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Cytokines, the Acute-Phase Response, and Resting Energy Expenditure in Cachectic Patients with Pancreatic Cancer

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Objective

To determine whether resting energy expenditure (REE) is increased in cachectic patients with pancreatic cancer and to define the relation of tumor necrosis factor (TNF) and interleukin-6 (IL-6) production to the acute-phase response and to REE.

Methods

Measurement of REE (indirect calorimetry) and assessment of body composition (bioelectrical impedance analysis) were done in 21 patients with unresectable pancreatic cancer and on 16 age-related controls. The systemic inflammatory response in peripheral blood of the cancer patients was assessed using the acute-phase protein, C-reactive protein, and the cytokines TNF and IL-6. Production of these cytokines by peripheral blood mononuclear cells *in vitro* was also measured.

Results

Patients with pancreatic cancer had an elevated REE when compared with controls (73.4 ± 5.0 *vs.* 53.5 ± 1.6 kcal/kg body cell mass; $p < 0.003$). Resting energy expenditure was significantly greater in cancer patients with an acute-phase response (C-reactive protein > 10 mg/L) than in those who did not have such a response (85.5 ± 10.0 [n = 9] *vs.* 64.3 ± 3.0 [n = 12] kcal/kg body cell mass; $p < 0.04$). Tumor necrosis factor was not detected in the serum of any of the cancer patients. Serum IL-6 was detected but levels were not significantly different among cancer patients with or without an acute-phase response. In contrast, spontaneous production of TNF and IL-6 by isolated peripheral blood mononuclear cells was significantly greater in cancer patients with an acute-phase response than in those without (TNF: 1231 ± 244 *vs.* 210 ± 54 pg/mL/10^5 cells; $p < 0.001$; IL-6: 11.5 ± 1.7 *vs.* 3.6 ± 1.4 ng/mL/10^5 cells; $p < 0.003$).

Conclusions

In pancreatic cancer at least a component of weight loss is due to increased REE. Furthermore, the presence of an acute-phase response identifies a group of patients who are markedly hypermetabolic. The serum concentration of TNF or IL-6 does not correlate with the presence of an acute-phase response, whereas rates of cytokine production by peripheral blood mononuclear cells are significantly greater in patients with such a response. This suggests that local rather than systemic cytokine production may be important in regulating the acute-phase response.

Weight loss is a common cause of morbidity in patients with cancer.[1,2] Such patients have a negative energy balance resulting from a reduced energy intake, an increased energy output, or a combination of the two. The extent of these changes should influence the nutritional support plan for individual patients. Unfortunately, substantial interindividual variation and difficulties with accurate documentation of food intake make determination of the precise role of reduced food intake difficult. Furthermore, when cancer patients are given intravenous nutritional support, the tissue gained appears to be abnormal,[3,4] suggesting that supplementation of intake alone may not abolish the cachetic state. Therefore investigators have targeted increased energy expenditure and abnormal metabolism in planning therapy for malnourished cancer patients.

Increased resting energy expenditure (REE) in patients with cancer has been studied. Results have been contradictory, with some investigators finding increased REE[5,6] whereas others have found no significant alteration.[7] Tumor type might influence REE. Fredrix and coworkers[8] found that patients with non–small-cell lung cancer had elevated REE, whereas those with gastric or colorectal cancer did not. In contrast, Dempsey and colleagues[9] found that patients with gastric cancer tended to be hypermetabolic, patients with esophageal or colorectal neoplasms were evenly distributed within the metabolic groups, and patients with pancreatic or hepatobiliary tumors were predominantly hypometabolic. Hansell and associates[7] concluded that REE was not increased in patients with gastric, colorectal, or non–small-cell bronchial cancer. Together these studies suggest that the metabolic response to cancer is highly variable. Furthermore, even within particular types of cancer, REE is increased in some patients but not in others.[10] Such heterogeneity does not appear to depend on tumor site or stage,[9,10] nor has it been related to nutritional status or duration of disease.[9]

