DDT Vol. 8, No. 18 September 2003

Catabolic mediators as targets for cancer cachexia

Josep M. Argilés, Rodrigo Moore-Carrasco, Sílvia Busquets and Francisco J. López-Soriano

The cachexia syndrome, characterized by a marked weight loss, anorexia, asthenia and anaemia, is invariably associated with the growth of a tumour and leads to a malnutrition status caused by the induction of anorexia or decreased food intake. In addition, the competition for nutrients between the tumour and the host results in an accelerated catabolism state, which promotes severe metabolic disturbances in the patient. The search for the cachectic factor(s) started a long time ago, and many scientific and economic efforts have been devoted to its discovery, but we are still a long way from a complete answer. The present review aims to evaluate the different molecular mechanisms and catabolic mediators (both humoural and tumoural) that are involved in cancer cachexia and to discuss their potential as targets for future clinical investigations.

Josep M. Argilés
Rodrigo Moore-Carrasco
Sílvia Busquets
Francisco J. López-Soriano*
Cancer Research Group
Departament de Bioquímica i Biologia Molecular
Facultat de Biologia
Universitat de Barcelona
Diagonal 645
08028-Barcelona, Spain
*e-mail:
fsoriano@porthos.bio.ub.es

▼ The development of cancer cachexia is, perhaps, the most common manifestation of advanced malignant disease. Cachexia occurs in the majority of terminally ill cancer patients and is responsible for 22% of deaths from cancer [1]. The abnormalities associated with the condition include anorexia, weight loss, muscle loss and atrophy, anaemia and alterations in carbohydrate, lipid and protein metabolism (reviewed in [2,3]). The degree of cachexia is inversely correlated with the survival time of the patient and always implies a poor prognosis. The aim of the present review is to summarize and update the role of catabolic mediators (cytokines and tumour-derived factors) in cancer cachexia; research based on these compounds might be significant in future clinical investigations.

Cytokines

Cytokines have a key role as the main humoural factors involved in cancer cachexia, thus, many of them might be responsible for the metabolic changes associated with cancer wasting. Figure 1 provides a clear overview of the humoral factors involved in cancer cachexia.

Interleukin-1 and anorexia

Anorexia accounts for the malnutrition that is invariably associated with cancer cachexia, but are cytokines involved in the induction of anorexia? Cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF) are thought to be involved in cancer-related anorexia, possibly by increasing the levels of corticotrophin-releasing hormone, a CNS neurotransmitter that suppresses food intake and the function of glucose-sensitive neurons, which also decreases food intake. However, the involvement of many other mediators has been proposed in cancer-induced anorexia. Leptin – a member of the cytokine family and a proposed adiposity signal for the long-term regulation of body weight by the brain – does not seem to have a role, at least in experimental models [4,5]. In human subjects, cancer anorexia does not seem to be caused by a dysregulation of leptin production [6] and, indeed, leptin concentrations are not elevated in weight-losing cancer patients [7,8] and are inversely related to the intensity of the inflammatory response [9] and the levels of inflammatory cytokines [10,11]. However, leptin levels are unreliable if they are not adjusted to body fat mass; concentrations of the peptide seem to be dependent only on the total amount of adipose tissue present in the patient.

Cytokines could have a role in cancer-induced anorexia because they modulate gastric motility and emptying, either in the gastrointestinal system itself or via the brain, by altering efferent signals that regulate satiety. IL-1, in particular, has been clearly associated with the induction

 1359-6446/03/$ – see front matter PII: S1359-6446(03)02826-5

of anorexia [12], by blocking neuropeptide Y (NPY)-induced feeding. The levels of this molecule (a feeding-stimulating peptide) are reduced in anorectic tumour-bearing rats and a correlation between food intake and brain-IL-1 has been found in anorectic rats with cancer [13]. The mechanism involved in the attenuation of NPY activity by cytokines might be related to an inhibition of cell firing rates, an inhibition of NPY synthesis or an attenuation of its postsynaptic effects [14]. Other mediators have been proposed, including changes in the circulating levels of free tryptophan [15]; these could induce changes in brain serotonin concentrations and, consequently, cause changes in food intake. Bing *et al.* have also suggested that some tumour-derived compounds might mediate the anorexia associated with tumour burden [16].

Tumour necrosis factor-α

Various experimental approaches have demonstrated that cytokines are able to induce weight loss. Nevertheless, the results obtained have to be carefully interpreted. Episodic TNF administration has proved unsuccessful at inducing cachexia in experimental animals; repetitive TNF administrations initially induce a cachectic effect, but tolerance to the cytokine soon develops and food intake and body weight return to normal [17]. Other studies have shown that escalating doses of TNF are necessary to maintain the cachectic effects. However, the site of production and action of the cytokine can modify its metabolic effects, as demonstrated in an animal model [18].

