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Symposium on ‘New perspectives in the anorexia–cachexia syndrome’

Isolation of a novel cancer cachectic factor

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Cachexia is common in a variety of neoplastic diseases, but is particularly prevalent in patients with pancreatic and gastric cancer, with 85 % of cases displaying weight loss (De Wys, 1985). Patients with cachexia have an unfavourable performance status, a decreased response to chemotherapy and a shorter survival time compared with cancer patients not displaying cachexia. A 30 % loss of body weight is frequently fatal, although a few patients may survive as much as 50 % loss of body weight.

Weight loss in cancer patients differs from that found in simple starvation where more than three-quarters of the weight loss arises from adipose tissue and only a small amount from skeletal muscle. In cachexia, weight is lost equally from adipose tissue and muscle such that for a given degree of weight loss there is more depletion of skeletal muscle in a cachectic cancer patient than a starving normal subject (Cohn *et al.* 1981). It is this loss of skeletal muscle mass which contributes most to the deterioration in performance status and early death.

Although anorexia is invariably present in the cachectic cancer patient this alone does not appear to be responsible for the loss of body weight. Thus, in both rat and man, loss of both muscle and adipose tissue often precedes the fall in food intake (Costa, 1977), and in malnourished cancer patients the measured food intake does not correlate with the degree of malnutrition (Costa *et al.* 1980). Studies aimed at increasing energy intake through dietary counselling failed to reverse cachexia and its adverse influence on clinical outcome (Grosvenor *et al.* 1989; Oversen *et al.* 1993). Even administration of total parenteral nutrition (TPN) has failed to give long-term stabilization of body weight (Evans *et al.* 1985). A transient weight gain has been observed with TPN, but this is due to an increase in fat and water rather than skeletal muscle mass. A similar result has been reported with the appetite stimulant megestrol acetate (Megace; Bristol-Meyers Squibb, Hounslow, Middlesex). In one study (Loprinzi *et al.* 1993a), Megace was reported to induce a weight gain of greater than 5 % in 15 % of the patients treated, although a recent study (McMillan *et al.* 1994) reported no change in body weight or body composition in gastrointestinal-cancer patients treated with Megace. Where weight gain was observed there was increased adipose tissue, but no increase in lean body mass (Loprinzi *et al.*

1993b). This suggests that depletion of skeletal muscle proteins during the process of cachexia is not simply due to undernutrition, but a much more complex metabolic problem.

ROLE OF CYTOKINES IN CANCER CACHEXIA

Tumour necrosis factor- α (TNF- α) was first recognized as the mediator of the anorexia-cachexia syndrome in trypanosome-infected rabbits and was given the name cachectin to indicate its role in the cachectic process (Beutler *et al.* 1985). Since then, other cytokines, in particular interleukins-1 and -6 (IL-1 and IL-6 respectively), interferon- γ and leukaemia-inhibiting factor, have all been suggested to play a role in the development of cancer cachexia. All cytokines produce a profound anorexia and weight loss. The effect on adipose tissue was thought to be mediated through an inhibition of the cleaving enzyme lipoprotein lipase (EC 3.1.1.34; LPL), which would prevent adipocytes from extracting fatty acids from plasma lipoproteins for storage. However, inhibition of LPL alone is unlikely to induce the massive depletion of body fat seen in cancer cachexia, since in type 1 hyperlipidaemia there is an inherited deficiency of LPL and yet the patients are not cachectic. Although *in vivo* studies show that muscle protein degradation is significantly increased by TNF- α (Flores *et al.* 1989), the effect must be indirect, since Goldberg *et al.* (1988) were unable to detect a catabolic effect after incubation of skeletal muscle with TNF- α *in vitro*. IL-6 (Garcia-Martinez *et al.* 1994) and IL-1 (Goldberg *et al.* 1988) have also been shown to be incapable of inducing muscle protein degradation *in vitro*.

It has been difficult to correlate serum levels of TNF- α with the extent of cachexia in cancer patients, although it is known that cachexia is not a local effect of a tumour. Thus, in patients with solid tumours and a weight loss of 8–40 % no TNF- α was detected in serum samples using an assay capable of detecting ≤ 40 pg/ml (Socher *et al.* 1988). However, in another study, serum levels of TNF- α were found to be increased from 7.8 to 16 pg/ml and correlated with the extent of disease (Scagliotti *et al.* 1995). Thompson *et al.* (1993) were also unable to detect elevated TNF- α levels in cancer patients and, furthermore, neither the total LPL enzyme activity nor the relative levels of mRNA for LPL were significantly different between cancer patients and controls. Thus, while elevated TNF- α levels may be present in some cancer patients, there is no absolute requirement for this cytokine for the development of cancer cachexia. In contrast to TNF- α , it has been much easier to detect elevated levels of IL-6 in cancer patients. IL-6 is the main cytokine involved with acute-phase protein (APP) synthesis, and patients with colon cancer and an ongoing APP response had an elevated circulating IL-6 concentration (Fearon *et al.* 1991). Unfortunately, all patients in this study had lost some weight and further studies are required to determine whether IL-6 is elevated in cachectic cancer patients. These results suggest that some other factors may be involved in the induction of cancer cachexia.