Researchers recently suggested that certain macrophage-derived mediators of the inflammatory response, such as interleukin-1, interleukin-6 (IL-6), and tumor necrosis factor (TNF), may play a role in the metabolic changes observed in cachectic cancer patients.[11–13] In particular, these cytokines may increase REE[14] and redirect host protein metabolism away from peripheral tissues and toward the liver with the development of an acute-phase response.[15,16] Such cytokines may be derived from the tumor itself[17] but probably are produced by host cells in response to the cancer.[18,19] A host mechanism of cachexia involving the inflammatory response might account for the heterogeneity of metabolic change and weight loss observed in patients with the same tumor types. In support of the hypothesis that an inflammatory response is important in the genesis of cachexia, Hyltander and coworkers[6] found a significant correlation between ESR and REE in a heterogeneous group of cancer patients (n = 106) using erythrocyte sedimentation rate as a marker of inflammation.

Our study tried to determine whether a homogeneous group of patients with pancreatic cancer who were losing weight had an elevated REE compared with age- and sex-matched healthy controls. In addition, we divided the patients with pancreatic cancer into those with or without an acute-phase protein response to determine whether this response was associated with a change in REE. We also examined the relation of the cytokines TNF and IL-6 to these changes and paid particular attention to both serum levels and peripheral blood mononuclear cell-secreted cytokine levels.

MATERIALS AND METHODS

Patients with Pancreatic Cancer

We included 21 patients with histologically proven pancreatic cancer who were losing weight. All patients had newly detected tumors and none had received systemic antineoplastic treatment. Seven patients had endobiliary stenting during endoscopic retrograde cholangio-pancreatography and 10 had surgical bypass. Four patients had tumors of the body or tail of the pancreas and did not need surgical or endoscopic relief of jaundice. No patient was studied within 1 month of operative or endoscopic treatment. At the time of study no patient was pyrexial, had clinical evidence of infection, or had an episode of cholangitis within the preceding month. No patient was clinically jaundiced at the time of study: The mean plasma bilirubin concentration of the patients with pancreatic cancer was 14.5 (SEM 2.1) μmol/L. All patients had been staged with a chest x-ray, upper abdominal ultrasonography, and abdominal computed tomography, whereas 12 had selective mesenteric angiography and 6 had laparoscopy. At the time of study, 7 patients had stage II disease, 8 had stage III disease, and 6 had stage IV disease (Union Internationale Contre le Cancer).

Control Patients

Sixteen age-matched volunteers who had been admitted to undergo minor elective surgical procedures (hernia repair or varicose vein surgery, for example) acted as

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controls. None had evidence of infection or inflammation. All patients (cancer and control) gave written informed consent and the study was approved by the local ethics committee.

Resting Energy Expenditure Measurements

We measured REE by indirect calorimetry using a ventilated hood system (Deltatrac Metabolic Monitor, Helsinki, Finland). We performed gas analyses using a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer. The mean relative error for the measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ using this system is less than 4%.[20] We measured REE between 8.00 and 9.00 A.M. after an overnight fast, with the patient at rest and having remained supine from the time of awakening. Before each measurement, the equipment was calibrated using gas containing 95% oxygen and 5% carbon dioxide (Deltatrac High-Accuracy Calibration Gas, Helsinki, Finland) at a known barometric pressure. Flow through the canopy was constant at 40 L/minute. We measured $\dot{V}O_2$ and $\dot{V}CO_2$ during a 20-minute period, and data were processed by an on-line micro-processor allowing conversion to mean REE using the abbreviated deWeir formula.[21]

Body Composition Analysis

We estimated fat-free mass and body cell mass using bioelectrical impedance analysis (BIA 101, RJL Systems, Detroit, MI) as previously described.[22]

Acute-Phase Protein Response and Serum Sampling

We obtained serum immediately after REE measurement and measured the concentration of C-reactive protein using an immunoturbimetric assay (Abbott TDX, Abbott Laboratories, Maidenhead, UK). Serum concentrations of C-reactive protein greater than 10 mg/L indicated the presence of an acute-phase protein response. In addition, aliquots of serum were frozen and stored at −70 C for subsequent measurement of the cytokines TNF and IL-6.

