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Targets in clinical oncology: the metabolic environment of the patient

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TABLE OF CONTENTS

1. ABSTRACT

Cancer cachexia is a syndrome characterized by a marked weight loss, anorexia, asthenia and anemia. The degree of cachexia is inversely correlated with the survival time of the patient and it always implies a poor prognosis. Lean body mass depletion is one of the main features of cachexia and it involves not only skeletal muscle but also

affects cardiac protein. The cachectic state is invariably associated with the presence and growth of the tumour and leads to a malnutrition status due to the induction of anorexia or decreased food intake. In addition, the competition for nutrients between the tumour 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. Unfortunately, at the clinical level, cachexia is not treated until the patient suffers from a considerable weight loss and wasting. Therefore, it is of great interest to analyze possible early markers of the syndrome. In the present review both metabolic and hormonal markers are described. Although the search for the cachectic factor(s) started a long time ago, and although many scientific and economic efforts have been devoted to its discovery, we are still a long way from fully understanding the underlying basis for this syndrome. The suggested mediators (associated with both depletion of fat stores and muscular tissue) can be divided into two categories: of tumour origin (produced and released by the neoplasm) and humoural factors (mainly cytokines). One of the aims of the present review is to summarize and evaluate the different catabolic mediators (both humoural and tumoural) involved in cancer cachexia, since they may represent targets for clinical investigations. Additionaly, an overview of the main therapeutic approaches for the treatment of the cachectic syndrome is presented.

2. INTRODUCTION: THE SANITARY COSTS OF CANCER CACHEXIA

Perhaps the most common manifestation of advanced malignant disease is the development of cancer cachexia. Indeed, cachexia occurs in the majority of cancer patients before death, and according to Warren it is responsible for the deaths of 22% of cancer patients (1). Although it is not an easy endeavour, the health costs associated with cancer cachexia are considerable. While we know that the actual costs of cancer treatment are tremendous (it has been reported around 190 billion US $ for 2002 in the USA (2)), those associated with cancer cachexia are much more difficult to estimate because they include both medical (hospitalisation, treatment and ambulatory care) and non-medical (loss of income, carer support, welfare support, ambulance transport and nursing homes) (3). Estimates from USA hospitals suggest that 6 billion US $ are expended for cachectic patients. The abnormalities associated with cancer cachexia include anorexia, weight loss, muscle loss and atrophy, anemia and alterations in carbohydrate, lipid and protein metabolism (see ref. 4 for a review). The degree of cachexia is inversely correlated with the survival time of the patient and it always implies a poor prognosis (5-7). Perhaps one of the most relevant characteristics of cachexia is that of asthenia (or lack of muscular strength), which reflects the great muscle waste that takes place in the cachectic cancer patient (8). Asthenia is also characterized by a general weakness as well as physical and mental fatigue (9). In addition, lean body mass depletion is one of the main trends of cachexia, and it involves not only skeletal muscle but it also affects cardiac proteins, resulting in important alterations in heart performance.

At the biochemical level, different explanations can be found to account for cancer-induced cachexia. First, the presence and growth of the tumour is invariably associated with a malnutrition status due to the induction of anorexia (decreased food intake). In addition, the competition for nutrients between the tumour and the host promotes important metabolic disturbances (Figure 1), which include a considerable nitrogen flow from the skeletal muscle to the liver. Amino acids are used there for both acute-phase protein (APP) synthesis and gluconeogenesis (4). Both tumoural and humoural (mainly cytokines) factors are associated with depletion of fat stores and muscular tissues. Indeed cells of the immune system release cytokines that act on multiple target cells such as bone marrow cells, myocytes, hepatocytes, adipocytes, endothelial cells and neurons, where they produce a complex cascade of biological responses leading to the wasting associated with cancer cachexia. Among the cytokines that have been involved in this cachectic response are tumour necrosis factor-α (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6) and interferon-γ (IFN-γ). Interestingly, these cytokines share the same metabolic effects and their activities are closely interrelated, showing in many cases synergistic effects.

The aim of the present review is to summarize and evaluate the different catabolic mediators (both humoural and tumoural) involved in cancer cachexia since they may represent targets for promising clinical investigations in the future. Additionally, an overview of the main therapeutic approaches for the treatment of the cachectic syndrome is presented.

3. A NEW LOOK AT METHODOLOGY

A plethora of different techniques have been used for the evaluation of cachectic patients. In fact, deciding on the most appropriate methodology for the evaluation of the cancer status is a major decision when undertaking new therapeutic anti-cachexia approaches. There are basically three different parameters to be evaluated: 1) nutritional status of the patient, 2) body composition and performance, and 3) quality of life.

3.1. Nutritional status of the patient

Nutritional screening and assessment during cancer may serve to improve the oncological outcome. In general, evaluation of nutritional status is a complicated task and it becomes even more difficult when cancer patients are involved. It is worth mentioning the work of Ottery *et al.* (10) where global assessment of nutritional status is undertaken by a patient-generated subjective questionnaire (PG-SGA). The utilization of this questionnaire has been successful and has facilitated nutritional intervention in cancer patients to help and/or reverse the progressive weight loss that is seen in more than 80% of oncological patients at some point in their disease. The Ottery assessment model combines the subjective self-evaluation of each patient together with fomenting "anabolic competence"; thus, optimal results are obtained by combining adequate nutrients to support the stimulated protein and energy goals, promoting exercise (particularly

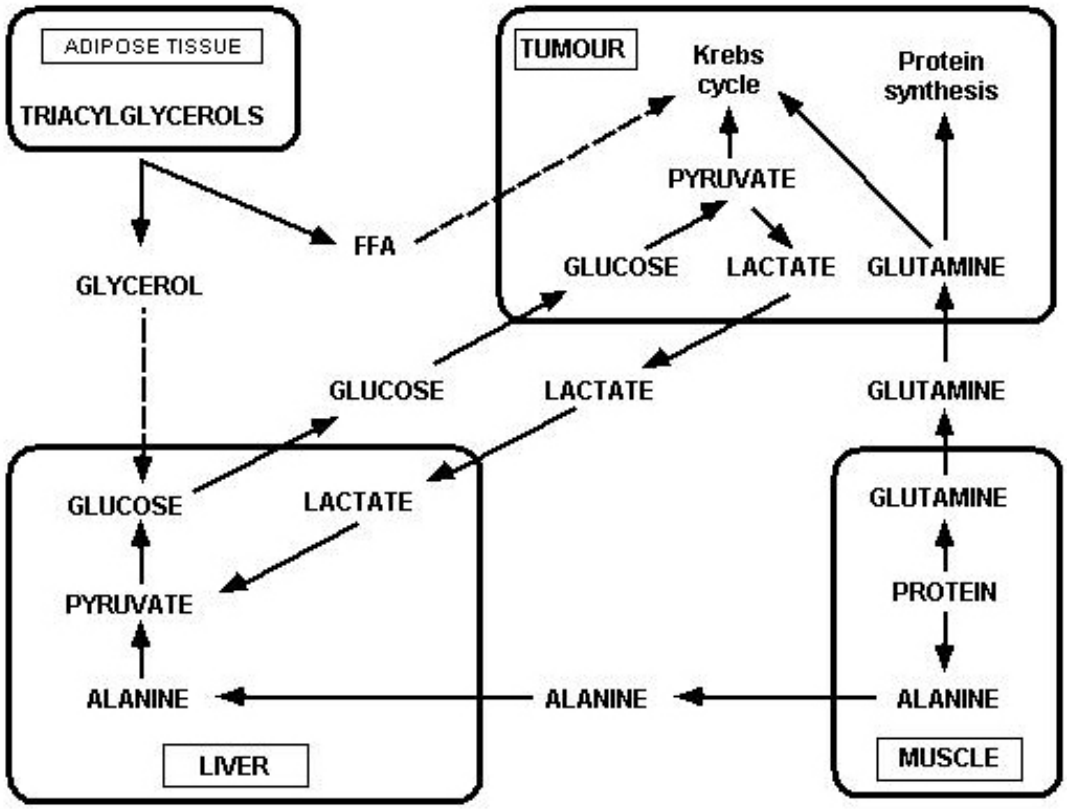

Figure 1. The metabolic "puzzle" between the tumour cells and the patient. The three main metabolic trends associated with cancer cachexia are: (1) lactate recycling between the liver and the tumoural mass, (2) lipid mobilization from adipose tissue, and (3) muscle waste basically caused by enhanced protein degradation.

progressive resistance exercise) and finally optimizing the hormonal milieu to fight both hormonal and cytokine mediated metabolic changes.

In conclusion, assessing nutritional status will allow: a) to identify patients who have, or are a risk of developing malnutrition and wasting, and b) to monitor the adequacy of nutritional therapy.

3.2. Body composition and performance

The evaluation of body composition, although simple in theory, can be complicated in practice (11). Thus body weight assessment is the simplest way of evaluating body weight; however, the concept of body composition should involve at least assessment of the free fatty mass (FFM) and fat mass (FM). In addition, sometimes it is also convenient to assess bone mineral content (BMC), total body water (TBW) and extracellular fluid. In comparison with simple ways of estimating body composition, body weight is easy to measure and has a high degree of precision. In addition, skinfold thicknesses and anthropometric methods provide an easy methodology for assessment especially for field studies or when working in rural and isolated locations. More sophisticated and expensive techniques include bioelectrical impedance (BIA), which allows FFM, FM and TBW estimation, and double labeled water, tracer dilution technique allowing for the estimation of TBW, extracellular fluid and energy expenditure. Perhaps the use of dual-energy X-ray absorptiometry (DEXA) is one of the most promising techniques allowing also measurement of BMC. This technique can be used alone or in combination with tracer techniques. Estimation of different minerals, total body potassium in particular, may also serve for estimation of FFM. Therefore the "goal standard" of body composition assessment has to include FFM, FM, BMC and TBW. Even more sophisticated methodologies such as computer tomography or magnetic resonance imaging are used for the assessment in isolated location.

In conclusion, there are three types of approaches for measuring body composition: 1) primary measures, basically TBW (which can be measured by densitometry or isotopes), and total body potassium (which can be measured by γ-spectrometry), also called body cell mass; 2) secondary measures: cellular water (which can be measured by BIA), and organ volume (which can be measured by DEXA); and finally 3) tertiary measures, which involve skeletal muscle mass measured by urine-creatinine (24 hours assessment). In addition, energy and nitrogen balance should also be estimated as part of body composition.

It is advisable that evaluation of body composition is accompanied by performance evaluation.