CATABOLIC FACTORS IN CANCER CACHEXIA

In order to study the mechanisms responsible for the catabolism of muscle and adipose tissue during the process of cancer cachexia, we have utilized a dimethylhydrazine-induced transplantable murine colon adenocarcinoma (MAC16; Bibby *et al.* 1987). This tumour induces weight loss when the tumour mass comprises more than 0.3 % of the host body weight and weight loss reaches 30 % when the tumour represents 3 % of the body weight (Beck & Tisdale, 1987). The reduction in host body weight occurs without a decrease in food and water intake, is proportional to the tumour mass and is reversible when the tumour is excised. Cachexia in this model differs from that expected with cytokines such as

TNF- α , which produces a decrease in body weight in direct proportion to a decreased food and water intake (Mahony *et al.* 1988). In addition, cachexia induced by the MAC16 tumour was accompanied by a marked hypotriacylglycerolaemia, whereas TNF- α (and other cytokines) produce hypertriacylglycerolaemia due to inhibition of LPL. These results, together with the inability to detect TNF- α production by the MAC16 tumour, suggested that some other factor(s) may be responsible for the cachexia.

Since the most obvious changes in body composition in mice bearing the MAC16 tumour were a depletion of adipose tissue triacylglycerols and skeletal muscle proteins, bioassays were developed to detect tumour products capable of direct catabolism of host tissues. Mediators of fat breakdown were detected by measurement of the release of glycerol or non-esterified fatty acids from freshly-isolated epididymal adipocytes (Beck & Tisdale, 1987), while protein breakdown was detected by the release of tyrosine from fresh gastrocnemius muscles under tension (Smith & Tisdale, 1993). Using these bioassays, there was evidence for the production by the MAC16 tumour of both lipid- and protein-mobilizing factors, while related tumours, which did not produce cachexia, showed little catabolic activity in these bioassays (Beck & Tisdale, 1987; Smith & Tisdale, 1993). This suggests that these factors may be responsible for the induction of cachexia. In addition, lipid-mobilizing activity was detected in the serum and urine of cancer patients with weight loss, while it was undetectable in patients with weight loss due to Alzheimer's disease (Groundwater *et al.* 1990). A linear relationship was observed between both the serum and urinary lipid-mobilizing activity and weight loss in cancer patients, when the total body weight loss did not exceed 20%. Moreover, patients showing a response to chemotherapy showed a decrease in plasma levels of lipid-mobilizing activity, suggesting that this catabolic factor emanated from the tumour (Beck *et al.* 1990a).

Other workers have also described tumour-derived lipid-mobilizing factors. Costa & Holland (1966) were the first to show that weight loss, and in particular fat depletion induced by Krebs-2 carcinoma cells in mice, could be reproduced with a non-viable preparation of the tumour. When serum from lymphoma-bearing mice was injected into normal mice it produced an immediate fat mobilization, providing further evidence for a circulatory catabolic factor (Kitada *et al.* 1980). The lipid-mobilizing factor was also present in tumour extracts and culture medium from the lymphoma cell line, showing that it was a direct product of the tumour, rather than being produced by host tissues. Another lipid-mobilizing factor, toxohormone L, was isolated from the ascitic fluid of patients with hepatoma and mice with sarcoma 180 (Masuno *et al.* 1981, 1984). The material was acidic with an isoelectric point at pH 4.7–4.8 and of molecular mass 70–75 kDa. Trypsin digestion of the active material produce a fragment of low molecular mass, which was still biologically active, suggesting that only part of the molecule may be responsible for the biological effects. A low-molecular-mass, acidic, lipid-mobilizing factor was also isolated from the human melanoma cell line, A375 (Taylor *et al.* 1992). The material stimulated lipolysis in normal murine adipocytes by a mechanism involving activation of triacylglycerol lipase (EC 3.1.1.3).

Thus, both human and murine tumours appear to be capable of elaborating lipid-mobilizing factors. The lipid-mobilizing factor present in serum (Beck *et al.* 1990b) and urine (Beck & Tisdale, 1991) of cachectic cancer patients has been shown to display identical molecular mass and chromatographic characteristics to that found in the MAC16 tumour, suggesting that the induction of cachexia in man and mouse may be mediated by the same factor.