Interleukin-6

Strassman and co-workers have shown that treatment with an anti-mouse interleukin-6 (IL-6) antibody was successful in reversing the key parameters of cachexia in colon adenocarcinoma tumour-bearing mice [19]. These results indicate that, at least in certain types of tumours, IL-6 could have a more direct involvement than TNF in the cachectic state. Similar results were obtained in a mouse model that reproduced the cachexia associated with multiple myeloma [20, 21] and in a murine model of intracerebral injection of human tumours [22]. Conversely, other studies, using a very similar mouse tumour model, have revealed that IL-6 is not involved in cachexia. Furthermore, studies using incubated rat skeletal muscle have clearly shown that IL-6 had no direct effect on muscle proteolysis.

Interferon-γ

Another interesting candidate for cachexia is interferon-γ (IFN-γ), which is produced by activated T and NK cells and displays biological activities that overlap with those of TNF. Using a monoclonal antibody against IFN-γ, Matthys *et al.* were able to reverse the wasting syndrome associated with

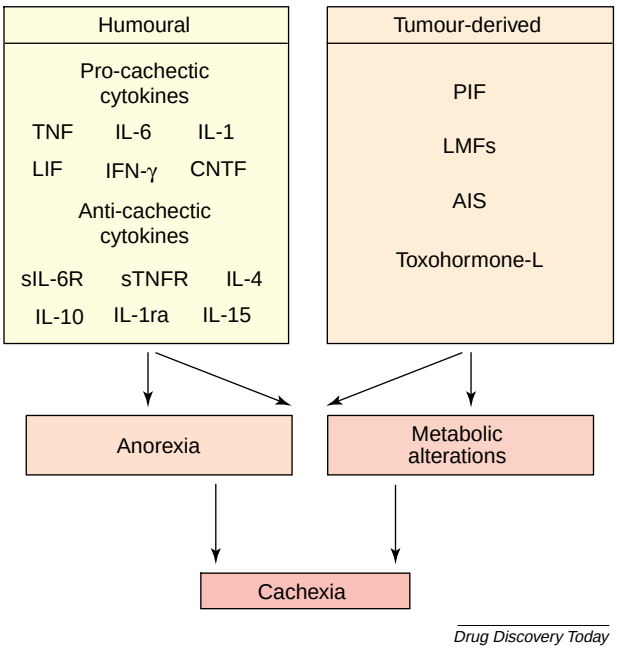

Figure 1. Catabolic mediators in cancer. Both tumour-derived and humoural factors (cytokines) are involved in mediating anorexia and metabolic changes that are characteristic of the cachectic state. Abbreviations: AIS, anaemia-inducing factor; CNTF, ciliary neurotrophic factor; IL, interleukin; LIF, leukaemia inhibitory factor; LMFs, lipid-metabolizing factors; PIF, proteolysis-inducing factor; TNF, tumour necrosis factor.

the growth of the Lewis lung carcinoma in mice [23], 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 that are 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.

Other cytokines

Other cytokines, such as leukaemia inhibitory factor (LIF), transforming growth factor-β or IL-1 have also been suggested as mediators of cachexia. Thus, mice engrafted with tumours secreting LIF develop severe cachexia. The anorectic and pyrogenic effects of IL-1 are well-known, but administration of IL-1 receptor-antagonist to tumour-bearing rats did not result in any improvement in the degree of cachexia, thus suggesting that the role of IL-1 in cancer cachexia is secondary to the actions of other mediators [24]. Interestingly, the levels of both IL-6 and LIF are raised in patients with different types of malignancies.

Ciliary neurotrophic factor (CNTF) is a member of the family of cytokines that includes IL-6 and LIF and is

produced predominantly by the glial cells of the peripheral nervous system; however, this cytokine also seems to be expressed in skeletal muscle. Henderson *et al.* [25] have demonstrated that CNTF induced potent cachectic effects and acute-phase proteins (independent of the induction of other cytokine family members) in mice that were implanted with C6 glioma cells, genetically modified to secrete this cytokine. However, the CNTF exerted divergent direct effects on *in vitro* muscle preparations, which were dependent on the dose and the time of exposure [26].

Cytokines and uncoupling proteins

Anorexia is not the only factor involved in cancer cachexia – it is clear that metabolic abnormalities leading to a hypermetabolic state must have an important role. Interestingly, low doses of TNF, injected peripherally or into the brain of laboratory animals, elicits rapid increases in metabolic rate that are not associated with increased metabolic activity but, rather, with an increase in blood flow and thermogenic activity – associated with uncoupling protein (UCP1) –of brown adipose tissue (BAT). During cachectic states, there is an increase in BAT thermogenesis, both in humans and experimental animals.

