reactants during cancer growth is still far from understood.

**Insulin Resistance**

In addition to having increased glucose production and glucose intolerance, cancer patients show a clear insulin resistance status that involves adipose tissue, skeletal muscle and liver. The increased hepatic glucose production is partially the result of a lack of inhibition of gluconeogenesis by insulin due to a certain degree of liver insulin resistance. Similarly, glucose utilization by skeletal muscle is reduced both in experimental animals and cancer patients, this being the result of clear insulin resistance. In addition, increases in counter-regulatory hormones, such as glucocorticoids or glucagon, also seem to be involved. As Tayek [24] pointed out: “the cancer patient behaves like the type II diabetic who is unable to maximize glucose uptake in the skeletal muscle in the presence of large amounts of insulin”. The decreased stimulation of glucose uptake does not seem to be the consequence of a defect in insulin binding but rather a postreceptor defect. The insulin resistance observed in skeletal muscle also affects glycogen synthesis, which is reduced in cancer patients. Insulin resistance in the tumor-bearing host may be overcome by the use of exogenous insulin, which in animal studies has improved the degree of cachexia as well as the response to anticancer therapy, including surgery and chemotherapy.

Pathological situations associated with a high TNF production show a state of peripheral insulin resistance. Among them, endotoxemia, cancer and trauma are normally associated with increases in circulating TNF and peripheral insulin resistance. Thus, the general clinical impression is that the insulin dose for diabetic patients must be increased if infection is present, thus suggesting an additional impairment to insulin resistance. Lang et al. [25], using a euglycemic clamp in septic rats, found that the septic state induced peripheral insulin resistance. It is well known that during infection there is a stimulation of macrophage TNF pro-
duction by means of either LPS or other endotoxins released by the infecting agent. In addition, chronic administration of TNF to rats induces systemic insulin resistance. Clinical administration of TNF to healthy humans has been reported to reduce insulin sensitivity by inducing hyperglycemia without lowering insulin levels.

**Other Mediators**

It is by no means intended here to give the idea that cytokines are the only molecules involved in cachexia. Many other compounds have been reported to play an important role in the cachectic state. Perhaps the first evidence of such compounds came from studies with Krebs-2 carcinoma cells in mice; inactive extracts of these cells could induce cachexia once injected in normal non-tumor-bearing mice [26]. Similarly, toxohormone L was isolated from the ascites fluid of patients with hepatoma and sarcoma-bearing mice [27] and induced lipid mobilization and immunosuppression and involution of the thymus. Extracts of thymic lymphoma, conditioned medium from thymic lymphoma cell lines, and serum from lymphoma-bearing mice cause lipid mobilization in experimental animals [28].

It is worth mentioning the studies of Todorov et al. [29] using a murine colon adenocarcinoma (an experimental tumor that induces cachexia with a small tumor burden and without causing hypophagia). They were able to purify and characterize a 24-kD proteoglycan (also present in urine of human cachectic patients) which perhaps accounts for the loss of adipose tissue and skeletal muscle mass found in tumor-bearing mice [29].

**Conclusions**

Under normal circumstances, maintenance of homeostasis in mammals, including humans, is guaranteed by a number of mechanisms. Deviations from this stable situation may occur, imposing a serious threat to the health of the organism. The organism responds to these challenges by a coordinated sequence of systemic and metabolic changes or by local changes such as inflammatory reactions. The macrophage-derived proinflammatory cytokines (IL-1, IL-6, TNF) play key roles in inducing these changes and are therefore involved in many pathophysiological conditions, not only immune and inflammatory reactions but also in the development of cachexia. In fact, the balance between these and the anti-inflammatory cytokines such as IL-1ra, IL-10 and TGF-β is pivotal for the fine tuning of many biochemical processes.

