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Metabolic disturbance and sensitivity to endotoxin in patients with advanced cancer: relationship to lymphocyte reactivity, tumour necrosis factor (TNF) production and survival

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SUMMARY

Increased TNF production and impaired lymphocyte function have been individually linked with metabolic disturbance, endotoxaemia and mortality in humans. The inter-relationship between these observations was investigated in humans with cancer. In 13 patients with metastatic colorectal cancer and seven healthy volunteers, observations ($n = 23$) included peripheral blood mononuclear cell (PBMC) TNF production, IL-2 production and phytohaemagglutinin (PHA) response; the acute-phase protein response (APPR) (serum C-reactive protein (CRP), albumin, CRP/albumin ratio), and survival. APPR correlated with survival (CRP, $r = -0{\cdot}689$, $P = 0{\cdot}006$; CRP/albumin, $r = -0{\cdot}758$, $P = 0{\cdot}002$; albumin, $r = 0{\cdot}655$, $P = 0{\cdot}011$), but not with TNF production. TNF production in response to *in vitro* endotoxin correlated with impaired lymphocyte function in patients ($r = 0{\cdot}567$, $P = 0{\cdot}043$) and in the whole group ($r = 0{\cdot}65$, $P = 0{\cdot}001$). The ratio (basal PBMC TNF production)/(lymphocyte function) correlated with CRP ($r = 0{\cdot}569$, $P = 0{\cdot}042$), CRP/albumin ($r = 0{\cdot}617$, $P = 0{\cdot}025$), endotoxin sensitivity ($r = 0{\cdot}567$, $P = 0{\cdot}043$) and survival ($r = -0{\cdot}545$, $P = 0{\cdot}038$) in patients, and the whole group ($P < 0{\cdot}002$). Impaired lymphocyte function may influence TNF production, endotoxin sensitivity and metabolic disturbance in humans with cancer. (r = Spearman correlation coefficient.)

Keywords tumour necrosis factor IL-2 lymphocyte acute-phase proteins survival cancer

INTRODUCTION

Many patients with cancer develop metabolic dysfunction consisting of refractory weight loss, decreased appetite and altered metabolism of body protein and fat (cachexia) [1]. They are also relatively susceptible to sepsis [2,3]. These two phenomena (cachexia and sepsis) account for a significant proportion of cancer-related deaths [2,3], but their development appears to be unrelated to tumour bulk [1,4], and is in fact linked to the activity of monokines, e.g. IL-1 and IL-6, particularly TNF/cachectin [4–7].

While there is significant evidence to implicate TNF and endotoxin in the induction of these phenomena and the associated acute-phase response [6–12], serum levels of TNF or endotoxin have not been correlated directly with parameters reflecting cachexia, the acute-phase response or sepsis [13,14]. This may be related to the fact that sensitivity to TNF and endotoxins may vary, particularly in the tumour state [15,16].

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In many conditions associated with sepsis and metabolic disturbance (e.g. cancer, burns, trauma, AIDS) in which TNF is thought to be an important mediator [17–19] lymphocyte function is frequently impaired [20–22]. Whether lymphocyte function alters sensitivity to, or production of TNF is unknown, but receptors for lymphocyte-derived substances, such as IL-2 and IL-4, are expressed on cells such as peripheral blood monocytes, which are highly potent in terms of TNF secretion [23,24]. Some of these cytokines, e.g. IL-4, may reduce monocyte production of TNF and IL-1 in response to *in vitro* endotoxin [24].

The present study was designed to investigate the relationships between lymphocyte function, TNF production, survival, and parameters reflecting cachexia and the acute-phase response of patients with advanced metastatic colorectal cancer. A further aim was to establish whether factors related to peripheral blood cell type and concentration, or the ability of these cells to produce TNF, might account for the expected differences in metabolic status and acute-phase protein response between older patients with metastatic cancer and healthy younger volunteers.