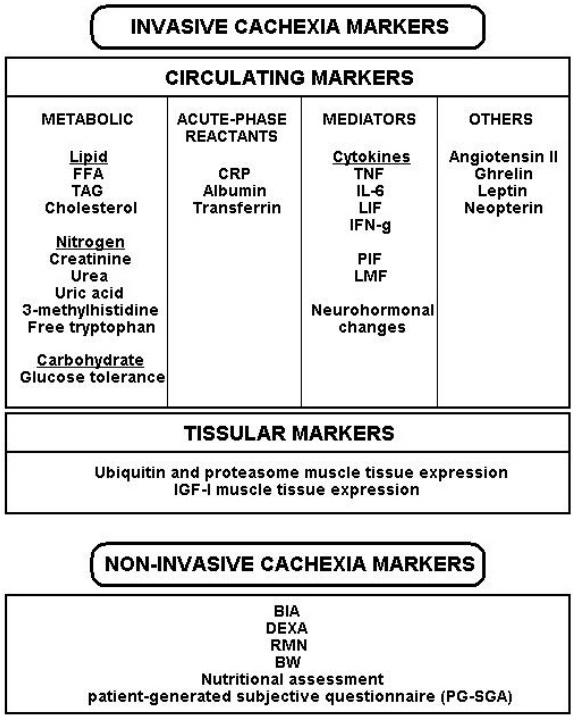

Figure 2. Main cachexia markers. Both circulating and tissular parameters can be used as a markers for cachexia. In addition, there are also non-invasive markers which may help to diagnose cancer-associated wasting.

This can be accomplished by different approaches such as grip-force evaluation, underwater weight lifting or treadmill performance. All of this accompanied by the Karnofski performance score.

3.3. Quality of life

Quality of life (QoL) represents the difference between the person's ideal state and their actual state. The intention in QoL measurement is to try to narrow the gap between the two. The obvious goal of any anti-cachexia therapy is both to improve survival and/or improve QoL. Unfortunately evaluation of the QoL in cancer patient is not an easy matter and some of the standard QoL questionnaires are not appropriate for cancer patients. In any case, QoL means different things to individuals in bed, groups in trials or populations when policy is being decided.

The main reasons for estimating QoL are: 1) choosing between treatment options when survival is equivocal or toxicity is high, 2) advanced cancer with palliation as an end-point, 3) health economic evaluation, and 4) prediction of prognosis. To choose the methodology for evaluating QoL in cancer patients should take into consideration that clinical trials sometimes require a sophisticated approach but routine monitoring in fact needs a simple one. Perhaps the best compromise is the EORTC core 30Q test (12). This is a well established score test which takes approximately ten minutes to fill out. There is a core of 30 questions but, in addition, it has a disease module, a treatment module and an issue module. There are functioning scales (eg: physical, role, emotional, cognitive, social), global scores (physical) and symptom scales.

In conclusion, nutritional assessment, body composition and QoL should always be monitored routinely in the evaluation of the cancer patient since they may provide excellent indicators for evaluating not only the efficacy of anticancer treatment but they may also provide very good decision-making elements for optimizing intervention.

4. EARLY DETECTION OF THE SYNDROME: CACHEXIA MARKERS

In most cases diagnosis of cancer is established after body weight loss has been occurring in a sustained way. As a result, treatment for cachexia starts at a very advanced stage of the wasting process when protein changes are almost irreversible. Bearing this in mind, it is very important to establish cachexia markers (ideally easy to measure) that might help to decide when to start the treatment. In addition, early prevention and treatment of cachexia will result in an increased efficacy of the anticancer treatment and in an improvement in the quality of the patient's life. In spite of this, not a single unequivocal cachexia marker has been described and here we report both circulating and tissular changes that may help to diagnose cancer-associated wasting (Figure 2).

From a therapeutic point of view, wasting during cancer is generally treated when a considerable amount of body weight loss is observed. However, the degree of weight loss may influence the treatment and sometimes a considerable loss of both body and muscle weight becomes irreversible. Bearing this in mind, considerable efforts have been devoted to finding a suitable marker for cachexia, before actual weight loss is observed. Unfortunately not a single unequivocal marker exists, therefore a combination of events (markers) should be taken into consideration.

Among the most relevant markers found in blood circulation, there are those related to changes in the metabolism of the patient. For instance, elevation in free fatty acids, triacilglycerols or cholesterol are lipidic markers (13-15). Taking into account nitrogen metabolites, we find that increases in creatinine, urea and uric acid are detected (16, 17). Perhaps a more specific nitrogen-related marker is 3-methylhistidine, an amino acid which is produced only when myofibrilar degradation occurs (18). Interestingly the levels of free tryptophan have also been proposed as a marker for cachexia-anorexia syndrome (19). Indeed increased plasma tryptophan levels (the precursor of serotonin) lead to increased cerebrospinal fluid tryptophan concentrations and increased ventromedial hypothalamic serotonin synthesis, which then mediates the occurrence of anorexia (20).

The last of the metabolic markers refers to a rather common phenomenon that affects a great number of cancer patients. This is the fact that during cancer marked glucose intolerance is observed, partly as a result of changes in insulin sensitivity (21).

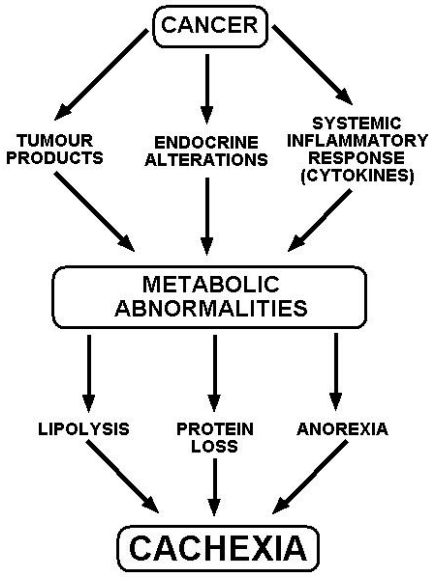

Figure 3. Cancer cachexia: factors involved. Cancer cachexia is a complex pathological condition characterized by many metabolic changes involving numerous organs. These changes are triggered by alterations in the hormonal milieu, release of different tumour factors and a systemic inflammatory reaction characterized by cytokine production and release.

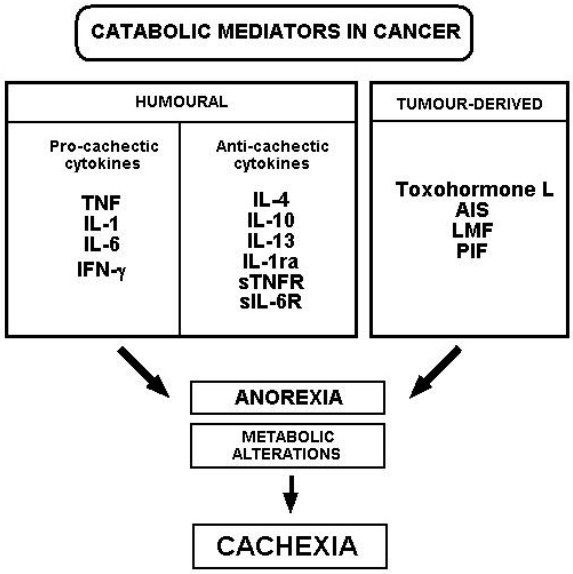

Figure 4. Catabolic mediators in cancer. Both tumour-derived and humoural (cytokines) factors are involved in mediating anorexia and metabolic changes, characteristic of the cachectic state.

Another interesting group of markers refers to the hepatic inflammatory response associated with the production of acute phase proteins (APP) (22). Among these proteins, C-reactive protein (CRP) (23, 24) and transferrin have been shown to be important indicators of the cachexia response and associated with poor prognosis. A drop in albumin concentration is also often observed in cachectin cancer patients. Taking into account that during cachexia there is an inflammatory catabolic response, many mediators of this response show increased circulating concentration. Among them are cytokines (TNF-α, IL-6, leukemia inhibitory factor (LIF), IFN-γ) (25,26) and tumour-produced factors (proteolysis-inducing factor (PIF), lipid-metabolizing factor (LMF)) (27,28).

Finally, other circulating markers include some peptides which are involved in the control of food intake such as leptin (29) and ghrelin (30). Neopterin (an indicator of ROS production) has also been suggested as a circulating marker during cancer (31) and wasting associated with several types of neoplasia (32). According to Anker (33) angiotensin II, generated through the action of ACE (angiotensin converting enzyme) on angiotensin I, is also an interesting marker for cachexia.

In spite of the fact that muscle biopsies often require surgery, they may provide an important tool for specific muscle tissue marking. From this point of view, activation of the ubiquitin-dependent proteolytic system occurs in human cancer patients before actual weight loss (34). Similar results are found with IGF-I (Paola Costelli, personal communication).

The above-referred invasive approaches can be combined with non-invasive methods such as BIA, DEXA, RMN and nutritional assessment questionnaires (35) (see *a new look at methodology* section).

5. CANCER: AN INFLAMMATORY DISORDER

The presence of the tumour clearly elicits a systemic inflammatory response that triggers anorexia as well as metabolic and neuroendocrine alterations. This systemic inflammatory response is triggered by different mediators either generated by the tumour or by non-tumoural cells in the patient. Mainly, two basic hypothesis can explain this phenomenon. First, the so-called endotoxic hypothesis, by which the tumour burden results in an enhanced translocation of intestinal bacteria into the peritoneum and, consequently, a release of endotoxine which finally triggers the cytokine cascade. Second, the tumour hypothesis involves either specific tumour-derived compounds or cytokines produced by the tumour which trigger the inflammatory response.

All together, the systemic inflammatory response generates many alterations that affect the patient's metabolism, activating among others, muscle protein breakdown, and consequently, wasting (Figure 3).

6. HUMOURAL MEDIATORS

Cytokines have a key role as the main humoural factors involved in cancer cachexia (Figure 4). Thus a large number of them may be responsible for the metabolic changes associated with cancer wasting.

6.1. Food intake

Anorexia may play an important role in accounting for malnutrition, invariably associated with

cancer cachexia. But, are cytokines involved in the induction of anorexia? The application of molecular and genetic techniques to the study of body weight regulation have produced exciting new insights into the physiological systems governing appetite, energy expenditure and metabolic signaling. 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 the feeding, while gastrointestinal distention prevents it. Concerning the possible involvement of cytokines in tumour-induced anorexia, although it is not precisely conclusive, several points have to be taken into consideration. Cytokines such as IL-1 (36) and TNF (37) have been proposed as involved in cancer-related anorexia, possibly by increasing the levels of corticotropin-releasing hormone (CRH), 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 are pieces of evidence in favour 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 the cases where 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 was manifested as a peripheral edema together with a decreased hematocrit. Secondly, in different experimental models, pair-feeding does not lead either to the same extent of weight loss or metabolic abnormalities as found in the tumour-bearing animals. In fact, tumour burden induces metabolic changes which do not resemble those of caloric restriction or starvation but rather those found in infection or injury. Thirdly, sometimes tumours 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 (depression) may contribute to the reduced food intake.