A complex interaction of pro-cachectic and anti-cachectic cytokines or cytokine-neutralizing molecules probably determines the critical presentation and
Table 2. Pro-cachectic and anti-cachectic cytokines

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<tr>
<th>Pro-cachectic</th>
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<tr>
<td>IL-1</td>
<td>sTNFρ</td>
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<tr>
<td>IL-6</td>
<td>IL-1ra</td>
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<td>TNF</td>
<td>IL-4</td>
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<td>IFN-γ</td>
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<td>LIF</td>
<td>IL-13</td>
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<td>CNTF</td>
<td>IL-15</td>
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course of cachexia (Table 2). Intervening in this sequence of events to modify the host responses may prove to be a beneficial treatment strategy for cachexia. Currently tested anti-proinflammatory cytokines have produced interesting results. Among the cytokines that can protect against TNF-mediated cachexia, IL-4 seems to be a good candidate since Th2 response-deficient IL-4 (–/–) mice were very susceptible during acute schistosomiasis, exhibiting severe acute cachexia followed by death. Similarly, monocyte chemoattractant protein-1 (MCP-1) seems to protect mice against endotoxin-induced mortality, possibly by increasing IL-10 and decreasing TNF. Indeed, administration of recombinant-derived MCP-1 to LPS-challenged mice supports this view. Similarly, administration of another cytokine related to the Th2 response, IL-13, also reduces TNF and increases survival against LPS challenge. It has been shown that while mice inoculated with an adenocarcinoma cell line expressing IL-6 developed major wasting and cachexia, the inoculation of IL-10-producing transfectant cells to mice did not cause anorexia or weight loss, suggesting that the cytokine was able to counteract cachexia by inhibition of IL-6 production by the tumor cells.

Hyperalimentation (either enteral or parenteral) of cancer patients has unfortunately proved to be an inefficient tool for fighting cachexia. It either leads to an increased accumulation of body fluids or to an acceleration of tumor growth without any increases in lean body mass. Consequently, future clinical and research efforts will be devoted to investigating the metabolic alterations induced in the host by the presence of the tumor and how this knowledge may be used more deeply in the clinical management of cancer patients. Particularly interesting is the idea of controlling protein metabolism in the patients essentially by means of blocking protein degradation in skeletal muscle. This would result both in preservation of the muscle mass and, to some extent, in a depletion of amino acids for tumor growth. Investigations are currently being undertaken in our laboratory to understand the regulation of the ATP-dependent proteolysis, the main proteolytic pathway activated during cancer cachexia in skeletal muscle.

Bearing in mind all the information presented here, it can indeed be concluded that no definite mediator of cancer cachexia has yet been identified. However, among all the possible mediators considered here, TNF is one of the most relevant...
candidates. Indeed, TNF can mimic most of the abnormalities found during cancer cachexia: weight loss, anorexia, increased thermogenesis, alterations in lipid metabolism and adipose tissue dissolution, insulin resistance and muscle waste including activation of protein breakdown and increased branched chain amino acid metabolism. However, TNF alone cannot explain all the cachectic metabolic alterations present in different types of human cancers and experimental tumors. Another important drawback is the fact that circulating TNF concentrations are not always elevated in cancer-bearing states and, although it may be argued that in those cases local tissue production of the cytokine may be high, cachexia does not seem to be a local tumor effect. Consequently, both tumor-produced and humoral factors must collaborate in the full induction of the cachectic state.

Because metabolic alterations often appear early after the onset of tumor growth, the scope of appropriate treatment, although not aimed at achieving immediate eradication of the tumor mass, could influence the course of the patient’s clinical state or, at least, prevent the steady erosion of dignity that the patient may feel in association with the syndrome. This would no doubt contribute to improving the patient’s quality of life and, possibly, prolong survival. Although exploration of the role that cytokines play in the host response to invasive stimuli is an endeavour that has been underway for many years, considerable controversy still exists over the mechanisms of lean tissue and body fat dissolution that occur in the patient with cancer or inflammation and whether humoral factors regulate this process. A better understanding of the role of cytokines interfering with the molecular mechanisms accounting for protein wasting in skeletal muscle is essential for the design of future effective therapeutic strategies. In any case, understanding the humoral response to inflammation and modifying cytokine actions pharmacologically may prove very effective and no doubt future research will concentrate on this interesting field.