One of the characteristics of tumour lipid-mobilizing factors which distinguishes them from the lipolytic hormones is a strong negative charge at physiological pH, and this

characteristic has been utilized in the purification of this factor from a MAC16 tumour homogenate. Using a combination of ion-exchange, exclusion and hydrophobic chromatography, a lipid-mobilizing factor has been isolated, representing 0.005% of the total protein present in the tumour (McDevitt *et al.* 1995). It was noted that animals transplanted with the MAC16 tumour, and with a delayed weight loss, contained in their serum antibodies that recognized a material of molecular mass 24 kDa on Western blots and which co-purified with the lipid-mobilizing factor. Such antibodies were not present in the serum of mice bearing a related tumour (MAC13) which did not induce cachexia, suggesting that the antibodies were directed against the factor inducing cachexia rather than the tumour itself.

Splenocytes from mice bearing the MAC16 tumour, and with a delayed weight loss, were fused with mouse myeloma cells to produce hybridomas. These hybridomas were cloned to produce antibody reactive to the material of molecular mass 24 kDa (Todorov *et al.* 1996b). The monoclonal antibody was then used to purify the material of molecular mass 24 kDa using a combination of affinity and hydrophobic chromatography to give a single species representing one part in 10^8 of the total protein present in the tumour (Todorov *et al.* 1996a). The material was shown to be a sulphated glycoprotein or proteoglycan containing a short peptide core with carbohydrate chains linked to both serine and asparagine residues. Using Western blotting with the MAC16 monoclonal antibody the material was found to be present in the urine of cachectic cancer patients, but absent from the urine of normal subjects, patients with weight loss due to major burns, multiple injuries or surgery-associated catabolism and sepsis, or from the urine of cancer patients with little or no weight loss. Material isolated from urine of cancer patients was found to be chemically, immunologically and functionally identical to that found in the MAC16 tumour.

In vivo studies showed that intravenous administration of the purified material of molecular mass 24 kDa to non-tumour-bearing mice produced a weight loss of 2 g over a 24 h period, without a reduction in food and water intake (Todorov *et al.* 1996a). Weight loss was attenuated by pretreatment with the MAC16 monoclonal antibody, showing the specificity of the effect. Body composition analysis showed the majority of weight was lost from the skeletal muscle mass and there was no change in body water. *In vitro* studies showed the material to be capable of inducing direct release of tyrosine from isolated gastrocnemius muscle, and this effect could also be blocked by the MAC16 monoclonal antibody. These results suggest that the 24 kDa material is responsible for protein catabolism during the process of cachexia. Although fewer studies have been carried out on protein catabolic factors than on lipid catabolic factors in cancer, Belizario *et al.* (1991) found evidence for circulatory skeletal-muscle proteolysis-inducing factors in serum samples of patients with weight loss greater than 10%. Although the effect appeared to be mediated in part by IL-1, this acted in cooperation with other unidentified factors.

Thus, tissue breakdown during the process of cancer cachexia is associated with tumour-produced catabolic factors which are present in the circulation, and appear to be distinct from the recognized cytokines. One outstanding question that still requires resolution is why do tumours produce such factors? Tumours have an increased requirement for certain amino acids and have specific requirements for others such as L-cysteine (Uren & Lazarus, 1979), methionine (Tisdale, 1980), tyrosine and phenylalanine (Demetrakopoulos & Brennan, 1982), serine and threonine (Pfizer & Regan, 1972). Also tumours have a poor capacity to synthesize their own lipids. Nutritional conditions which lead to catabolism of host adipose tissues in rats, such as an acute fast (Sauer & Dauchy, 1987a) and acute streptozotocin-induced diabetes (Sauer & Dauchy, 1987b), result in a

stimulation of tumour growth, suggesting that the products from host fat stores may be limiting for tumour growth *in vivo*. It is most likely that the mitogenic effect results from the release of polyunsaturated fatty acids such as linoleic and arachidonic acids. Metabolites derived from both lipoxygenase and cyclooxygenase pathways of these essential fatty acids have been shown to transduce growth-related signals and regulate cell proliferation (Bandyopadhyay *et al.* 1988; Glasgow & Eling, 1990). In addition, products of both the 12- and 15-lipoxygenase pathways of arachidonic acid appear to play an important physiological role in regulating apoptosis (Tang *et al.* 1996).

CONCLUSION

In addition to the cytokines, tumours elaborate catabolic factors which cause direct breakdown of host lipid and skeletal muscle protein stores. The importance of such factors to tumour homeostasis is indicated by the conservation in structure of these factors between mouse and man. A knowledge of the mechanism of the induction of cachexia should lead to the development of therapeutic agents capable of inhibiting the steps in the process. One such agent, the polyunsaturated fatty acid eicosapentaenoic acid, is capable of direct inhibition of lipid (Tisdale & Beck, 1991) and protein (Smith & Tisdale, 1993) catabolic factors, and has been shown to induce weight gain and stabilization of energy expenditure in patients with unresectable pancreatic cancer (Wigmore *et al.* 1996). Attacking the catabolic process of cachexia should lead not only to new anti-cachectic agents, but also offers the promise of anti-tumour activity, assuming the products of breakdown of host tissues are used by the tumour to maintain its growth and integrity.

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