Concerning the mechanisms involved in cancer-induced anorexia, leptin does not seem to play a role (at least in experimental models) (38). Many mediators have been proposed, including changes in the circulating levels of free tryptophan; these may induced changes in serotonin brain concentrations and, consequently, cause changes in food intake (20). Bing *et al.* (39) have also suggested that some tumour-derived compound may mediate the anorexia associated with tumour burden.

6.2. Hypermetabolism

Different experimental approaches have demonstrated that cytokines are able to induce weight loss. Nevertheless, the results obtained have to be carefully interpreted. Thus, episodic TNF administration has proved unsuccessful at inducing cachexia in experimental animals (40). 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. Others studies have shown that escalating doses of TNF are necessary to maintain the cachectic effects. However, a very elegant approach involving the implantation of CHO cells which were transfected with the human TNF gene in *nude* mice seems to indicate that TNF may have an important role in the induction of cachexia (41).

Raised concentrations of TNF have been detected in the serum of about 70% of patients with parasitic infections and septicemia, which are pathological states where 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.* (42) found that 50% of serum samples from cancer patients had a positive response for TNF ELISA, 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 plasma in tumour-bearing mice but, in contrast, other studies have found considerable amounts of TNF in the blood of tumour-bearing rats. These divergent findings may be due to different sensitivities of the assay methods, stability of TNF on storage, short half-life of TNF *in vivo* or localized paracrine production of TNF.

Strassman *et al.* (43) 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 tumour-bearing mice. These results seem to indicate that, at least in certain types of tumours, 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 tumour model (44). In addition, studies using incubated rat skeletal muscle have clearly shown that IL-6 had no direct effect on muscle proteolysis (45).

Another interesting candidate for cachexia is IFN-γ, which is produced by activated T and NK cells and possesses biological activities that overlap with those of TNF. Matthys *et al.* (46), 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 the tumour-bearing mice and is instrumental in bringing about some of the metabolic changes characteristic of cancer cachexia. The same group has also demonstrated that severe cachexia develops rapidly in *nude* mice inoculated with CHO cells constitutively producing IFN-γ, as a result of the transfection of the corresponding gene.

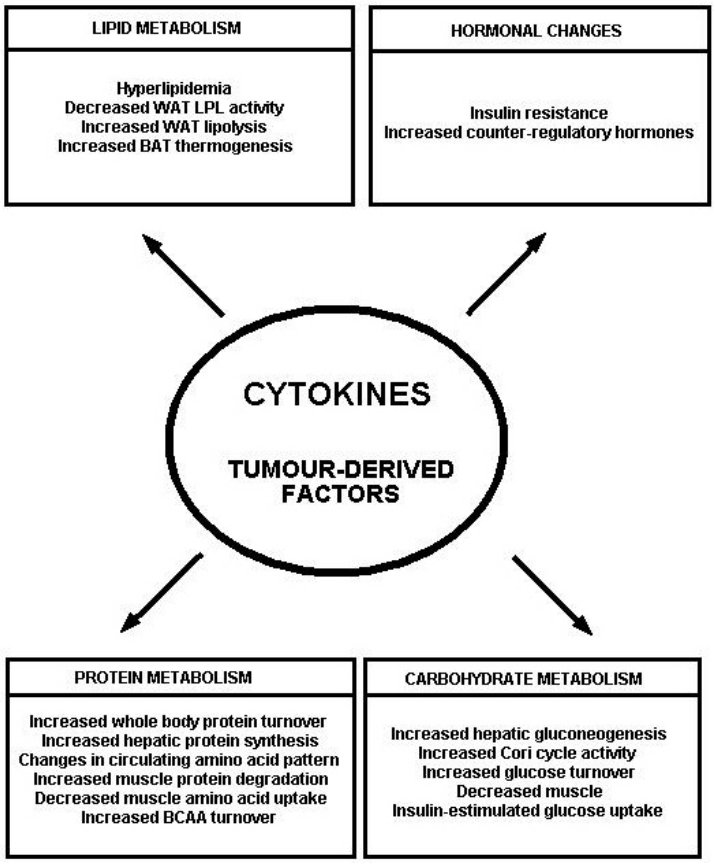

Figure 5. Cytokines can mimic most metabolic alterations. Most of the metabolic alterations present during cancer cachexia can be mimicked by pro-inflammatory cytokines. WAT: white adipose tissue.

Other cytokines, such as the LIF, transforming growth factor-β (TGF-β) or IL-1 have also been suggested as mediators of cachexia. Thus, mice engrafted with tumours secreting LIF develop severe cachexia. Concerning IL-1, although its anorectic and pyrogenic effects are well-known, administration of IL-1 receptor antagonist (IL-1ra) to tumour-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.

Cyliary neurotrophic factor (CNTF) is a member of the family of cytokines which include IL-6 and LIF and which 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.* (47) have demonstrated that CNTF induced potent cachectic effects and APP (independent of the induction of other cytokine family members) in mice implanted with C6 glioma cells, genetically modified to secrete this cytokine. The cytokine, however, exerted divergent direct effects on *in vitro* muscle preparations, which were dependent on the dose and the time of exposure (48).

Bearing all this in mind, it may be concluded that although TNF has 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.

Since anorexia is not the only factor involved in cancer cachexia, it becomes clear that metabolic abnormalities leading to a hypermetabolic state must have a very important role. Basically, the tumour-bearing host is energetically more inefficient than in the normal non-tumour bearing state, and this leads to an increased energy expenditure that, together with the decreased food intake, has 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 viceversa. 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 (Figure 5). Thus, the activity of futile cycles, such as the Cori cycle (glucose to lactate to

glucose) or lactate recycling that takes place between the tumour and the host, are certainly involved in generating energetic inefficiency. Indeed the gluconeogenic utilization of the tumour-derived lactate is a very inefficient metabolic process consuming 6 molecules of ATP per cycle but it is essential for compensating tumour acidosis. IL-6 seems to be clearly involved in stimulating gluconeogenesis in cultured hepatocytes. Yasmineh and Theologides (49), however, have shown that TNF does not seem responsible for the increase in gluconeogenesis observed in the tumour-bearing host. In addition, Christ and Nath (50) 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.* (51) have clearly shown that TNF action in cultured myocytes is linked with an important activation of a futile cycle. Thus, the cytokine stimulates 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. Protein synthesis and degradation (protein turnover) constitutes another wasteful metabolic process which is activated by TNF. Another futile cycle is that which involves an abnormal function of the Na^+-K^+-ATPase. The enzyme complex works by pumping Na^+ out of the cells at a certain energetic cost in order to fulfil the demands of the active transport. Alterations in the stoichiometry (more ATP per Na^+ translocated) have been observed in Ehrlich ascites tumour cells.

Non-shivering thermogenesis takes place in brown adipose tissue (BAT). Brown adipocytes contain numerous mitochondria and are characterized by the presence of a 32 kDa 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, non-shivering thermogenesis due to BAT activity may be a very important factor contributing to the decreased energy efficiency found in the cachectic state.

Until recently, the UCP1 protein (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 tumour growth and that TNF is able to mimic the increase in gene expression (52).

6.3. Lipid metabolism

Loss of whole body fat is a typical feature of cancer cachexia (Figure 5). Increased fat breakdown results in release of both glycerol (a gluconeogenic substrate) and fatty acids which may be oxidized, thus supplying energy for the gluconeogenic pathway. These changes are 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 (53) 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 of adipose tissue LPL activity in 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 elevation may, in part, be the result of stimulation of lipolysis in adipose tissue with subsequent increased secretion of very-low density lipoproteins (VLDL) 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 have a prominent role in cancer cachexia. In cultured adipocytes, IL-1, TNF, IFN-γ and LIF were all shown to decrease LPL activity. Similarly, IL-1 and IFN-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 of 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.* (54) 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 tumour-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 VLDL production. *In vivo* administration of IL-1, IL-6 and IFN-γ to mice also produces a rapid increase in hepatic lipogenesis. Interleukin-4 (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 tumour-bearing rodents (55). In addition, studies involving TNF p55 receptor-deficient mice have shown that the cytokine via p55 receptor is involved in the alterations in lipid metabolism associated with the implantation of a cachexia-induced tumour (56). In conclusion, it may be suggested that TNF, together with perturbations in the hormonal homeostasis, is likely to play an important role in forcing the metabolic balance of the adipocyte towards the catabolic side.

6.4. Muscle changes

One of the main characteristics of cancer cachexia is asthenia or lack of strength, being directly related to the muscle waste observed in cachectic states (Figure 5). 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 synthesis 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 tumour growth and that there is a great increase in protein degradation both studied *in vivo* and *in vitro*. In addition, we have identified the proteolytic mechanism which is involved in skeletal muscle during cancer cachexia (57).

A large body of evidence suggests that TNF participates in the 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-1 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 *in vivo* administration of TNF have shown an increase in nitrogen efflux from skeletal muscle of non-weight losing humans with disseminated cancer. Flores *et al.* (58), by infusing ^{14}C-leucine to rats, showed that chronic TNF administration significantly enhanced muscle protein breakdown. Goodman (59), measuring both tyrosine and 3-methylhistidine release by incubated rat muscles of animals 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 tumour growth, muscle wasting is associated with the activation of non-lysosomal ubiquitin-dependent proteases (57) and that this activation seems to be mediated via TNF (60). 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. Following interaction with its receptor the cytokine seems to eventually acts via enhancing NF-κB binding activity (61). During cancer cachexia this binding activity is also decreased, this being associated with a decreased myoD expression, the main transcription factor in muscle differentiation (62). In fact, NF-κB antisense therapy seems to be able to revert cachexia in experimental animals without altering tumour growth (63).

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 states. In addition to

the massive muscle protein loss, during cancer cachexia muscle DNA is also decreased, this leading to DNA fragmentation and, thus, apoptosis (64). Interestingly, TNF can mimic the apoptotic response in muscle of healthy animals (65). In conclusion, muscle protein degradation and apoptosis are 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.