Isolation and Culture of Peripheral Blood Mononuclear Cells)

Peripheral blood was obtained under aseptic conditions on the day of REE measurement. Blood was layered on a hypaque gradient (Histopaque 1077, Sigma Chemical Co., Poole, UK) and centrifuged at 1200 rpm for 25 minutes. Cells were removed from the interface and washed 3 times in cell culture medium (Roswell Park Memorial Institute [RPMI] medium 1640, ICN/Flow Laboratories, Irvine, UK) with penicillin/streptomycin (Sigma Chemical Co.) added. Cells were resuspended, stained with trypan blue, and counted using a hemocytometer. Cells were then cultured in 96-well, flat-bottomed tissue culture plates at a concentration of 10^5 cells/well in 120 μL cell culture medium (RPMI 1640) with penicillin/streptomycin, glutamine (2 mmol/L) (Sigma), and 10% fetal calf serum (ICN/Flow Laboratories) in the presence or absence of 10 μg/mL endotoxin (Lipopolysaccharide from *E. coli* 0127:B8, Sigma Chemical Co.). For estimation of IL-6 and TNF production, supernatants from endotoxin-stimulated or unstimulated PBMC cultures were removed after 24 hours and stored at −70 C for subsequent analysis. All cultures were performed in a humidified incubator at 37 C in the presence of 5% CO_2.

Tumor Necrosis Factor Production by Peripheral Blood Mononuclear Cells

Tumor necrosis factor production from peripheral blood mononuclear cell (PBMC) supernatants was determined using the TNF-sensitive L929 fibroblast cell line.[23] Briefly, L929 cells were plated on 96-well plates at a concentration of 4×10^4 cells per well in D-MEM medium (ICN/Flow Laboratories) supplemented with glutamine, penicillin/streptomycin (Sigma Chemical Co.), and 5% fetal calf serum (ICN/Flow Laboratories). After incubation for 16 hours, the medium was removed and samples were placed in the wells and serially diluted in twofold dilutions from 1:4 to 1:8192. Actinomycin D was added to give a final concentration of 1 μg/mL in each well. After an additional 24-hour incubation, the medium was removed and any remaining viable cells were fixed with 5% formaldehyde for 10 minutes. After washing, cells were stained with a 0.5% aqueous solution of crystal violet for 10 minutes and after another wash the dye was eluted with 33% aqueous acetic acid and read at 570nm on a Dynatech 5000 microplate reader (Dynatech Laboratories, Billingshurst, West Sussex, UK). Dilution curves were performed in duplicate and values were calculated using a computer program for probit analysis (provided by Dr. Gillian Raab, Department of Statistics, University of Edinburgh). The lower limit of sensitivity of the assay was 15 pg/mL and the specificity was confirmed by using a neutralizing anti-TNF antibody.

Serum Tumor Necrosis Factor Measurement

Serum samples were tested for TNF with a sandwich-type enzyme-linked immunosorbent assay (ELISA) using paired antibodies against TNF (Boehringer Mann-

heim, Lewes, East Sussex, UK). Briefly, serum samples were diluted 1:5 and incubated in 96-well ELISA plates coated with mouse monoclonal antihuman TNF antibody. The plates were washed and incubated sequentially with the second peroxidase-labeled mouse monoclonal antihuman TNF antibody and with the peroxidase substrate o-phenylenediamine dihydrochloride (0.5 mg/mL) (Sigma Chemical Co.) in citric acid/sodium phosphate buffer (pH = 5.2). The reaction was stopped with 4 mmol/L H_2SO_4 and the plates were read at 490 nm using a Dynatech 5000 microplate reader. Wells were set up in triplicate and the TNF concentrations in the samples were calculated using the AssayZap computer program (Biosoft, Cambridge, UK). The lower limit of sensitivity of the assay was 5 pg/mL.

Measurement of Serum Interleukin-6 and Peripheral Blood Mononuclear Cell Interleukin-6 Production

Both serum and supernatants were tested for IL-6 using a sandwich ELISA similar to that used for TNF. In this case, plates were coated with mouse monoclonal antibodies against IL-6 (Boehringer Mannheim) and after incubation with the samples, wells were sequentially incubated with goat polyclonal (non–enzyme labeled) anti–IL-6 antibody (British Biotechnology, Abingdon, UK), polyclonal rabbit anti-goat–IgG peroxidase-labeled antibody (Sigma Chemical Co.), and the peroxidase substrate o-phenylenediamine dihydrochloride in citric acid/sodium phosphate buffer. The reaction was stopped, plates were read, and concentrations of IL-6 were determined as with the TNF ELISA. The lower limit of sensitivity of the assay was 15 pg/mL.