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PATIENTS AND METHODS

Subjects and protocol

With the approval of the Hospital Ethics Committee and informed patient consent, the study group consisted of 13 patients (11 males), median age 50 years (range 39–73 years), with biopsy-proven liver metastases, each of whom had a primary resection for colorectal cancer at least 6 months before the study. All had received chemotherapy (5-fluorouracil via the hepatic artery) 1 week before the study, and metastases accounted for a significant proportion of liver volume. All were out-patients with no evidence of gastrointestinal obstruction or clinical infection, and all were consuming a normal diet. Seven healthy male laboratory staff were also studied (median age 31 years, range 24–41 years). Three of these had experienced minor weight gain ($< 3\%$) over the previous 6 months.

Blood samples were drawn in the early morning from fasted patients and volunteers. For logistical reasons, the study was performed over 2 study days separated by 9 weeks. One patient and two volunteers were studied on both study days, and resulting data therefore consisted of 23 observations for most of the parameters studied.

Serum albumin, serum C-reactive protein (CRP) and CRP/albumin ratios were used as indices of the acute-phase protein response [25]. Routine haematology tests were performed. Weight change over the previous 6 months was assessed by questionnaire and patient records, and survival from day of study was noted for all patients.

Assessment of lymphocyte function

Two assays of *in vitro* lymphocyte function were used in the study, IL-2 production and phytohaemagglutinin (PHA)-induced blastogenesis.

Generation of IL-2 supernatants

Heparinized peripheral blood (20 ml) was separated over Ficoll as previously described [26]. Unless otherwise stated, reagents for cell washing and culture were obtained from Sigma Chemical Co. (Poole, UK). Cells were counted and concentrations adjusted to 1×10^6 cells/ml in medium with added fetal calf serum (FCS; 5%). Cells (100 μl) were then plated in microtitre plates and 20 μl of PHA (62·5 μg/ml) were added to triplicate wells and the final concentration in stimulus and control wells made up to 250 μl volumes with media. Following a 24-h incubation under standard conditions [26], supernatants were withdrawn and frozen at -20°C.

IL-2 assay

IL-2 concentration was determined using the CTLL-2 cell bioassay [27]. Cell viability was determined using a colorimetric method [28]. An IL-2 standard of 2·5 U/ml was incorporated in the assay for determination of sample IL-2 concentration. All samples were assayed on the same day.

Lymphocyte blastogenesis

Mononuclear cells were isolated by Ficoll density centrifugation [26] as above and concentrations adjusted to 1×10^6 cells/ml in medium with added FCS (5%). Cells (100 μl) were then dispensed in microtitre plates. PHA (20 μl of 125 μg/ml) was added to each well at a final concentration of 10 μg/ml. Media were added to attain equal well volumes of 250 μl/well. Each assay was performed in triplicate and background wells, without PHA, were also used for each sample. Cultures were incubated under standard conditions [26]. Tritiated thymidine (^{3}H-TdR), 1 μCi/well in 20 μl media, was added after 72 h and the plates removed after a further 16 h and frozen at -20°C. Cells were later harvested and uptake of ^{3}H-TdR determined on a beta-counter (LKB Instruments, Gaithersburg, MD). The mean response in ct/min for triplicate samples without PHA was subtracted from the mean of samples with PHA to yield the PHA response.

To eliminate interassay variation between both study days, percentage PHA response of each subject with respect to the mean of the five healthy volunteers studied on that day, was used as an index, rather than PHA response in ct/min, which may be subject to daily variation.

Percentage volunteer PHA response for each subject was calculated by the formula: % volunteer PHA response = ((PHA response in ct/min for each subject)/(mean PHA response in ct/min for volunteers studied on the day)) $\times 100$.

Peripheral blood mononuclear cell TNF production

Peripheral blood mononuclear cells (PBMC) were separated as above and plated in microtitre plates at 1×10^6 cells/well in 200 μl medium (as above). *Escherichia coli* endotoxin 055-B5 (or saline) (Difco Labs, Detroit, MI), 20 μl to yield a final concentration of 1·25 μg/ml was added to each of triplicate wells to generate TNF-containing supernatants. Samples were incubated under the same conditions as above for 24 h, at which time cell-free supernatants were collected and frozen at -20°C.

In seven of the subjects (four patients and three volunteers) TNF production in response to a higher concentration of endotoxin (5 μg/ml) was also measured.