6.5. Liver inflamatory response

The result of the enhanced muscle proteolysis is a large release of amino acids from skeletal muscle, which takes place specially as alanine and glutamine. 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 tumour to sustain both its energy and nitrogen demands, alanine is mainly channelled to the liver for both gluconeogenesis and protein synthesis. Increased hepatic production of acute-phase proteins (APP) has been suggested to be partly responsible for the catabolism of skeletal muscle protein, the essential amino acids being indeed required for APP synthesis. Despite the increased synthesis of APP, hypoalbuminemia is common in cancer patients, although this does not appear to be due to a decreased in albumin synthesis (66).

The acute-phase response is a systemic reaction to tissue injury, typically observed during infection, inflammation or trauma, characterized by the increased production of a series of hepatocyte-derived plasma proteins known as acute-phase reactants (including CRP, serum amyloid A (SAA), α1-antitrypsin, fibrinogen, and complement factors B and C3) and by decreased circulating concentrations of albumin and transferrin. An APP response is observed in a significant proportion of patients with the type of cancer frequently associated with weight loss (i.e. pancreas, lung, esophagus). The proportion of pancreatic patients exhibiting an acute-phase response increases with disease progression (67). For many years investigators have been searching for mediators involved in the regulation of APP synthesis. Interestingly the cytokines IL-6, IL-1 and TNF are now regarded as the major mediators of APP induction in the liver (68). In fact, APP can be divided into two groups: type I and type II. Type I proteins include SAA, CRP, 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, OSM (oncostatin M), CNTF and CT-1 (cardiotrophin-1). Unfortunately, the role of APP during cancer growth is still far from understood.

7. TUMOUR-DERIVED COMPOUNDS

In addition to humoural factors, tumour-derived molecules have also been proposed as mediators of cancer cachexia (Figure 4). Thus, several compounds have been reported to have an important role in mimicking the metabolic changes associated with the cachectic state.

Perhaps the first evidence of tumour-derived catabolic factors came from studies with Krebs-2 carcinoma cells in mice; inactive extracts of these cells could induce cachexia once injected in normal non-tumour-bearing mice (69). Similarly, Kitada *et al.* (70) purified a low molecular weight proteinaceous material from extract of thymic lymphoma in AKR mice that showed lipolytic activity in rat adipocyte suspensions. Thus, extracts of thymic lymphoma, conditioned medium from thymic lymphoma cell lines, and serum from lymphoma-bearing mice, cause lipid mobilization in experimental animals (70). Toxohormone L, a polypeptide of approximately 75 kDa, was isolated from the ascites fluid of patients with hepatoma and sarcoma-bearing mice (71), and induces lipid mobilization immunosuppression and involution of the thymus. A suppresive effect on feeding has also been observed by Okabe *et al.* (72) suggesting that the anorexia and cachexia present in the cancer patient may be related to the inhibitory effect of toxohormone L on food intake.

Tisdale's group at the University of Aston (UK) have described and characterized a LMF that was originally purified from a cachexia-inducing mouse colon adenocarcinoma (MAC16), but that has been further found in the urine of cancer patients (73). Indeed, LMF causes immediate release of glycerol in incubated epididymal adipocytes; this lipolytic induction is associated with an increase in the intracellular levels of cyclic AMP (cAMP), possibly formed in response to activation of adenylate cyclase (74). In turn, release of these fatty acids may also influence tumour growth. In addition, following administration of LMF to experimental animals, serum levels of glycerol and 3-hydroxybutyrate are increased as well as oxygen uptake by interscapular BAT, providing evidence of increased lipid mobilization and utilization (75). In fact, LMF is a 43 kDa glycoprotein, homologous to the plasma protease Zn-α2-glycoprotein (ZAG) in amino acid sequence, electrophoretic mobility and immunoreactivity. The cristal structure of ZAG resembles a class I major histocompatibility complex (MHC) heavy-chain although it does not bind the class I light chain α2-microglobulin. Instead of a peptide, the ZAG groove contains a nonpeptidic compound that may be implicated in lipid metabolism under pathological conditions. Hirai *et al.* (75) also suggest that LMF can have a role in initiating hepatic glycogenolysis during experimental cancer cachexia again through an increase in cAMP in liver. The decrease in liver glycogen due to the increased energy demand of the tumour-bearing state and the increasing weight loss are indeed in good correlation.

Anemia-inducing factor (AIS) is a protein of approximately 50 kDa secreted by malignant tumour tissue that depresses erythrocyte and immunocompetent cell functions. Experimental data show that energy metabolism in red blood cells is affected by AIS, the pyruvate kinase activity and ATP concentrations being lowered and the transmembrane glucose influx being suppressed. Moreover, in patients with terminal cancer, the osmotic resistance and the deformability of erythrocytes were also decreased. In addition, AIS is able to reduce food intake, body weight and body fat in rabbits and may, therefore, be a crucial catabolic mediator involved in the enhanced lipolytic activity (76).

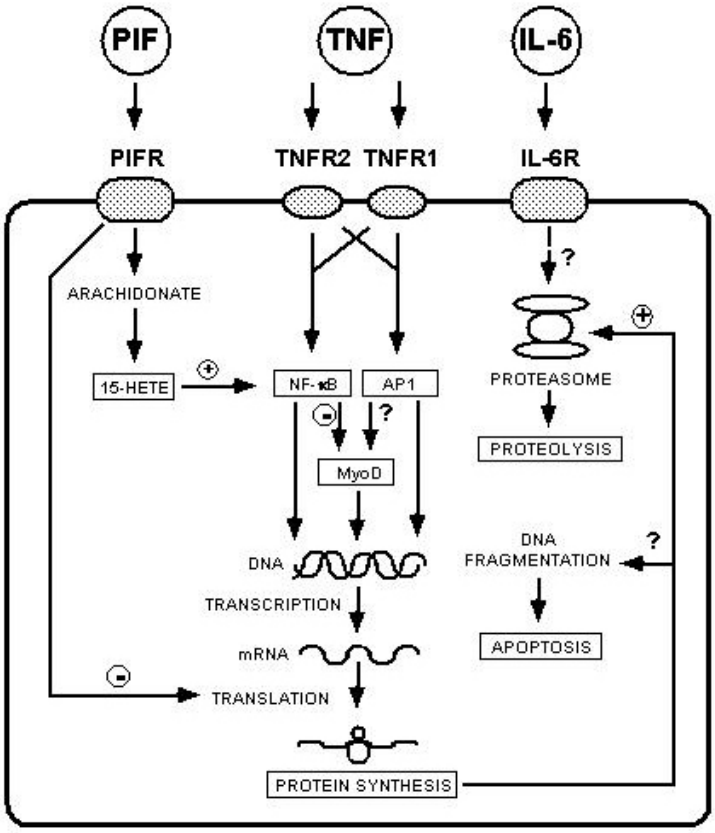

Figure 6. Interactions between PIF and pro-inflammatory (catabolic) cytokines. Both humoural (TNF and IL-6) and tumoural (PIF) factors have been shown to be able to activate intracellular muscle proteolysis by different mechanisms, possibly sharing common pathways.

Todorov *et al.* (77) have purified and characterized a 24 kDa sulphated proteoglycan, present both in experimental animals (78) and in the urine of cachectic patients (79), which seems to account for increased muscle protein degradation and decreased protein synthesis (78). Unlike some cytokines, this compound known as PIF produces direct proteolysis not only *in vivo* but also in isolated muscle preparations (78). Moreover, it appears to have a direct inhibitory effect on the glucose consumption by skeletal muscle, since uptake of 2-deoxyglucose by C2C12 myoblasts *in vitro* is also inhibited by PIF (79). The proteolytic mechanism activated by the proteoglycan is an ATP-proteasome dependent pathway (80). Thus, evidence for increased levels of ubiquitin-conjugated proteins, 14 kDa ubiquitin carrier protein E2 and C8 and C9 proteasome subunits in atrophying gastrocnemius muscle, have been reported. When injected into healthy animals, PIF is capable of mimicking the muscle waste associated with experimental cancer cachexia. *In vitro* studies on C2C12 myoblasts have shown that eicosapentaenoic acid (EPA) is able to block PIF action on proteolysis, in addition to suggesting that PIF acts intracellularly via the arachidonate metabolite 15-HETE (15-hydroxyeicosatetraenoic acid) (81). Nevertheless, EPA only significantly reduced protein degradation but did not attenuate the inhibitory effect of PIF, blocking the translation process of protein synthases (81).

PIF has also been shown to stimulate liver APP synthesis, a process characteristically linked with the inflammatory response observed in cancer patients. Using hepatocyte primary cultures, Watchorn *et al.* (82) have shown that PIF induces APP synthesis by activating both NF-κB and STAT3 transcription factors. These factors are required for the promotion of cell growth and differentiation during early embryogenesis, like PIF, which is also expressed during the embryonic period E8 to E9, may play a crucial stage in the patterning and eventual development of skeletal muscle in the mouse. In conclusion, PIF may have a constitutive role in normal states and became altered or overproduced during cancer cachexia and, therefore, induce important effects in both muscle protein catabolism and APP synthesis in this pathological state.

8. INTRACELLULAR SIGNALING

At the moment there are few studies describing the involvement of different transcriptional factors in muscle wasting (Figure 6). Penner *et al.* (83) reported an increase in both NF-κB and AP-1 transcription factors during sepsis in experimental animals. Recent data from our laboratory do not support an involvement of NF-κB in skeletal muscle during cancer cachexia (unpublished data), however, tumour burden results in a significant increase in

the binding activity of AP-1. Interestingly, inhibition of NF-κB is not able to revert muscle wasting in cachectic tumour-bearing animals (84); however, inhibition of AP-1 results in a partial reversal of protein degradation in skeletal muscle associated with tumour growth (unpublished data). The increase in NFκB observed in skeletal muscle during sepsis can be mimicked by TNF. Indeed, TNF addition to C2C12 muscle cultures results in a short-term increase in NF-κB (85, 86). Whether or not this increase in NF-κB promoted by TNF is associated with increased proteolysis and/or increased apoptosis in skeletal muscle remains to be established. In relation with AP-1 activation, TNF has been shown to increase c-jun expression in C2C12 cells (87). Interestingly, overexpression of c-jun mimics the observed effect of TNF upon differentiation; indeed, it results in decreased myoblast differentiation (88). Tumour mediators, PIF in particular, also seem to be able to increase NF-κB expression in cultured muscle cells, this possibly being linked with increased proteolysis (89). Other transcriptional factors that have been reported to be involved in muscle changes associated with catabolic conditions include c/EBPβ and δ (which are increased in skeletal muscle during sepsis (90)), PW-1 and PGC-1. TNF decreases MyoD content in cultured myoblasts (91) and blocks differentiation by a mechanism which seems to be independent of NF-κB and which involves PW-1, a transcriptional factor related to p53-induced apoptosis (92). The action of the cytokines on muscle cells, therefore, seems to rely most likely on satellite cells blocking muscle differentiation or, in other words, regeneration.