Statistical Analysis

Groups of patients were compared using a two-tailed Student's unpaired t test. Results are presented as mean ± SEM and differences were significant when the chance of their occurrence by sampling error was less than 1 in 20 ($p < 0.05$).

RESULTS

The characteristics of the 21 patients with pancreatic cancer who were losing weight and the 16 healthy controls are shown in Table 1. The mean age of the pancreatic cancer patients (57 years) and controls (55 years) did not differ significantly. There was a predominance of men in both groups. The pancreatic cancer patients had lost, on average, 18% of their preillness stable weight, whereas the controls were weight stable. The weight loss of the pancreatic cancer patients was reflected in a significant reduction in their total body weight, fat-free mass, and body cell mass when compared with controls (Table 1). In comparison with the healthy controls, mean REE was significantly greater in pancreatic cancer patients when expressed in relation to total body weight (33% greater), fat-free mass (28% greater), or body cell mass (37% greater) (Table 2).

Table 1. DEMOGRAPHIC DETAILS AND BODY COMPOSITION OF WEIGHT-LOSING PANCREATIC CANCER PATIENTS AND HEALTHY CONTROLS

Demographic	Pancreatic Cancer Patients (n = 21)	Healthy Controls (n = 16)	p Value
Age (yrs)	57 ± 2	55 ± 3	NS
Sex (M:F)	14:7	11:5	NS
Weight (kg)	59.3 ± 2.8	72.4 ± 3.6	0.0064
Weight loss (%)	18 ± 2	0	0.0001
Fat free mass (kg)	49.0 ± 2.4	57.0 ± 2.0	0.022
Body cell mass (kg)	21.7 ± 1.3	26.1 ± 1.1	0.0182

Mean ± SEM.
NS = not statistically significant.

To determine the influence of acute-phase response on energy expenditure, we grouped cancer patients according to the presence or absence of acute-phase response. Nine cancer patients had evidence of an acute-phase response (45%) and 12 did not. The mean age, weight, and sex distribution of the two groups of patients were not significantly different (Table 3). Although weight loss and reduction in fat-free mass and body cell mass were greater in patients with a positive acute-phase response, this was not statistically significant. The mean serum C-reactive protein concentration in pancreatic cancer patients with a detectable acute-phase response was 72 mg/L, and this was associated with a significant reduction in serum albumin concentration (Table 3).

The mean REE of pancreatic cancer patients with an

Table 2. RESTING ENERGY EXPENDITURE (REE) VALUES IN PANCREATIC CANCER PATIENTS AND CONTROLS

	Pancreatic Cancer Patients (n = 21)	Healthy Controls (n = 16)	p Value
REE (kcal/kg body weight)	25.9 ± 1.2	19.4 ± 0.7	0.0001
REE (kcal/kg fat free mass)	31.5 ± 1.7	24.6 ± 1.0	0.0023
REE (kcal/kg body cell mass)	73.4 ± 5.0	53.5 ± 1.6	0.002

Mean values ± SEM.

Table 3. CHARACTERISTICS OF PANCREATIC CANCER PATIENTS WITH AN ACUTE PHASE PROTEIN RESPONSE (APPR) (C-REACTIVE PROTEIN ≥ 10 mg/L) COMPARED WITH PANCREATIC CANCER PATIENTS WITH NO RESPONSE (C-REACTIVE PROTEIN < 10 mg/L)

	Pancreatic Cancer with +ve APPR (n = 9)	Pancreatic Cancer with −ve APPR (n = 12)	p Value
Age (ys)	57 ± 3	57 ± 3	NS
Sex (M:F)	6:3	8:4	NS
Weight (kg)	58.5 ± 4.9	59.9 ± 3.4	NS
Weight loss (%)	20 ± 3	16 ± 2	NS
Fat free mass (kg)	46.7 ± 3.6	50.7 ± 3.3	NS
Body cell mass (kg)	20.9 ± 2.5	22.2 ± 1.5	NS
Serum C-reactive protein (mg/L)	72 ± 20	<10	0.0011
Serum albumin (g/L)	33 ± 1	40 ± 1	0.005