TNF bioassay

Supernatant cytotoxicity was titrated against the TNF-sensitive cell line WEHI 164 clone 13 [29] using a recombinant human TNF preparation as a standard. PBMC TNF production was then calculated in relation to the standard TNF preparation. The stimulation index (SI) for TNF production was calculated by the formula: SI for TNF (S/U) = amount of TNF generated in endotoxin-stimulated cultures/amount of TNF generated in cultures with saline (basal TNF production).

Statistical analysis

Dose–response curves from the IL-2 and TNF bioassays were analysed using the curve fitting program 'ALLFIT' [30]. All parameters are expressed as medians with range of values and were compared between patients and volunteers using the Mann–Whitney test. Spearman tests were used to evaluate correlation coefficients. Significance was implied for $P < 0.05$.

RESULTS

Survival

Median patient survival was 144 days (range 5–679 days). Survival duration was significantly associated with increased serum albumin, decreased serum CRP, decreased CRP/albumin, and decreased leucocyte count (Table 1, Fig. 1). Survival did not correlate with weight loss (Table 1).

Table 1. Relationship between immune and metabolic function, and survival in patients ($n = 13$) with metastatic colorectal cancer

	r value	*P*
CRP/albumin (ratio)	−0·758	0·002*
CRP (mg/*l*)	−0·689	0·006*
Albumin (g/*l*)	0·655	0·011*
Leucocyte count ($\times 10^9/l$)	−0·67	0·012*
Basal PBMC TNF (pg/ml per million PBMC)	−0·534	0·049*
Weight loss (%)	0·15	0·608

r = Spearman correlation coefficient.

* Implies statistical significance.

CRP, C-reactive protein; PBMC, peripheral blood mononuclear cells.

Weight loss, serum albumin and CRP values

Though all patients had similar tumours, large variations were noted in terms of weight loss (3–25%), serum CRP (8–180 mg/*l*) and albumin values (18–42 g/*l*). Median serum albumin levels for patients (31 g/*l*) were significantly reduced, and serum CRP concentrations (54 mg/*l*) and CRP/albumin ratios (2·1) were increased, compared with normal reference ranges for this population (albumin (39–46 g/*l*), CRP (< 10 mg/*l*), CRP/albumin ($< 0·28$)) and with volunteers.

Although weight loss correlated with decreased serum albumin levels ($r = -0·62$, $P < 0·018$), significant correlations were not observed between weight loss and CRP ($P = 0·549$) or CRP/albumin ratios ($P = 0·399$) in patients.

Haematology

Peripheral blood percentages of neutrophils, lymphocytes, monocytes, leucocytes or monocyte/lymphocyte ratios were not significantly different between patients and volunteers.

Cell culture concentrations

Percentages of monocytes and lymphocytes in Ficoll-prepared mononuclear cell populations were calculated from peripheral blood indices, and values are given as for those of peripheral blood, less neutrophil percentage, as the percentage of polymorphs after Ficoll preparation was found to amount to $< 5\%$ of the total cell number. No significant differences were observed between patients and volunteers.

IL-2 production

IL-2 production varied in patients (0·34 (0·6–1·2 U/ml)) and volunteers (0·9 (0·1–6 U/ml)) but was significantly less in patients ($P = 0·03$). In patients, IL-2 production failed to correlate with leucocyte count ($P = 0·871$), percentage of lymphocytes ($P = 0·19$), survival ($P = 0·288$), serum albumin ($P = 0·254$), CRP ($r = -0·477$, $P = 0·08$) or CRP/albumin ratio ($r = -0·534$, $P = 0·06$).

Lymphocyte blastogenesis

PHA responses correlated with IL-2 production for the whole group ($r = 0·589$, $P < 0·004$) and for patients ($r = 0·683$, $P < 0·01$), and was significantly less in patients (38 (9–80%)) compared with volunteers (100 (60–154)) ($P < 0·0005$). No correlations were observed in patients between PHA responses and CRP ($P = 0·396$), CRP/albumin ratio ($P = 0·323$), albumin ($P = 0·766$), basal TNF production ($P = 0·52$), percent lymphocytes ($P = 0·169$), leucocyte count ($P = 0·199$), or survival ($P = 0·499$).