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 that is generated during respiration across the inner mitochondrial membrane, thereby, uncoupling respiration from ATP synthesis. However, two additional proteins sharing this same function, UCP2 and UCP3, have been described. While UCP2 is expressed ubiquitously, UCP3 is expressed specifically and abundantly in human and rodent skeletal muscle and also in rodent BAT. Both UCP2 and UCP3 mRNAs are elevated in skeletal muscle during tumour growth and TNF is able to mimic the increase in gene expression [27]. TNF is also able to induce uncoupling of mitochondrial respiration, as shown recently in isolated mitochondria [28].

Cytokines and metabolic abnormalities

Several cytokines have been shown to mimic many of the metabolic abnormalities found in the cancer patient during cachexia. Among these metabolic disturbances, changes in lipid metabolism, skeletal muscle proteolysis and apoptosis and acute-phase protein synthesis have been described – (reviewed in [29]). Administration of TNF to rats results in an increased skeletal muscle proteolysis that is associated with an increase in gene expression and higher levels of free and conjugated ubiquitin, both in experimental animals [30] and humans [31]. Also, the *in vivo* action of TNF during cancer cachexia does not seem to be mediated by IL-1 or glucocorticoids. 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 [32] seems to mediate most of the changes concerning nitrogen metabolism that are associated with cachectic states. In addition to the massive muscle-protein loss, muscle DNA is also decreased during cancer cachexia (similar to that observed in skeletal muscle of chronic heart failure in patients suffering from cardiac cachexia [33]) leading to DNA fragmentation and apoptosis [34,35]. Interestingly, TNF can mimic the apoptotic response in muscle of healthy animals [36].

Tumour-derived factors

Aside from humoural factors, tumour-derived molecules have also been proposed as mediators of cancer cachexia; tumour-produced cytokines might even have a more important role in the anorexia-cachexia syndrome than those produced by the host [37]. First, cancer cells are capable of producing cytokines constitutively. These cytokines might act on the cancer cells in an autocrine manner or on the supporting tissues, such as fibroblasts and blood vessels, to produce an environment that is conducive to cancer growth [38]. As well as tumour-produced cytokines, several compounds have been reported to have an important role in mimicking the metabolic changes associated with the cachectic state. Figure 1 gives an overview of these tumour-derived factors.

Perhaps the first evidence of tumour-derived catabolic factors came from studies of Krebs-2 carcinoma cells in mice; inactive extracts of these cells could induce cachexia when injected in normal non-tumour-bearing mice [39]. Similarly, Kitada *et al.* purified a low-molecular-weight proteinaceous material (<10 kD) 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 [40]. Toxohormone-L, a polypeptide of ~75 kD was isolated from the ascites fluid of patients with hepatoma and sarcoma-bearing mice [41] and was found to induce lipid mobilization, immunosuppression and involution of the thymus.

Michael Tisdale's group at the University of Aston, Uk (http://www.aston.ac.uk) have described and characterized a lipid-metabolizing factor (LMF) that is able to induce lipolysis in adipose tissue – this is associated with stimulation of adenylate cyclase activity [42]. This factor was originally purified from the cachexia-inducing mouse colon adenocarcinoma, MAC16, but has also been found in the urine of cancer patients, suggesting that it is able to induce lipid

mobilization and catabolism in cachectic cancer patients [43]. LMF is homologous to the plasma protease, Zn-α2-glycoprotein, in its amino acid sequence, electrophoretic mobility and immunoreactivity. The 2.8 Å crystal structure of Zn-α2-glycoprotein resembles a class I major histocompatibility complex (MHC) heavy chain, although it does not bind the class I light chain, β2-microglobulin. The Zn-α2-glycoprotein structure includes a large groove analogous to class I MHC peptide-binding grooves but instead of a peptide, the Zn-α2-glycoprotein groove contains a nonpeptidic compound that might be implicated in lipid metabolism under pathological conditions. Hirai and coworkers also suggest that LMF has a role in initiating hepatic glycogenolysis during experimental cancer cachexia through an increase in cyclic AMP in liver [44].

Anaemia-inducing factor (AIS) is a protein of ~50 kDa that is secreted by malignant tumour tissue, thus depressing erythrocyte and immunocompetent cell functions. This protein is able to reduce food intake, body weight and body fat in rabbits. In addition, it shows an important lipolytic activity [45].