In addition, the transcription factor PGC-1 has been associated with the activation of UCP-2 and UCP-3 and increased oxygen consumption by cytokines in cultured myotubes (93). This transcriptional factor is involved as an activator of PPAR-γ in the expression of uncoupling proteins.

Finally, concerning therapeutical strategies based on the events related to trancriptional factors in muscle wasting, several points can be raised. First, Kawamura *et al.* (94, 95) reported that the use of an oligonucleotide that competes with NF-κB-binding site can revert cachexia in a mouse experimental model without affecting the growth of primary tumour. This treatment, however, reduces the metastatic capacity in colon-26 adenocarcinoma model. In spite of this, administration of curcumine to tumour-bearing rats was unable to block muscle wasting, therefore suggesting the non-involvement of NF-κB in the cachectic response in this tumour model (84). As we have previously said, AP-1 is involved in muscle wasting during sepsis (83) and also in cancer (unpublished data). Interestingly, administration of an inhibitor of both (NF-κB and AP-1) results in a partial blockade of muscle wasting in rats bearing the AH-130 Yoshida ascites hepatoma, a highly cachectic rat tumour (unpublished data).

9. THE PHARMACOLOGICAL TREATMENT OF CACHEXIA

In clinical terms, anorexia means decreased appetite resulting in decreased food intake, fatigue, changes in body image and weight loss. In addition to anorexia, cachexia includes asthenia, anemia and loss of fat tissue and skeletal muscle, associated with abnormalities in protein, lipid and carbohydrate metabolism (see ref. 4 for review).

Cytokines, neuroendocrine changes and tumour mediators are involved in appetite depression in cachexia. Additional factors contributing to the anorectic state are altered taste perception, therapy-induced side effects, depressed motor activity, possible mechanical interference on the gastrointestinal tract and, of course, psychological factors. Indeed, patients with cachexia often experience psychological distress as a result of the uncertainties of the disease, its diagnosis, its treatment, its anticipated and final outcome. This psychological state, which often involves depression, is bound to affect food intake.

Although anorexia represents a very important factor in the development of cachexia, it has to be pointed out that in many cases the use of total parenteral nutrition does not stop the loss of body weight (96). It seems, therefore, quite evident that metabolic disturbances present in the host (increased energy inefficiency, insulin resistance and abnormal carbohydrate metabolism, adipose tissue dissolution and hypertriglyceridemia, and muscle wasting) have a definitive role in the development of cachexia (4). Anorexia seems to be the effect rather than the cause of weight loss during catabolic states as has been indicated (4); cachexia perpetuates and worsens itself through a mechanism involving anorexia, in a sort of positive feed-back mechanism which usually leads to death.

Bearing in mind the fact that both anorexia and metabolic disturbances are involved, the development of different therapeutic strategies has focused on these two factors. Several pharmacological and nutritional approaches have been used (Figure 7).

9.1. Increasing food intake

9.1.1. Pharmacologically

9.1.1.1. Progesterone derivatives

Megestrol acetate (MA) and medroxyprogesterone (MPA) are synthetic, orally active derivatives of the naturally occurring hormone progesterone. In humans these compounds have been found to improve appetite, caloric intake and nutritional status in several clinical trials (97-99). In the case of MA, the reason for the associated weight gain is mostly unknown, although it has been postulated that the effect is partially mediated by neuropeptide Y, a potent central appetite stimulant (100). On the other hand, MPA has been shown to reduce the *in vitro* production of serotonin and cytokines (IL-1, IL-6 and TNF) by peripheral blood mononuclear cells of cancer patients (101). All of these humoural factors have been implicated in the cachectic-anorexic response. The human trials involving progesterone derivatives have on the whole demonstrated, with few exceptions, a favourable effect on appetite and weight gain (which is no doubt of positive value for the body image); however, this has been mainly attributed to an increased fat mass, not to muscle mass (102, 103). Weight gain is also due to edema and,

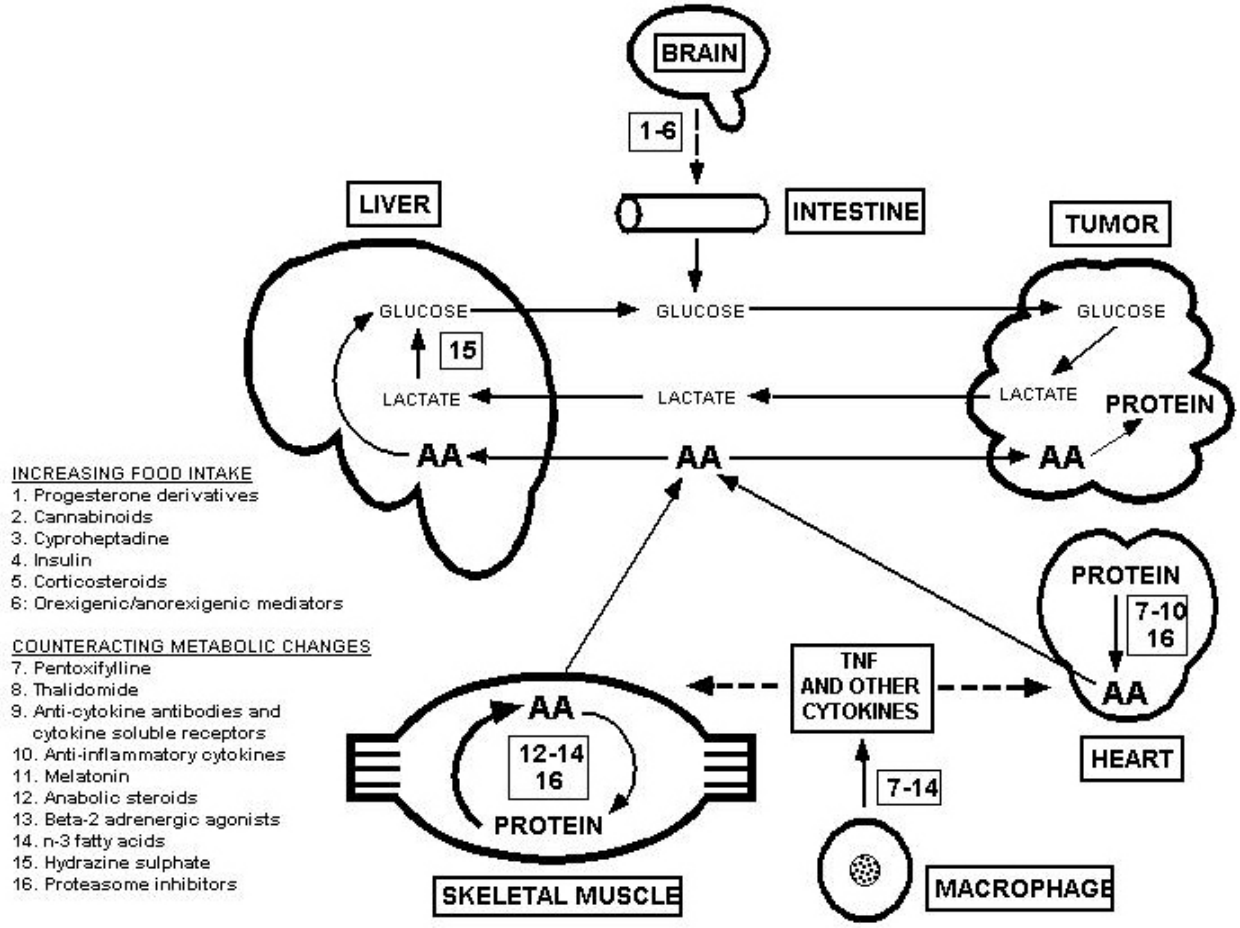

Figure 7. Metabolic targets of the different drugs employed for the treatment of cachexia. Therapeutical approaches to fight cachexia are based on either counteracting anorexia, by means of increasing food intake, or neutralizing metabolic abnormalities associated with the cachectic state. Among the optimal targets, blocking protein degradation in skeletal muscle is a promising strategy that will depend on the discovery of specific inhibitors of the proteasome system in skeletal muscle.

therefore, no significant effects are reported using the Karnovsky index – a performance score method used to describe how well a patient can go about their daily activities (104). If anorexia, nausea and a negative body image are major concerns, and if the patient has a life expectancy of more than three months, progesterone derivatives are a reasonable treatment option. On the other hand, if the central problem is fatigue and a low Karnovsky index, these drugs are not likely to be of significant help. In addition, in experimental animals MA treatment results simply in a gain of body water rather than a real increase in lean body mass; this weight gain is also associated with the doubling of the tumour mass (105).

9.1.1.2. Cannabinoids

Cannabinoids, which are present in marijuana and its derivatives, have a definitive effect on weight gain and, bearing this in mind, have been used to increase food intake in cancer patients. Plasse *et al.* (106) carried out a clinical study using one of these compounds, dronabinol (Δ9-tetrahydrocannabinol), to see if appetite enhancement could be disassociated from mood effects. Although the patients continued to lose weight following treatment, the rate of weight loss decreased at all the doses tested, symptomatic improvement being noted in both mood and appetite (107). The mechanism by which cannabinoids exert their effects has yet to be clarified. It was postulated that they may act via endorphin receptors or by inhibiting prostaglandin synthesis (107). Other reports suggest that the marijuana derivative may act by inhibiting cytokine production/secretion (108-110).

In different clinical trials, although the drug was originally used as an antiemetic in cancer patients suffering chemotherapy-induced nausea (111), it has been shown that almost 20% of the patients receiving dronabinol experienced side effects (euphoria, dizziness, somnolence and confusion), resulting in a dose reduction or less frequently, withdrawal of the treatment.

9.1.1.3. Cyproheptadine

Considerable evidence, both in humans and experimental animals, suggests that anorexia may be mediated by an increased serotonergic activity in the brain

(112, 113). Taking this into consideration, attempts to block serotonin activity during cancer cachexia have involved the use of cyproheptadine, a serotonin antagonist with antihistaminic properties, usually used for the treatment of allergies. Although initial clinical data suggested that it had appetite- and weight-enhancing effects in both patients without cancer and patients with cancer-related cachexia, it did not prevent progressive weight loss in patients with advanced malignant disease (114). Future clinical trials with other antiserotonergic drugs are necessary to define the role of the serotonergic system in the development of cancer cachexia.