References

Discussion

*Dr. Baracos:* You present a huge palette of potential mediators and I think somebody in the clinical arena would be looking to you for some focus. I don’t know if you’re a betting man, but if you had to bet your research efforts on a single most likely mechanism from all of those that you’ve described, which one would it be?

*Dr. Argiles:* That’s a difficult question. One answer is a dangerous thing, always. But the anti-tumor necrosis factor (TNF) approach could lead to interesting results. We know that anti-TNF trials have given very poor results in sepsis, but they haven’t been tried in cancer cachexia. Anti-TNF treatment is very successful in other pathologies where there is also cachexia, and in other abnormalities such as rheumatoid arthritis. So I would focus on trying to counteract TNF to start with, while at the same time also testing anti-inflammatory cytokines such as IL-15, to counteract some of the effects that they may be involved in.

*Dr. Tisdale:* There has been a clinical trial of pentoxyphyllin in cancer cachexia, which showed no beneficial effect [1]. I think the other question that needs to be answered in relation to cytokine involvement in cancer cachexia relates not only to the circulating level of the cytokine but also to a signature that a cytokine has been present in the tissue. A signature in adipose tissue is the inhibition of lipoprotein lipase (LPL) expression. In the only study that I’ve seen where people have looked at LPL expression in adipose tissue, the expected decrease was not found [2]. Would you like to comment on those two points?

*Dr. Argiles:* I wasn’t aware of this clinical trial of pentoxyphyllin in cachexia, but I don’t think pentoxyphyllin is the best drug to use in that situation. There are more effective inhibitors of TNF synthesis, and so I don’t think we can conclude that inhibition of TNF synthesis would not be effective just because one clinical trial with pentoxyphyllin has failed. Concerning your second question, was it LPL expression that was measured, or activity? LPL measurement can be awkward.

*Dr. Tisdale:* They measured the mRNA.

*Dr. Argiles:* As you know, mRNA values may not always reflect actual activity, and this could have been the case here. Certainly when we inject TNF into an animal, we do have effects on adipose tissue, and in our animals, where I claim that cytokines are involved, LPL is one of the most responsive things.

*Dr. Tisdale:* I was just referring to clinical studies. There’s also a clinical study going on in Alberta, Canada, on thalidomide for cancer cachexia. It will be interesting to see the results of that trial.

*Dr. Argiles:* There again, I don’t know whether thalidomide is a particularly good inhibitor of TNF. Maybe there are other more specific inhibitors that would be better for such clinical trials. Some compounds that are being developed by drug companies not only inhibit TNF but also increase IL-10, which is an anti-inflammatory cytokine. If I was responsible for a clinical trial trying to block TNF, I would use a more specific inhibitor that both decreases TNF and increases IL-10, rather than using thalidomide or pentoxyphyllin.

*Dr. Kho:* How could you explain the lack of effect of pentoxyphyllin? And do you know of any other TNF inhibitors that have few side effects which might be used in a clinical study?

*Dr. Argiles:* A lack of effect of these anti-TNF treatments could be due to a lack of inhibition of TNF production at the right site. Maybe these compounds are unable to inhibit TNF that is being produced in muscle tissue for some reason. It is possible that most of the TNF that is responsible for the cachexia might not be produced by the immune system as one would expect, but by muscle, which represents 50% of body weight, and the compound might just not be able to get into the muscle to inhibit the action of the cytokines. An intracellular TNF receptor has been described recently [3] and like receptor 2 it is bound to the mitochondria. Thus TNF produced inside the cell could bind to this receptor and have proteolysis-inducing effects. We are investigating this possibility at the moment.
Concerning other possible inhibitors, at present we are testing some of the compounds which block TNF and activate IL-10, but I’m not authorized to give you any results yet.