Mean values ± SEM.
NS = not statistically significant.

acute-phase response was significantly greater than in those without when REE was expressed in relation to total body weight (21% greater), fat-free mass (28% greater), or body cell mass (33% greater) (Table 4). The REE of pancreatic cancer patients without a positive acute-phase response, although less than that of patients with a positive response, was significantly greater than that observed in the healthy controls (REE/kg body cell mass 20% greater) (Tables 2 and 4).

None of the pancreatic cancer patients had detectable levels of serum TNF. Although IL-6 was detectable in the serum of both groups of cancer patients, the difference between those with an acute-phase protein response (117 ± 23 pg/mL) and those without (105 ± 10 pg/mL) was not significant (Table 5). In contrast, the spontaneous secretion of both of these cytokines by isolated PBMCs was significantly greater in patients who had a positive inflammatory response (IL-6:11.5 ± 1.7 ng/mL *vs.* 3.6 ± 1.4 ng/mL; TNF:1231 ± 244 pg/mL *vs.* 210 ± 54 pg/mL). The levels of these cytokines secreted by PBMCs in response to endotoxin-stimulation was also greater in the group of patients with an acute-phase protein response, although these differences failed to reach statistical significance (Table 5).

Table 4. RESTING ENERGY EXPENDITURE (REE) VALUES IN PANCREATIC CANCER PATIENTS WITH OR WITHOUT AN ACUTE PHASE PROTEIN RESPONSE (APPR)

	Pancreatic Cancer with +ve APPR (n = 9)	Pancreatic Cancer with −ve APPR (n = 12)	p Value
REE (kcal/kg body weight)	28.7 ± 1.8*	23.8 ± 1.3*	0.035
REE (kcal/kg fat free mass)	36.0 ± 2.7*	28.1 ± 1.5	0.014
REE (kcal/kg body cell mass)	85.5 ± 10.0*	64.3 ± 3.0*	0.033

Mean values ± SEM.
* Values significantly greater than those of healthy controls $p < 0.004$ (see Table 2).

DISCUSSION

Previous studies suggested that REE may be elevated in patients with specific types of tumors. In particular, patients with lung cancer or sarcoma have increased energy expenditure.[10,24] The findings regarding gastrointestinal malignancy are, however, more controversial.[7,9,25] In this study we evaluated a group of 21 pancreatic cancer patients who were all losing weight at the time of study and therefore had a negative energy balance. When compared with weight-stable healthy controls, the mean energy expenditure of the pancreatic cancer patients expressed in relation to their total body weight was increased by approximately 33% (Table 2). This observation is particularly significant because anorexia is a common symptom in patients with pancreatic cancer, and the normal response to semistarvation is a decrease in REE. Our findings are consistent with the theory that accelerated weight loss in cancer cachexia is due to specific metabolic alterations leading to a state of uncompensated semistarvation.[26]

Abnormalities in carbohydrate, fat, and protein metabolism have been documented in cachectic cancer patients.[27–31] However, it is still not clear which, if any, of these abnormalities lead to increased energy expenditure and contribute to weight loss, nor is the mechanism behind such metabolic changes known. Evidence suggests

Table 5. SERUM LEVELS AND SPONTANEOUS AND ENDOTOXIN STIMULATED PBMC-SECRETED LEVELS OF TNF AND IL6 OF PANCREATIC CANCER PATIENTS WITH AN ACUTE PHASE PROTEIN RESPONSE (APPR) (C-REACTIVE PROTEIN ≥10 mg/L) COMPARED WITH PANCREATIC CANCER PATIENTS WITH NO RESPONSE (C-REACTIVE PROTEIN < 10 mg/L)