9.1.1.4. Insulin

Some of the main alterations associated with tumour burden include glucose intolerance, increased gluconeogenesis and Cori cycle activity (liver recycling of tumour-generated lactate), and accelerated lipolysis and protein catabolism. These metabolic changes are accompanied by insulin resistance and a blunted insulin secretory response to hyperglycaemia (see ref. 4 for review). Taking this into consideration, in the tumour-bearing host cachexia may be overcome by the use of exogenous insulin and, indeed, in animal studies insulin administration has improved the degree of food intake and muscle wasting (115, 116). Body compositional analysis also indicated significant host preservation of nitrogen, fat and potassium (117). However, care should be taken in the use of insulin since the body weight gain observed was associated with an increase in tumour weight (118).

9.1.1.5. Corticosteroids

This group of hormones was one of the first pharmacological approaches for the treatment of cancer anorexia. Indeed, they have been used to increase food intake in cancer patients, and a number of uncontrolled studies show that some of the symptoms in cancer patients (such as anorexia and asthenia) can be partially mitigated by corticosteroid treatment, giving the patient an increased sensation of well-being. Both dexamethasone and prednisolone have been used in different trials, and seem to act as a result of their euphoriant activity or through inhibition of prostaglandin metabolism (119). Although corticosteroid treatment has been associated with low toxicity in several trials, prolonged treatment seems to lead to weakness, delirium, osteoporosis, and immunosuppression, all of which are commonly present in advanced cancer patients (120).

Although their anti-inflammatory action of corticosteroids is well recognized, most likely through the inhibition of TNF release (121), they also have been involved in TNF-induced muscle proteolysis characteristic of cachexia (122). In addition, it has been postulated that these hormones may also be responsible for muscle wasting during sepsis (123). However, studies involving the treatment with RU486 (a glucocorticoid antagonist) of experimental animals bearing cachectic tumours, suggest that glucocorticoids are not involved in skeletal muscle wasting associated with cancer cachexia (124).

Although costicosteroids seem to improve the quality of life of terminal cancer patients and can be used as palliative therapy, unfortunately corticosteroid treatment does not seem to have any significant effects in the reduction of mortality (119).

9.1.1.6. Orexigenic and anorexigenic mediators

The network of mediators involved in the control of food intake in pathophysiological states has proved to be extremely complex. Several studies have dealt with the role of leptin in cancer-induced anorexia. López-Soriano *et al.* (38) have described lower circulating levels together with decreased adipose tissue mRNA content for leptin in cachectic tumour-bearing animals. In the same way, both in advanced lung (125) and colon cancer (126) patients, serum leptin levels are also reduced, suggesting that cancer anorexia and cachexia are not solely due to a dysregulation of leptin production. However, elevated circulating leptin levels have been described in patients with advanced chronic heart failure (127), indicating a possible participation for leptin in the catabolic state leading to the development of cardiac cachexia.

The orexigenic mediator ghrelin (a novel endogenous ligand for the GH secretagogue receptor) has recently been reported as having a key role in increasing appetite and, therefore, food intake. Interestingly the circulating levels of ghrelin have been reported to be increased in patients with chronic heart failure and muscle wasting (128) and in patients with cancer cachexia (129). Again, although this may seem contradictory, it could represent a compensatory mechanism during negative energy balance (129, 130), as a similar situation is found during starvation. In fact, treatment of both tumour-cachectic bearing animals and rats affected by heart failure results in an improvement of muscle wasting (131-133). In conclusion, ghrelin could be a good therapeutic strategy for the treatment of several types of cachexia in the future, but additional studies are required to understand the role of ghrelin in the extremely complicated network of signals that control food intake.

9.1.2. Nutritionally

9.1.2.1. Enteral and total parenteral nutrition

Enteral nutrition is a reasonable option as an alternative to the oral route in patients with a functional bowel. From this point of view, it can be useful in some patients with advanced head and neck tumours or oesophageal carcinoma who are not able to swallow properly but still have an appetite and a good performance status (119).

Total parenteral nutrition has been extensively used in malnourished cancer patients who were unable to receive either oral or enteral nutrition. Its clinical use, however, has been subject to conflicting results. While some studies have reported beneficial effects from this type of nutritional therapy (including wound healing, reduction in sepsis and increased responsiveness to chemotherapy) (134, 135), others, after evaluating the influence of total parenteral nutrition upon treatment sequelae, have concluded that these benefits are limited to improvements of operative mortality and major surgical complications (136).

Concerning the potential differences between the two types of nutritional support, it has to be pointed out that enteral feeding is above all more economical, and randomized trials with patients undergoing major surgery indicate that they lead to similar results concerning nitrogen balance, plasma proteins, body cell mass or body weight (96).

Altogether, the nutritional strategies represent "tools" for the treatment of anorexia and cachexia, and in combination with a pharmacological approach can lead to interesting and promising results in the near future.

9.2. Counteracting metabolic alterations

As previously mentioned, the strategy of counteracting anorexia, either pharmacologically or nutritionally, has led to rather disappointing results in the treatment of cancer cachexia. It is basically for this reason that the strategies mentioned below rely on neutralizing the metabolic changes induced by the tumour, which are, ultimately, responsible for the weight loss.

9.2.1. Cytokine-mediated

The presence of the tumour results in important changes in hormone plasma concentrations and also hormonal responsiveness by various tissues. Specifically, still unidentified invasive stimuli from tumours generate an important release of cytokines by cells of the immune system. These cytokines act on multiple target sites such as bone marrow, myocytes, hepatocytes, adipocytes, endothelial cells and neurons, where they produce a complex cascade of biological responses leading to the wasting associated with cancer cachexia. The cytokines that have been implicated in this cachectic response are TNF, IL-1, IL-6 and IFN-γ. Interestingly, these cytokines share the same metabolic effects and their activities are closely interrelated. In many cases these cytokines exhibit synergic effects when administered together (137). Therefore, therapeutic strategies have been based on either blocking their synthesis or their action (138).

9.2.1.1. Blocking synthesis

9.2.1.1.1. Pentoxifylline

Pentoxifylline, a methylxanthine derivative, is a phosphodiesterase inhibitor that inhibits TNF synthesis by decreasing gene transcription. This drug was originally used for the treatment of various types of vascular insufficiency because of its haemorheological activity, thought to be based on its ability to reduce blood viscosity and increase the filterability of blood cells. While several studies using animal models suggest that pentoxifylline is able to decrease the cytokine-induced toxicity of antineoplasic agents while preserving antitumour treatment efficacy (139), clinical studies have shown that the drug failed to improve appetite or to increase the weight of cachectic patients (140).

9.2.1.1.2. Thalidomide

Thalidomide (α-N-phthalimidoglutaramide) is a drug unfortunately associated with tragedy. Indeed, its use as a sedative in pregnant women caused over 10,000 cases of severe malformations in newborn children. However, a certain revival has affected the drug since it has been demonstrated to suppress TNF production in monocytes *in vitro* (141) and to normalize elevated TNF levels *in vivo* (142). Apparently thalidomide activity is due to a selective destabilization of the TNF mRNA (143). The drug has certainly been used to counteract high cytokine levels in tuberculosis patients (144). A significant improvement in quality of life and weight gain occurs in AIDS patients given relatively low doses of thalidomide (145). Its use in cancer cachexia remains to be established, but it could potentially have a role in counteracting TNF-mediated metabolic changes. In addition, thalidomide therapy has been shown to improve insomnia and restlessness as well as nausea in advanced cancer patients, and it has improved appetite as well, resulting in an enhanced feeling of well-being in one-half to two-thirds of patients studied (146). Indeed, a recent pilot study carried out in 10 patients affected by oesophageal cancer revealed that thalidomide was able to reverse the loss of weight and lean body mass over a 2-week trial period (147).

9.2.1.2. Blocking action

9.2.1.2.1. Antibodies and soluble receptors

The use of anti-cytokine antibodies (either mono or polyclonal) and cytokine receptor antagonists or soluble receptors has led to very interesting results. Thus, in rats bearing the Yoshida AH-130 ascites hepatoma (a highly cachectic tumour), anti-TNF therapy resulted in a partial reversal of the abnormalities associated with both lipid (148) and protein metabolism (149). In humans, however, clinical trials using anti-TNF treatment have led to poor results in reversing the protein catabolism associated with sepsis (150). Furthermore clinical trials involving anti-TNF strategies, such as etanercept (fusion protein directing against p75 TNF receptor) or infliximab (monoclonal antibody against TNF) in chronic heart failure have led to a poor clinical outcome (151).

Concerning IL-6, experimental models have proven that the use of antibodies is highly effective in preventing tumour-induced waste (152). Strassman *et al.* (43) have demonstrated that the experimental drug suramine (which prevents the binding of IL-6 to its cell surface receptor, as demonstrated by radioreceptor-binding assay and affinity binding experiments) partially blocks (up to 60%) the catabolic effects associated with the growth of the colon-26 adenocarcinoma in mice. Concerning other cytokines, anti-IFN-γ therapy has also been efffective in reverting cachexia in mice bearing the Lewis lung carcinoma (46) but there is a lack of clinical data. On the other hand, blocking IL-1 actions by means of the IL-1 receptor antagonist (IL-1ra) in tumour-bearing rats had no effect on either body weight or reversal of metabolic changes (153).

It has to be pointed out here that the routine use of anti-cytokine antibodies is, at present, too expensive due to the fact that this type of therapy requires a very large number of antibody molecules in order to block cytokine action completely.

9.2.1.2.2. Anti-inflammatory cytokines

The degree of the cachectic syndrome is dependent not only on the production of the above-

mentioned cytokines, known as catabolic pro-inflammatory cytokines, but also on the so-called anti-inflammatory cytokines, such as interleukins-4, -10 and -12 (IL-4, IL-10, IL-12).

Mori *et al.* (154) have demonstrated that the administration of IL-12 to mice bearing the colon-26 carcinoma alleviates the body weight loss and other abnormalities associated with cachexia, such as adipose tissue wasting and hypoglycaemia. The anticachectic properties are seen at low doses of IL-12, insufficient to inhibit tumour growth. The effects of IL-12 seem to be dependent on an important decrease of IL-6 (154), a cytokine which has been responsible for the cachexia associated with this tumour model (155, 156). A similar action has been described for IFN-γ. Administration of this cytokine promoted a decrease in both IL-6 mRNA expression in the tumour and serum IL-6 levels, resulting in an amelioration of the cachexia in a murine model of malignant mesothelioma (157).

Interleukin-15 (IL-15) has been reported to be an anabolic factor for skeletal muscle (158) and very recent experiments carried out in our laboratory clearly demonstrate that the cytokine is able to reverse most of the abnormalities associated with cancer cachexia in a rat tumour model (159).