**Dr. Go:** In relation to what you said about multiple factors in cachexia and multiple factors in obesity, it is striking how when I go to a pancreatic meeting everyone talks about cachectic factors, while when I go to an obesity meeting, they all talk about obesity factors! There seems to be no cross fertilization between the two groups.

I have two questions. First, are all these cytokines depressed in obesity or increased in obesity? Second, in the obesity field people talk about leptin, about neuropeptide Y, about cholecystokinin, about β3-adrenergic receptors, and so on. What happens to these in relation to your cachectic factors? Are they also altered in cachexia? I’m looking at the yin-yang theory. Are we really talking about the same thing but in different guise?

**Dr. Argiles:** You have formulated two very interesting questions. As I pointed out, I do believe that cachexia and obesity are, to some extent, the two sides of the same coin – in other words, a malfunction of the regulation of body weight, energy intake, and energy expenditure. First of all, what happens with leptin? I didn’t present any data but we do have information about leptin during tumor growth [4]. We studied leptin levels and leptin gene expression in adipose tissue in our carcinoma models to see if there was a link between anorexia following the implantation of the tumor and changes in leptin. Obviously we expected to find an increase in leptin, but to our surprise we found that levels of leptin and leptin expression were both decreased from day 2. So this was one of the first events that took place in those animals, and unexpectedly leptin went down. There appeared to be no link with anorexia. So our conclusion was that the anorexia of tumor-bearing animals is not linked with leptin. There have also been studies in humans that have found little variation in leptin levels between tumor-bearing subjects and non-tumor-bearing subjects [5]. Leptin seems to be more an indicator of the mass of adipose tissue than anything else. That’s probably why you get decreased leptin levels during tumor growth, because there is a reduction in adipose tissue in these circumstances.

Now in relation to your first question, paradoxically TNF expression is increased in adipose tissue in obesity. You might argue that this is not possible, as we’ve tried to convince you that TNF is a cachectic factor. However, a few years ago Spiegelman’s group showed that obese animals, for example ob-ob mice and Zucker fatty rats, show increased adipose tissue TNF expression. This seems to be related to insulin resistance, because circulating TNF levels in those models of obesity are low. It seems that although the gene is expressed and maybe the protein is made, it isn’t actually released from the adipocyte. What it does is generate insulin resistance. But you’re right, it is surprising that in obesity we have an increased TNF expression in adipose tissue, and I am claiming here that during cachexia there is increased TNF production in muscle.

**Dr. Go:** When we look at adipocyte function or at the relation of the exocrine/endocrine pancreas to the adipocyte, we must not neglect the hypothalamic area because of the feedback loop. I think the satiety center becomes very important both in cachexia and in obesity. So we need to put the whole loop together to be able to sort out some of this paradox. Unfortunately not enough studies have been done in this area; for example, I still don’t know what neuropeptide Y is doing in the hypothalamic area in the regulation of TNF in adipocytes. I think that if we want to solve the cachexia and obesity problem, we need to bring the brain back into the equation.

**Dr. Nitenberg:** My question is a clinical one. The patient with cachexia could be in one of two situations: either receiving curative treatment or receiving palliative treatment. Do you think that treatment aimed at reversing cachexia is likely to have a potentiating effect on curative treatment? In other words, are there any studies that show that treating cachexia while also giving specific curative treatment gives better results than curative treatment alone? And in the second situation, do you think there is any point in giving palliative anti-cachexia treatment?
Dr. Argiles: I think that tolerance of any anti-tumor treatment will be better if cachexia is counteracted, even when anti-cachexia treatment has toxic side effects, so I would definitely go for that. Survival is likely to be increased. In relation to palliative care, I believe that anything which improves the quality of life is worthwhile, and this certainly applies to anti-cachexia treatment.

References