Todorov *et al.* [46] have purified and characterized a 24 kDa proteoglycan, present both in experimental animals [47] and in the urine of cachectic patients [48] that seems to account for increased muscle-protein degradation and decreased protein synthesis [47]. This compound, known as PIF (proteolysis-inducing factor), is able to activate protein degradation specifically, although it also stimulates the ATP-proteasome-dependent pathway [49]. The compound, when injected into healthy animals, is able to mimic the muscle-wastage associated with experimental cancer cachexia. *In vitro* studies on C2C12 myoblasts have shown that eicosapentaenoic acid is able to block PIF action on proteolysis and have suggested that PIF acts intracellularly, via the araquidonate metabolite 15-hydroxyeicosatetraenoic acid (15-HETE) [50]. PIF is also able to increase NFκB expression in cultured cells [51] (Figure 2). In conclusion, PIF might have a constitutive function in normal states and become altered or overproduced during cancer cachexia, therefore, inducing significant effects in both muscle protein catabolism and acute phase protein (APP) synthesis in this pathological state.

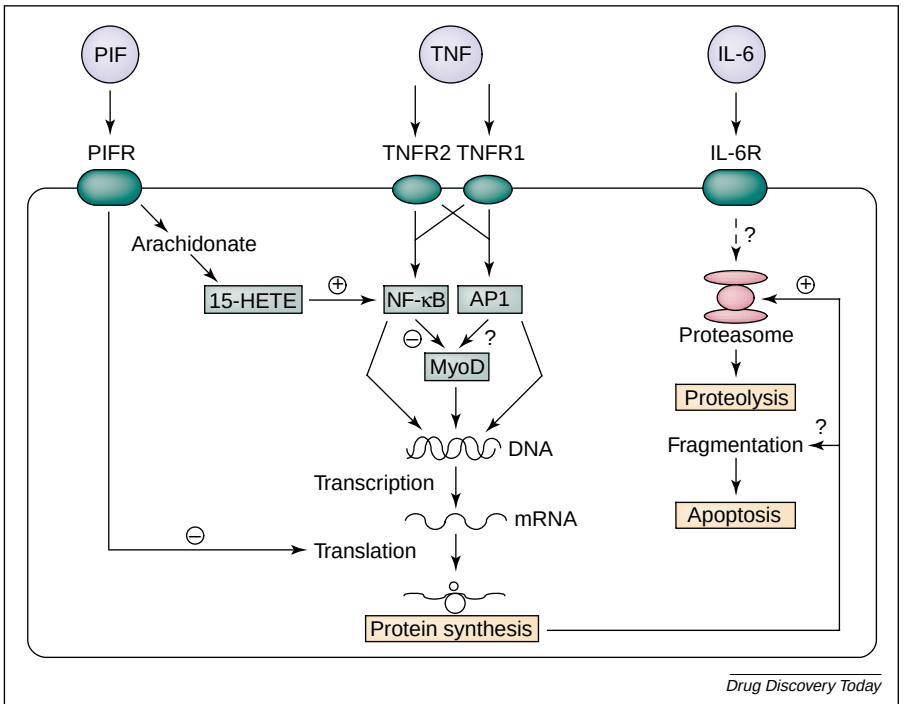

Figure 2. Interactions between pro-inflammatory cytokines and PIF (proteolysis-inducing factor) after binding with receptors in the cell mebrane. Both humoural [tumour necrosis factor (TNF) and interleukin-6 (IL-6)] and tumoural (PIF) factors are able to activate intracellular muscle proteolysis by different mechanisms, possibly sharing common pathways. Abbreviations: 15-HETE, 15-hydroxyeicosatetraenoic acid; AP1, MyoD and NFκB, transcription factors.

Transcription factors

There are currently few studies describing the involvement of transcription factors in muscle wasting. Penner *et al.* have reported an increase in both NFκB and AP-1 transcription factors during sepsis in experimental animals [52]. However, recent data do not support an involvement of NFκB in skeletal muscle during cancer cachexia (unpublished data). Tumour burden does result in a significant increase in the binding activity of AP-1. Interestingly, inhibition of NFκB is not able to revert muscle wasting in cachectic tumour-bearing animals [53], although 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 that is observed in skeletal muscle during sepsis can be mimicked by TNF; addition of TNF to C2C12 muscle cultures results in a short-term increase in NFκB [54,55]. Whether or not this TNF-promoted increase in NFκB is associated with increased proteolysis and/or increased apoptosis in skeletal muscle, remains to be established.

In relation to AP-1 activation, TNF has been shown to increase c-jun expression in C2C12 cells [56]. Notably, overexpression of c-jun mimics the observed effect of TNF upon differentiation, resulting in decreased myoblast differentiation

[57]. Tumour mediators, PIF in particular, also seem able to increase NFκB expression in cultured muscle cells, which has a possible link with increased proteolysis [51] (Figure. 2). Other transcription factors that have been reported to be involved in the muscle changes associated with catabolic conditions include c/EBPβ and δ (which are increased in skeletal muscle during sepsis [58]), PW-1 and PGC-1. Here, TNF decreases MyoD content in cultured myoblasts [59] and blocks diferentiation via a mechanism that seems to be independent of NFκB and involves PW-1, a transcriptional factor related to p53-induced apoptosis [60]. The action of the cytokines on muscle cells, therefore, is 'most likely' to be based on satellite cells blocking muscle differentiation or, in other words, blocking regeneration.