9.2.2. Hormone-mediated

9.2.2.1. Growth hormone

Administration of growth hormone (GH) results in an increase in whole body and skeletal muscle protein synthesis (160-162). Studies with experimental animals have shown that administration of recombinant rat GH to methylcholanthrene-induced sarcoma-bearing rats resulted in considerable stimulation of protein synthesis without changing tumour growth, protein degradation or host composition. Conversely, Wolf *et al.* (163) have reported improvements in whole body protein balance in cancer patients receiving GH. Very interestingly, the same research group (164) has demonstrated that exogenous GH can attenuate weight loss and preserve host body composition in tumour-bearing rats undergoing chemotherapy with doxorubicin without stimulating tumour growth.

9.2.2.2. Insulin-like growth factor-I

Insulin-like growth factor-I (IGF-I), also know as somatomedin-c, mediates many of the anabolic properties of GH (165) and appears to be involved in the regulation of protein turnover (166). Other studies have shown an important role in muscle cell proliferation and differentiation (167). Interestingly, during catabolic states (such as sepsis) antagonism of IGF-I bioactivity has been reported (168). In the particular case of cardiac cachexia, decreased circulation IGF-I levels have been reported in wasting congestive heart failure patients (169). Since IGF-I stimulates amino acid uptake and protein synthesis (165), it is potentially a good candidate to counteract the changes that take place in skeletal muscle during cancer cachexia. In addition, IGF-I also decreases lipolysis (165), a metabolic pathway that is activated in adipose tissue during cancer cachexia. Ng *et al.* (170) have shown, using a methylcholanthrene-induced rat sarcoma, that IGF-I treatment by continuous subcutaneous administration resulted in host preservation of lean tissue, attenuating muscle protein loss. Interestingly, the treatment did not stimulate tumour growth (as previously mentioned, IGF-I has mitogenic properties on certain cell types) but, conversely, the proportion of aneuploid cells and S-phase fraction in tumours was reduced, implying additional beneficial effects of the IGF-I treatment (170). In spite of the complexity of the IGF-1 family (which includes IGF-I-binding proteins that counteract the action of the growth factor), it seems that overexpression of IGF-I leads to a phenotype which is characterized by both increased muscle mass and increased muscle regeneration capacity. Bearing this in mind, gene therapy approaches involving local IGF-I increases in cachectic skeletal muscle may prove advantageous and cardiotrophic effects of growth factors would be avoided. Future research in this area is, therefore, promising (171).

9.2.2.3. Melatonin

Recent studies have shown the existence of reciprocal links between cytokine activity and immunomodulating neurohormones or neuropeptides. In particular, the pineal hormone melatonin appears to influence cytokine activity during tumour growth. Lissoni *et al.* (172) evaluated the effects of melatonin therapy on patients with solid metastatic tumours, demonstrating that there is indeed an inhibitory action of melatonin on TNF release, since the treatment resulted in a considerable decrease in the circulating levels of this cytokine.

The same research group investigated the relationship between melatonin, TNF and cancer-related weight loss in a group of 100 untreatable metastatic solid tumour patients (173), and showed that the melatonin-treated group were subjected to a lower weight loss as compared with the placebo-treated group, therefore concluding that the pineal hormone may be effective in the treatment of neoplastic cachexia by decreasing TNF blood concentrations.

Another clinical study (174) clearly shows that the concomitant administration of melatonin and cisplatin plus etoposide may improve the efficacy of chemotherapy in terms of survival time. Additionally, melatonin treatment seems to reduce the chemotherapeutic toxicity in patients in poor clinical condition (174).

9.2.2.4. Somatostatin

Cancer cachexia is associated with a decreased insulin/glucagon ratio. Some studies have postulated that the decrease in this hormone index is largely responsible for the progressive catabolism characteristic of cancer cachexia. Taking this into consideration, Bartlett *et al.* (175) studied the effects of somatostatin together with insulin and GH as a combined therapy with the aim of increasing the insulin/glucagon ratio. Animals inoculated subcutaneously with the MAC-33 (a spontaneously metastasizing mammary adenocarcinoma), which received the combined therapy, showed increased host carcass

weight, together with increased hamstring muscle weight and protein content, as compared with tumour-bearing animals that received no treatment (175). The advantage of the inclusion of somatostatin in the treatment relies on the fact that insulin treatment alone leads to limited success in treating cancer cachexia due to insulin-induced hypoglycaemia and subsequent glucagon secretion. Thus, administration of somatostatin leads to a decrease in the levels of glucagon. It is therefore becoming increasingly evident that a single pharmacological strategy is unlikely to reverse cachexia completely, and that future attempts to revert the progressive catabolism present in the tumour-bearing host will involve combinations of several drugs.

9.2.3. Others

9.2.3.1. Anabolic steroids

Although the treatment with derivatives of gonadal steroids has important side effects such as masculinization, fluid retention and hepatic toxicity, they promote nitrogen protein accumulation, and from this point of view they could be used to counteract the progressive nitrogen loss associated with cancer cachexia. Nandrolone decanoate treatment resulted in a decrease in weight loss in patients affected by non-small cell lung carcinoma (176).

9.2.3.2. β_2-adrenergic agonists

These molecules are potentially very interesting since they have important effects on protein metabolism in skeletal muscle, favouring protein deposition. We have described how β_2-agonists, such as clenbuterol and formoterol, suppress the activation of muscle proteolysis (through their action on the ubiquitin-dependent proteolytic system) during tumour growth (177-179). In a similar manner, β_2-agonists are also able to suppress the increase in branched-chain amino acid oxidation that takes place in skeletal muscle during cancer cachexia (180). Investigations are being carried out in our laboratory to evaluate usefulness of these compounds in ameliorating cancer cachexia in different experimental models.

9.2.3.3. n-3 fatty acids

n-3-Polyunsaturated fatty acids (PUFA), present in large amounts in fish oil, have been proposed as very active in reducing either tumour growth (181, 183) or the associated tissue wasting, particularly that of the adipose mass (183). In fact, the interest in n-3-PUFA originated from the observation that populations consuming a diet rich in such constituents showed the lowest incidence of certain types of cancer. Tisdale and Beck have shown that administration of eicosapentaenoic acid (EPA) to mice bearing a murine colon adenocarcinoma (MAC-16) resulted in a reversal of tumour-induced cachexia without changes in food intake (184). In this study, the effect of EPA concerned not only the adipose tissue (where it counteracted the action of a lipid mobilizing factor) but also in skeletal muscle, where it decreased the rate of protein degradation. Conversely, using a cachectic rat tumour model, we have not been able to see any effects of EPA treatment on cachexia improvement (185). Wigmore *et al.* (186) conducted human studies treating a group of pancreatic cancer patients with fish oil capsules (containing 18% EPA and 12% docosahexaenoic acid) for three months, and observed an increase in body weight, a reduction of APP (C-reactive protein) production, and a stabilisation of the resting energy expenditure. Therefore, future studies are required to ascertain the molecular mechanism(s) involved in the anticachectic action of these compounds.

9.2.3.4. Amino acids and nucleotides

Peripheral muscle proteolysis, as occurs in cancer cachexia, serves to mobilize amino acids required for the synthesis of liver and tumour protein (see ref. 4 for review). Therefore, the administration of exogenous amino acids may theoretically serve as a protein-sparing metabolic fuel by providing substrates for both muscle metabolism and gluconeogenesis. Based on this, branched-chain amino acids (BCAA: leucine, isoleucine and valine) have been used in parenteral nutrition with the aim of improving nitrogen balance and, particularly, muscle protein metabolism. Tayek *et al.*. (187), in a prospective, randomized, crossover trial involving patients with advanced intra-abdominal adenocarcinoma, concluded that BCAA-enriched total parenteral nutrition resulted in an improved protein accretion and albumin synthesis. Similarly, studies with tumour-bearing animals show that high BCAA concentrations in total parenteral nutrition have beneficial effects on host protein metabolism (188). Cangiano *et al.* (189) have proposed that BCAA administration would also serve to counteract the anorexia associated with tumour growth. The authors postulated that increased hypothalamic serotonergic activity is one of the pathogenic mechanisms leading to the development of cancer anorexia. In fact, free tryptophan (the precursor of brain serotonin) is increased during cancer (19, 190), and BCAA may act by competing for the same transport system as tryptophan across the blood-brain barrier (191). This hypothesis has been tested in anorectic cancer patients receiving an oral supplementation of BCAA with encouraging results since the treatment decreased the severity of the anorexia in the treated patients (189).

Glutamine-enriched solutions have also been used in total parenteral nutrition with the aim of enhancing immunoregulation of tumour growth (192), and compensating for the uptake of the amino acid by the tumour. Indeed, tumour cells are major glutamine consumers (for both protein synthesis and oxidation) (193), and therefore lead to host glutamine depletion (194), which results in a decreased host immune response and gastrointestinal mucosal integrity (192). In patients undergoing bone marrow transplantation for haematological malignancies, glutamine supplementation was found to be beneficial, improving nitrogen balance and diminishing the incidence of clinical infection as compared to the standard parenteral nutrition therapy (195). It could be argued that glutamine supplementation may facilitate tumour growth since it is one of the preferred substrates for fast-growing tumours. However, evidence obtained in experimental models demonstrates that glutamine supplementation improves the tumouricidal effectiveness of methotrexate while reducing its toxicity (196). As Laviano *et al.* (197) suggest, this could be due to the glutamine-

induced increased number of tumour cells in S-phase, a cell cycle phase during which they are more susceptible to chemotherapy.

Increasing evidence suggests that an abnormal cysteine and glutathione metabolism plays a decisive role in the development of catabolic conditions and associated immunological dysfunctions (198). Indeed, the increased glycolytic activity and lactate production that takes place during cancer (199) causes an acidification of the muscle cells which may result in a decreased transport of glutamate and therefore in an impaired glutathione metabolism (198). The acidification is aggravated by the fact that the temporary increase of intracellular pyruvate causes an increased rate of cysteine/pyruvate transaminase and, consequently, an increased cysteine degradation into sulphate and protons (198). Increased intracellular sulphate levels have indeed been found in skeletal muscle of tumour-bearing mice (200). This was associated with a decrease of the glutathione level in the skeletal muscle tissue, indicating that the cysteine catabolism was increased at the expense of glutathione biosynthesis at this time (198). Bearing all this in mind, Dröge *et al.* (198) suggest that N-acetylcysteine can be used to increase the availability of cysteine in the treatment of catabolic states such as cancer cachexia. In fact, preliminary studies support a positive role for this amino acid derivative in HIV-infected patients.