	Pancreatic Cancer with +ve APPR (n = 9)	Pancreatic Cancer with −ve APPR (n = 12)	p Value
Serum TNF (pg/mL)	ND	ND	NS
Serum IL6 (pg/mL)	117 ± 24	105 ± 10	NS
Spontaneous PBMC TNF (pg/mL)	1231 ± 244	210 ± 54	0.0001
PBMC + Endotoxin TNF (pg/mL)	4731 ± 1526	1833 ± 508	0.0501
Spontaneous PBMC IL6 (ng/mL)	11.5 ± 1.7	3.6 ± 1.4	0.0024
PBMC + Endotoxin IL6 (ng/mL)	37.4 ± 8.4	25.0 ± 4.5	0.1696

Mean values ± SEM.
ND = not detected; NS = not statistically significant.

that some proinflammatory cytokines (for example, IL-1, IL-6, and TNF) act as mediators of metabolic change in cancer patients.[12,17,32,33] For example, acute administration of TNF in humans leads to an increase in acute-phase protein synthesis, elevation of REE, promotion of lipolysis and proteolysis, and anorexia,[14,15] features that are all observed in patients with cancer cachexia.

In this study, 45% of the 21 pancreatic cancer patients had evidence of an acute-phase protein response (Table 3), and this was associated with a significantly higher REE than in cancer patients with no response (Table 4). It was not clear, however, which cytokines are responsible for such changes. Tumor necrosis factor was not detected in the serum of any patients, and IL-6, although present, was not significantly higher in patients with an acute-phase protein response. In contrast, the spontaneous secretion of both TNF and IL-6 by PBMCs was significantly greater in patients with an inflammatory response. These findings suggest that local production of cytokines by macrophages in tissues such as the liver may be of much greater importance in metabolic regulation than circulating levels in the serum. However, further investigation is needed to prove that such upregulated cytokine production is directly responsible for the metabolic changes we observed in these patients.

In support of the contention that cytokines are key mediators in cancer cachexia, Tracey and coworkers[12] showed that repeated injections of TNF into rats results in a syndrome characterized by marked weight loss, anorexia, increased catabolism and anemia, and Oliff and colleagues[17] found that implantation of tumor cells transfected with the TNF gene into nude mice resulted in marked cachexia. Sherry and associates,[34] using anti-TNF antibodies in cachectic tumor-bearing mice, reduced anorexia and some aspects of tissue wasting, although they were unable to attenuate the acute-phase protein response. Thus, although TNF may be important in some murine models of cancer cachexia, it is not responsible for all the metabolic changes observed. Yoneda and colleagues[18] showed that the marked cachexia and leukocytosis seen in nude mice bearing a human maxillary squamous carcinoma is associated with elevated levels of serum TNF and that the cachexia can be reversed by anti-TNF antibodies. However, the cytokine abnormalities in this model appeared to originate in the host rather than from the tumor because they could be reversed by splenectomy. Recently Matthys and associates[35] induced marked wasting after inoculation of gamma interferon-producing tumor cells into nude mice, and antibodies against gamma interferon were shown to attenuate the development of cachexia in a rat cachexia model.[36] Similarly, IL-6 levels are elevated in the serum of tumor-bearing mice[37] and of patients with advanced cancer.[30] Furthermore, antibody to murine IL-6 suppresses development of key features of cachexia in mice with C26IVX carcinoma.[19] Because the presence of one cytokine frequently leads to the production of another,[38] it is difficult to determine which mediator is most important. Nevertheless, these findings suggest that the cytokine network contributes significantly in the development of cancer cachexia.

In our study, an acute-phase response identified a subgroup of pancreatic cancer patients with a particularly elevated REE (Table 4) and an associated increase in the PBMC production of IL-6 and TNF (Table 5). Nevertheless, even pancreatic cancer patients without an acute-phase response had elevated REE when compared with controls (Tables 2 and 4). Cancer patients with an acute-phase response lost on average 20% of their preillness stable weight, a loss that was not significantly greater than that seen in patients without an acute-phase response (16%) (Table 3). Such observations clearly suggest

that factors other than the mediators associated with the development of an overt inflammatory response must contribute to weight loss in some patients. The findings of our study suggest, however, that an inflammatory response may contribute to significant hypermetabolism in some patients with pancreatic cancer and provide support for a trial of agents that might reduce the inflammatory response in these persons.

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