Finally, the transcription factor, PGC-1, is associated with the activation of UCP-2 and UCP-3 and increased oxygen consumption by cytokines in cultured myotubes [61]. This transcription factor is involved as an activator of PPAR-γ in the expression of uncoupling proteins.

Strategies to fight cachexia

Both anorexia and metabolic disturbances are involved in cancer cachexia, and therefore, the development of different therapeutic strategies has focused on these two factors. Unfortunately, counteracting anorexia, either pharmacologically or nutritionally, has led to rather disappointing results in the treatment of cancer cachexia. For this reason, among others, the strategies that follow rely on neutralizing the metabolic changes induced by the tumour, which are ultimately responsible for the weight loss. Therefore, taking into account the involvement of cytokines in cachexia, different therapeutic strategies are currently based on blocking their synthesis or mechanism of action.

TNF synthesis inhibitors

Different TNF synthesis inhibitors have been used with a therapeutic aim. Pentoxifylline, a methylxantine derivative, is able to decrease the cytokine-induced toxicity of antineoplasic agents, while preserving the efficacy of antitumour treatment in several animal models; however, the drug failed to improve the appetite or to increase the weight of cachectic patients in clinical studies. In addition, pentoxifylline was not able to reduce TNF levels in patients with idiopathic dilated cardiomyopathy [62]. Rolipram is a type-IV phosphodiesterase inhibitor that has been shown to decrease TNF production by lipopolysaccharide (LPS)-stimulated human monocytes via a mechanism analogous to that of pentoxifylline. Rolipram could provide therapeutic activity in disease states where TNF seems to be involved in the pathogenesis, such as endotoxic shock. Thalidomide (α-N-phthalimidoglutaramide) is another compound that suppresses TNF production in monocytes *in vitro* and normalizes elevated TNF levels *in vivo*. Its use in cancer cachexia remains to be established but it could potentially function in counteracting TNF-mediated metabolic changes [63].

Anti-cytokine antibodies

The use of anti-cytokine antibodies (either mono- or polyclonal) and cytokine receptor antagonists, or soluble receptors, has led to interesting results. 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 and protein metabolism [64]. In humans, however, clinical trials using anti-TNF treatment have produced poor results in reverting the protein waste associated with sepsis [65]. Concerning IL-6, experimental models have proved that the use of antibodies is highly effective in preventing tumour-induced waste. Strassman and co-workers 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 the catabolic effects associated with the growth of the colon-26 adenocarcinoma in mice, such as body, heart and epididymal fat weight-loss and hypoglycemia [19]. In humans, administration of an anti-IL-6 monoclonal antibody to patients with AIDS who are suffering from an immunoblastic or a polymorphic large-cell cell lymphoma, had a positive effect on fever and cachexia.

Anti-IFN-γ therapy has also been effective in reverting cachexia in mice bearing the Lewis lung carcinoma [23] but there is a lack of clinical data. It must be noted that the routine use of anti-cytokine antibodies is, at present, too expensive because this type of therapy requires many antibody molecules to block cytokine action completely.

Anti-inflammatory cytokines

The appearance of the cachexia syndrome is dependent not only on the production of the afore-mentioned cytokines, known as catabolic pro-inflammatory cytokines, but also on the so-called anti-inflammatory cytokines, such as IL-4 or IL-10. Mori *et al.* [66] 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, a cytokine that has been shown to be responsible for the cachexia associated with this tumour model. A similar action has been described for INF-α. 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. IL-15 has been reported to be an anabolic factor for skeletal muscle [67], and recent experiments demonstrate that the cytokine is able to reverse most of the abnormalities associated with cancer cachexia in a rat tumour model [68].

Additional anti-inflammatory strategies to influence cytokine levels during cachexia include the use of cyclo-oxygenase-2 inhibitors [69,70]. These compunds, in addition to decreasing cytokine levels in cancer, result in an improvement of weight loss and cachexia.

Strategies based on trancription factors

Transcription factors are another potential target to be considered in the fight against cancer cachexia [71]. On examination of therapeutic strategies based on trancription factor-events in muscle wasting, several points emerge. First, Kawamura *et al.* [72,73] reported that the use of an oligonucleotide that competes with the NFκB-binding site can revert cachexia in a mouse experimental model without affecting the growth of the primary tumour. This treatment, however, reduces the methastasic capacity in the colon-26 adenocarcinoma model. However, administration of curcumine to tumour-bearing rats was unable to halt muscle wasting, therefore the involvement of NFκB in the cachectic response in this tumour model can be discarded [53].

As discussed previously, AP-1 is clearly involved in muscle wasting during sepsis [52] and also in cancer. Interestingly, administration of an inhibitor of both NFκB 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).