Concerning nucleotides, animal studies have shown that their use as supplements in parenteral nutrition improves immune response, natural-killer cell activity and resistance to infections (201). It is for these reasons that they deserve to be tested in tumour models since they could well have a positive effect on the treatment of cancer patients receiving chemotherapy.

9.2.3.5. Hydrazine sulphate

Among the compounds that counteract metabolic changes, it is particularly interesting to take into consideration hydrazine sulphate, an inhibitor of gluconeogenesis from lactate and amino acids. Gold (202) introduced its use as an anticancer treatment based on his theory that the abnormalities of carbohydrate metabolism (including increased gluconeogenesis and enhanced Cori cycle activity) are the central causes of tumour-induced cachexia. Based on Gold's hypothesis, a study involving small-cell lung carcinoma indicates that hydrazine treatment can improve parameters of carbohydrate metabolism and prolong survival (203). Similarly, Tayek *et al.* (204), after performing a double-blind trial with malnourished patients of lung cancer, concluded that the administration of the drug reduced amino acid flux, favourably influencing the metabolic abnormalities of cachexia. Further clinical trials are being performed in order to see the utility of this low toxicity drug against cancer cachexia.

9.2.3.6. Prostaglandin and NO inhibitors

The effects of prostaglandins on cell growth have been studied both *in vitro* (205) and *in vivo* (206, 207), and it has been proposed that cell growth may be controlled by the interconversion of different types of prostaglandins. In fact, large amounts of these compounds are found both in tumour tissue and plasma from cancer patients (208). Taking all this into consideration, several studies have examined the role of cyclooxigenase inhibitors on tumour growth and cachexia. The results obtained are clearly contradictory. While Homem-De-Bittencourt *et al.* (209) report that indomethacin, ibuprofen and aspirin markedly inhibit tumour growth and reduce anorexia in rats bearing the Walker-256 carcinosarcoma, McCarthy and Daun (210) (using the same rat tumour model) also report a decrease in tumour weight but this was not associated with a reduction of anorexia or body weight loss. Hussey and Tisdale (211) have recently studied the effects of the COX-2 inhibitor meloxican on tumour growth and cachexia in the murine adenocarcinoma MAC16. The results suggest that the inhibitor is able to effectively attenuate cachexia, possibly by exercising a direct effect on skeletal muscle protein degradation.

Inhibition of nitric oxide production by specific blockade of the syntase (NOS) resulted in a a decreased muscle wasting in a model of cachexia. Interestingly, the decrease in body weight, the muscle wasting and skeletal muscle molecular abnormalities were prevented by the use of both N-nitro-L-arginine (a NOS inhibitor) and antioxidants (212). Therefore, further studies with other tumour models are needed before any serious conclusions can be drawn about the beneficial effects of prostaglandin inhibitors on cancer cachexia.

9.2.3.7. ACE inhibitors

In chronic heart failure, inhibition of the angiotensin converting enzime (ACE) by administration of enalapril reduces the risk of weight loss and it is linked to improved survival (213). Preliminary results demonstrate increased subcutaneous fat (increased skin fold thickness), greater muscle bulk (increased mid-upper arm and tight circumferences) together with a significant elevation in plasma albumin and the hematocrit (214). In fact, these ACE inhibitors, like captopril, seem to act by decreasing the production of TNF in mononuclear cells, suggesting a mechanism to account for the beneficial effects (related to body weight) observed in cardiac cachexia (215).

9.2.3.8. Antianemic drugs

Both erythropoietin and epoetin-α have been used to counteract the anemia observed in cachectic cancer patients. Clinical trials have shown that epoetin-α in combination with indomethacin results in an hematocrit improvement together with increased body weight and hepatic inflammatory response (216-218).

9.2.3.9. ATP and creatine

Weight maintenance is a balance of energy supplements. In fact during catabolic conditions, illness often increases energy demands. It is for this reason that administration of ATP, a directly hydrolizable source of energy, could potentially tip the balance towards weight gain and preservation of lean body mass. Several clinical data support this observation (219-221).

Using a similar principle, perhaps more directly linked with skeletal muscle, creatine administration may result in an increase in skeletal muscle phosphocreatine content which may, in turn, protect the tissue during catabolic conditions.

9.2.3.10. 5'deoxy-5-fluorouridine

5'deoxy-5-fluorouridine (5'-dFUrd) is a cytostatic agent that is converted upon metabolization on the active metabolite 5-fluorouracil (5-FUra) by uridine and thymidine phosphorylases, which are very active in tumour (222, 223). Tanaka *et al.* (224) have shown that treatment of mice bearing the colon-26 adenocarcinoma with 5'-dFUrd results in an improvement of the metabolic abnormalities induced by this tumour: hypoglycaemia, hormone imbalance, hepatic malfunction and elevation of acute-phase reactants (224). The same authors concluded that the anticachectic action of the drug is independent of its antiproliferative action (225), making it a candidate for the treatment of cancer cachexia in addition to its chemotherapeutic potential.

9.2.3.11. Proteasome inhibitors

As previously stated, enhanced protein degradation in skeletal muscle during cachexia involves activation of the ubiquitin/proteasome system in muscle. Therefore, inhibitors of this proteolytic system such as peptide aldehyde, lactacystin and β-lactone (which effectively can block up to 90% of degradation of normal proteins and short-lived proteins in the cells) are potential drugs for the treatment of muscle wasting (226). However, the toxicity of such compounds is fairly high since they are not specific inhibitors of the proteolytic system in muscle tissue (227). Bearing this in mind, a substance that can specifically block myofibrillar protein degradation in skeletal muscle is still waiting to be discovered.

10. TOWARDS THE IDEAL THERAPY: A MULTIDISCIPLINARY APPROACH

When trying to establish an effective anticachexia therapy, one should be aware of the different approaches which have been mentioned earlier in order to implement the best combination of treatments. It's quite clear that a single intervention may have limited efficiency; however, the combination of different approaches (avoiding, of course, high toxicity) will, no doubt, give the best results. From this point of view, the most effective therapy has to be both anabolic and anticatabolic. Unfortunately, nutrition alone has proved to be little efficienty in the treatment of cancer cachexia. Indeed, except for those patients whose intestinal function is affected, anorexia is not always present in the cachectic cancer patient. In any case, different studies report that total parenteral nutrition is not enough to stop the wasting process in the cancer patient. In spite of this, the cancer patient needs a considerable energy and nitrogen intake to provide biosynthesis concomitant with the growth of the tumour; this is a clear anabolic component. However, therapeutic strategies have to be also anticatabolic, targeted at stopping proteolytic mechanisms involving muscle tissue that finally lead to wasting. From this point of view a very low toxicity approach involves the use of nutraceuticals such as PUFA and antioxidants that have been shown to interfere with the intracellular signals that trigger the ubiquitin-proteasome proteolytic system. Indeed, EPA in particular has already been introduced in different clinical trials for the treatment of cancer-cachectic patients, with reasonably good results for pancreatic cancer patients. Combination of EPA and antioxidant has also been analyzed in clinical (228). Unfortunately the efficacy of this anticatabolic approach is not ideal: therefore we suggest that optimal available treatment should also incorporate drugs which act anticatabolically. For instance, β-2 agonists have been tried with great success in different models of experimental cancer cachexia. COX-2 inhibitors such as ibuprofen and indometacin have been used for the treatment of cachectic-cancer-patients (see above).

We therefore conclude that the ideal therapy should involve a multivariant approach including nutrition as well as the use of nutraceutical and also anticatabolic drugs.

11. CONCLUSIONS AND FUTURE RESEARCH

Cancer cachexia is a complex pathological condition characterized by many metabolic changes involving numerous organs. These changes are triggered by alterations in the hormonal milieu, release of different tumour factors and a systemic inflammatory reaction characterized by cytokine production and release. In fact, the macrophage-derived pro-inflammatory cytokines (IL-1, IL-6, TNF) have key roles in inducing metabolic changes associated with 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-1 receptor antagonist (IL-1ra), interleukin-10 (IL-10) and TGF-β is pivotal for the fine tuning of many biochemical processes. For instance, in chronic myelogenous leukemia, high cellular (leukocyte) levels of IL-1 beta and low levels of IL-ra are seen in advanced disease and correlate with reduced survival (55).

A complex interaction of pro-cachectic and anti-cachectic cytokines or cytokine-neutralizing molecules probably determines the critical presentation and course of cachexia (Figure 8). 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, possible 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 with Th2 response, interleukin-13 (IL-13), also reduces TNF and increases survival against LPS challenge. It has been shown that while mice inoculated

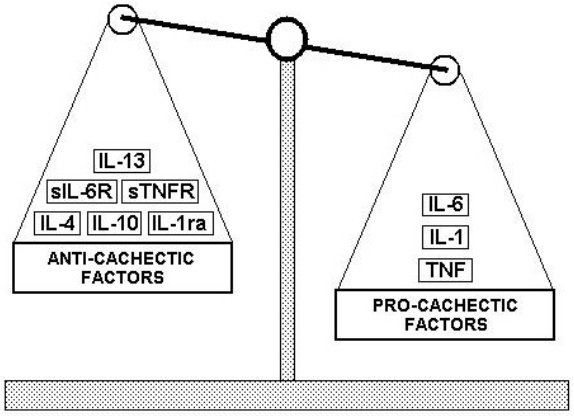

Figure 8. Cytokine balance and the cachectic syndrome. The balance between pro-inflammatory (pro-cachectic), their soluble receptors and the anti-inflammatory (anti-cachectic) cytokines plays a key role in the development of the cachectic syndrome.

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 tumour cells.

Tumour-derived factors have also been shown to represent important molecules involved in the process of cancer cachexia. Thus, both LMF and PIF can mimic the main metabolic events associated with fat and muscle protein depletion in experimental cancer cachexia. In addition, these factors have been found in the urine of cachectic cancer patients, therefore suggesting an important role in human cancer.

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 (BCAA) metabolism. However, TNF alone cannot explain all the cachectic metabolic alterations present in different types of human cancers and experimental tumours. Another important drawback is the fact that TNF circulating 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 tumour effect. Consequently, both tumour-produced and humoural factors must collaborate in the full induction of the cachectic state.

In conclusion, and because metabolic alterations often appear early after the onset of tumour growth, the scope of appropriate treatment, although not aimed at achieving immediate eradication of the tumour 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 either cancer or inflammation and whether humoural 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 humoural response to inflammation and modifying cytokine actions pharmacologically may prove very effective, and no doubt future research will concentrate on this interesting field.

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