Conclusion

Metabolic alterations often appear soon after the onset of tumour growth, therefore, the treatment for such alterations could influence the patient's clinical state, despite the fact that this treatment is not aimed at immediate eradication of the tumour mass. It could contribute to the improvement in quality of life and, possibly, prolong survival. Although exploration of the role of cytokines in the host response to invasive stimuli has been underway for many years, there is still considerable controversy 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 – both host and tumour-derived [37] – in the molecular mechanisms of protein wasting in skeletal muscle is essential for the design of therapeutic strategies. Understanding the humoural response to cancer and modifying cytokine actions pharmacologically could prove to be beneficial and future research will inevitably concentrate on this interesting field. Finally, understanding the intracellular signalling mechanisms, particulary transcription factors, might be crucial for the design of effective therapeutic approaches in the near future.

References

1 Warren, S. (1932) The immediate cause of death in cancer. *Am. J. Med. Sci.* 184, 610–613
2 Fearon, K. and Moses, A. (2002) Cancer cachexia. *Int. J. Cardiol.* 85, 73–81
3 Costelli, P. and Baccino, F.M. (2000) Cancer cachexia: from experimental models to patient management. *Curr. Opin. Clin. Nutr. Metab. Care* 3, 177–181
4 López-Soriano, J. *et al.* (1999) Leptin and tumour growth in the rat. *Int. J. Cancer* 81, 726–729
5 Sato, T. *et al.* (2002) Does leptin really influence cancer anorexia? *Nutrition* 18, 82–83
6 Kowalczuk, A. *et al.* (2001) Plasma concentration of leptin, neuropeptide Y and tumour necrosis factor alpha in patients with cancers, before and after radio- and chemotherapy. *Pol. Arch. Med. Wewn.* 106, 657–668
7 Tessitore, L. *et al.* (2000) Leptin expression in colorectal and breast cancer patients. *Int. J. Mol. Med.* 5, 421–426
8 Brown, D.R. *et al.* (2001) Weight loss is not associated with hyperleptinemia in humans with pancreatic cancer. *J. Clin. Endocrinol. Metab.* 86, 162–166
9 Aleman, M.R. *et al.* (2002) Leptin role in advanced lung cancer. A mediator of the acute phase response or a marker of the status of nutrition? *Cytokine* 19, 21–26
10 Mantovani, G. *et al.* (2000) Serum levels of leptin and proinflammatory cytokines in patients with advanced-stage cancer at different sites. *J. Mol. Med.* 78, 554–561
11 Mantovani, G. *et al.* (2001) Serum values of proinflammatory cytokines are inversely correlated with serum leptin levels in patients with advanced stage cancer at different sites. *J. Mol. Med.* 79, 406–414
12 Plata-Salaman, C.R. (2000) Central nervous system mechanisms contributing to the cachexia-anorexia syndrome. *Nutrition* 16, 1009–1012
13 Chance, W.T. *et al.* (1994) Hypothalamic concentration and release of neuropeptide Y into microdialysates is reduced in anorectic tumor-bearing rats. *Life Sci.* 54, 1869–1874
14 King, P.J. *et al.* (2000) Effect of cytokines on hypothalamic neuropeptide Y release *in vitro*. *Peptides* 21, 143–146
15 Laviano, A. *et al.* (2002) Neurochemical mechanisms for cancer anorexia. *Nutrition* 18, 100–105
16 Bing, C. *et al.* (2001) Cachexia in MAC16 adenocarcinoma: suppression of hunger despite normal regulation of leptin, insulin and hypothalamic neuropeptide Y. *J. Neurochem.* 79, 1004–1012
17 Sheppard, B.C. *et al.* (1990) Prolonged survival of tumor-bearing rats with repetitive low-dose recombinant tumor necrosis factor. *Cancer Res.* 50, 3928–3933
18 Tracey, K.J. *et al.* (1990) Metabolic effects of cachectin/tumor necrosis factor are modified by site of production. Cachectin/tumor necrosis factor-secreting tumor in skeletal muscle induces chronic cachexia, while implantation in brain induces predominantly acute anorexia. *J. Clin. Invest.* 86, 2014–2024
19 Strassmann, G. *et al.* (1993) Suramin interfers with interleukin-6 receptor binding *in vitro* and inhibits colon-26-mediated experimental cancer cachexia *in vivo*. *J. Clin. Invest.* 92, 2152–2159
20 Barton, B.E. *et al.* (2000) A model that reproduces syndromes associated with human multiple myeloma in nonirradiated SCID mice. *Proc. Soc. Exp. Biol. Med.* 223, 190–197
21 Barton, B.E. and Murphy, T.F. (2001) Cancer cachexia is mediated in part by the induction of IL-6-like cytokines from the spleen. *Cytokine* 16, 251–257

22 Negri, D.R. *et al.* (2001) Role of cytokines in cancer cachexia in a murine model of intracerebral injection of human tumours. *Cytokine* 15, 27–38
23 Matthys, P. *et al.* (1991) Anti-interferon-γ antibody treatment, growth of Lewis lung tumours in mice and tumour-associated cachexia. *Eur. J. Cancer* 27, 182–187
24 Costelli, P. et al. (1995) Interleukin-1 receptor antagonist (IL-1ra) is unable to reverse cachexia in rats bearing an ascites hepatoma (Yoshida AH-130). Cancer Lett. 95, 33–38
25 Henderson, J.T. *et al.* (1996) Physiological effects of CNTF-induced wasting. *Cytokine* 8, 784–793
26 Wang, M.C. and Forsberg, N.E. (2000) Effects of ciliary neurotrophic factor (CNTF) on protein turnover in cultured muscle cells. *Cytokine* 12, 41–48
27 Busquets, S. *et al.* (1998) In the rat, TNF-α administration results in an increase in both UCP2 and UCP3 mRNAs in skeletal muscle: a possible mechanism for cytokine-induced thermogenesis? *FEBS Lett.* 440, 348–350
28 Busquets, S. *et al.* (2003) Tumour necrosis factor-alpha uncouples respiration in isolated rat mitochondria. *Cytokine* 22, 1–4
29 Argilés, J.M. and López-Soriano, F.J. (1998) Catabolic proinflammatory cytokines. *Curr. Opin. Clin. Nutr. Metab. Care* 1, 245–251
30 Bossola, M. *et al.* (2001) Increased muscle ubiquitin mRNA levels in gastric cancer patients. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280, R1518–R1523
31 Baracos, V.E. (2000) Regulation of skeletal-muscle-protein turnover in cancer-associated cachexia. *Nutrition* 16, 1015–1018
32 Alvarez, B. *et al.* (2002) Tumor necrosis factor-alpha exerts interleukin-6-dependent and -independent effects on cultured skeletal muscle cells. *Biochim. Biophys. Acta* 1542, 66–72
33 Sharma, R. and Anker, S.D. (2002) Cytokines, apoptosis and cachexia: the potential for TNF antagonism. *Int. J. Cardiol.* 85, 161–171
34 Van Royen, M. *et al.* (2000) DNA fragmentation occurs in skeletal muscle during tumour growth: a link with cancer cachexia? *Biochem. Biophys. Res. Commun.* 270, 533–537
35 Belizario, J.E. *et al.* (2001) Cleavage of caspases-1, -3, -6, -8 and -9 substrates by proteases in skeletal muscles from mice undergoing cancer cachexia. *Br. J. Cancer* 84, 1135–1140
36 Carbó, N. *et al.* (2002) TNF-α is involved in activating DNA fragmentation in murine skeletal muscle. *Br. J. Cancer* 86, 1012–1016
37 Cahlin, C. *et al.* (2000) Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon-gamma, and tumor necrosis factor alpha evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia. *Cancer Res.* 60, 5488–5493
38 Dunlop, R.J. and Campbell, C.W. (2000) Cytokines and advanced cancer. *J. Pain Symptom Manage.* 20, 214–232
39 Costa, G. and Holland, J.F. (1962) Effects of Krebs-2 carcinoma on the lipid metabolism of male Swiss mice. *Cancer Res.* 22, 1081–1083
40 Kitada, S. *et al.* (1981) Characterization of a lipid mobilizing factor from tumors. *Prog. Lipid Res.* 20, 823–826
41 Masuno, H. *et al.* (1981) Purification and characterization of a lipolytic factor (toxohormone L) from cell free fluid of ascites sarcoma 180. *Cancer Res.* 41, 284–288
42 Khan, S. and Tisdale, M.J. (1999) Catabolism of adipose tissue by a tumour-produced lipid metabolising factor. *Int. J. Cancer* 80, 444–447
43 Hirai, K. *et al.* (1998) Biological evaluation of a lipid-mobilising factor isolated from the urine of cancer patients. *Cancer Res.* 58, 2359–2365
44 Hirai, K. *et al.* (1997) Mechanism of depletion of liver glycogen in cancer cachexia. *Biochem. Biophys. Res. Commun.* 241, 49–52
45 Ishiko, O. *et al.* (1999) Lipolytic activity of anaemia-inducing susbtance from tumor-bearing rabbits. *Nutr. Cancer* 33, 201–205
46 Todorov, P. *et al.* (1996) Characterization of a cancer cachectic factor. *Nature* 379, 739–742
47 Lorite, M.J. *et al.* (1997) Induction of muscle protein degradation by a tumour factor. *Br. J. Cancer* 76, 1035–1040
48 Wigmore, S.J. *et al.* (2000) Characteristics of patients with pancreatic cancer expressing a novel cancer cachexia factor. *Br. J. Surg.* 87, 53–58
49 Lorite, M.J. *et al.* (1998) Mechanism of muscle protein degradation induced by a cancer cachectic factor. *Br. J. Cancer* 78, 850–856
50 Smith, H.J. *et al.* (1999) Effects of a cancer cachectic factor on protein synthesis/degradation in murine C2C12 myoblasts: modulation by eicosapentaenoic acid. *Cancer Res.* 59, 5507–5513
51 Tisdale, M.J. (2000) Protein loss in cancer cachexia. *Science* 289, 2293–2294
52 Penner, C.G. *et al.* (2001) The transcription factors NF-kappaB and AP-1 are differentially regulated in skeletal muscle during sepsis. *Biochem. Biophys. Res. Commun.* 281, 1331–1336
53 Busquets, S. *et al.* (2001) Curcumin, a natural product present in turmeric, decreases tumor growth but does not behave as an anticachectic compound in a rat model. *Cancer Lett.* 167, 33–38
54 Fernandez-Celemin, L. *et al.* (2002) Inhibition of muscle insulin-like growth factor I expression by tumor necrosis factor-alpha. *Am. J. Physiol. Endocrinol. Metab.* 283, E1279–E1290
55 Li, Y.P. *et al.* (1998) Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-kappaB activation in response to tumor necrosis factor alpha. *FASEB J.* 12, 871–880
56 Brenner, D.A. *et al.* (1989) Prolonged activation of jun and collagenase genes by tumor necrosis factor-alpha. *Nature* 337, 661–663
57 Thinakaran, G. *et al.* (1993) Expression of c-jun/AP-1 during myogenic differentiation in mouse C2C12 myoblasts. *FEBS Lett.* 319, 271–276
58 Penner, G. *et al.* (2002) C/EBP DNA-binding activity is upregulated by a glucocorticoid-dependent mechanism in septic muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282, R439–R444
59 Guttridge, D.C. *et al.* (2000) NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science* 289, 2363–2366
60 Coletti, D. *et al.* (2002) TNFalpha inhibits skeletal myogenesis through a PW1-dependent pathway by recruitment of caspase pathways. *EMBO J.* 21, 631–642
61 Puigserver, P. *et al.* (2001) Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. *Mol. Cell* 8, 971–982
62 Skudicky, D. *et al.* (2001) Beneficial effects of pentoxifylline in patients with idiopathic dilated cardiomyopathy treated with angiotensin-converting enzime inhibitors and carvedilol: results of a randomized study. *Circulation* 103, 1083–1088
63 Peuckmann, V. *et al.* (2000) Potential novel uses of thalidomide: focus on palliative care. *Drugs* 60, 273–292
64 Costelli, P. *et al.* (1993) Tumor necrosis factor-α mediates changes in tissue protein turnover in a rat cancer cachexia model. *J. Clin. Invest.* 92, 2783–2789
65 Reinhart, K. *et al.* (1996) Assessment of the safety and efficacy of the monoclonal anti-tumor necrosis factor antibody fragment, MAK 195F, in patients with sepsis and septic shock: a multicenter, randomized, placebo-controlled, dose-ranging study. *Crit. Care Med.* 24, 733–742
66 Mori, K. *et al.* (1996) Murine interleukin-12 prevents the development of cancer cachexia in a murine model. *Int. J. Cancer* 67, 849–855
67 Quinn, L.S. *et al.* (1995) Interleukin-15: a novel anabolic cytokine for skeletal muscle. *Endocrinology* 136, 3669–3672
68 Carbó, N. *et al.* (2000) Interleukin-15 antagonizes muscle protein waste in tumour-bearing rats. *Br. J. Cancer* 83, 526–531
69 Okamoto, T. (2002) NSAID zaltoprofen improves the decrease in body weight in rodent sickness behavior models: proposed new applications of NSAID. *Int. J. Mol. Med.* 9, 369–372
70 Niu, Q. *et al.* (2001) Cytokines in experimental cancer cachexia. *Zhonghua Zhong Liu Za Zhi* 23, 382–384
71 Mitch, W.E. and Price, S.R. (2001) Transcription factors and muscle cachexia: is there a therapeutic target? *Lancet* 357, 734–735
72 Kawamura, I. *et al.* (1999) Intratumoral injection of oligonucleotides to the NF kappa B binding site inhibits cachexia in a mouse tumor model. *Gene Ther.* 6, 91–97
73 Kawamura, I. *et al.* (2001) Intravenous injection of oligodeoxynucleotides to the NF-kappaB binding site inhibits hepatic metastasis of M5076 reticulosarcoma in mice. *Gene Ther.* 8, 905–912