TNF production

In both patients and volunteers, basal PBMC TNF production was variable and was not significantly different between patients and volunteers (Table 2). For the group as a whole (patients and volunteers), basal PBMC TNF production failed to correlate with the proportion of monocytes ($P = 0·358$) or lymphocytes ($P = 0·355$) in the culture pool, nor did it correlate with these indices in either group alone.

In patients, basal TNF production correlated weakly with survival ($r = -0·534$, $P = 0·049$) and leucocyte count ($r = 0·676$, $P = 0·011$), but not with CRP ($P = 0·276$), CRP/albumin ($P = 0·125$), weight loss ($P = 0·964$), serum albumin ($P = 0·111$), percentage circulating lymphocytes ($P = 0·237$) or neutrophils ($P = 0·319$).

TNF production in response to in vitro *endotoxin*

There was no significant difference in TNF production in response to *in vitro* endotoxin (1·25 μg/ml) (endotoxin sensitivity) between patients and volunteers (Table 2), nor was endotoxin sensitivity related to the proportion of monocytes in blood ($P = 0·75$) or culture ($P = 0·318$) in the whole group. In a significant number of patients ($n = 5$) and controls ($n = 6$), no increase in TNF production was noted in response to endotoxin challenge.

In the four patients and three controls in whom enough cells were present to examine responses to higher concentrations of endotoxin (5 μg/ml), SIs in patients (1·15 (0·92–1·98 pg/ml)) were similar to those of volunteers (0·93 (0·28–2·02 pg/ml)).

For all subjects, depressed lymphocyte function was associated with increased sensitivity to *in vitro* endotoxin, reflected in correlations between both the change in TNF production in response to endotoxin and the SI for TNF, and decreased IL-2

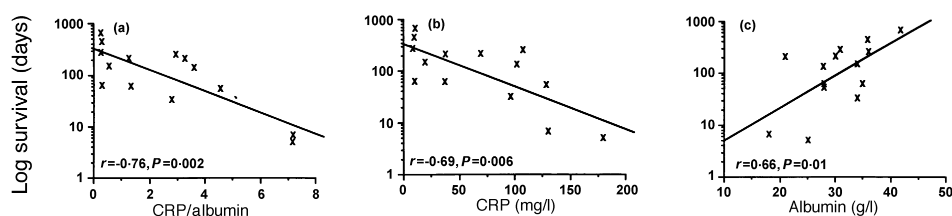

Fig. 1. Association between survival and ratio C-reactive protein (CRP)/albumin (a), CRP (b) and albumin (c) in the patient group. r = Spearman correlation coefficient between absolute values. Survival is indicated in log form for illustration purposes.

Table 2. Peripheral blood mononuclear cell (PBMC) TNF production

	Patients ($n = 14$)	Volunteers ($n = 9$)	P
In vitro *TNF production (basal)*			
pg/ml per million PBMC	119 (16–2100)	49 (19–402)	0·16
pg/ml per million monos	774 (112–10 500)	320 (9–1158)	0·10
In vitro *TNF production (endotoxin-stimulated)*			
Change in TNF production			
(pg/ml per million PBMC)	+23 (−77 to +770)	−7 (−3 to +15)	0·18
(pg/ml per million monos)	+76 (−98 to +3850)	−22 (−192 to 47)	0·08
Stimulation index for TNF	1·1 (0·6–1·9)	0·9 (0·3–1·4)	0·23
Comparison of (basal TNF/lymphocyte function)			
TNF/IL-2 ratio	426 (17–7211)	114 (8–231)	0·001*
TNF/PHA ratio	5·3 (0·2–38·7)	0·5 (0·2–6·8)	0·001*

n, Observations; stimulation index for TNF = PBMC TNF production in response to *in vitro* endotoxin (1·25 μg/ml) divided by PBMC TNF production without endotoxin; (pg/ml per million) = pg of TNF per ml of supernatant per million cells in culture; +, an increase in production; −, a decrease in production.

* Implies statistical significance by Mann–Whitney test.

production and reduced PHA response (Table 3, Fig. 2). In patients alone the SI for TNF also correlated with impaired PHA response ($r = -0{\cdot}567$, $P = 0{\cdot}043$).

When higher concentrations of endotoxin (5 μg/ml) were used, SIs also correlated significantly with impaired lymphocyte function, i.e. IL-2 production ($r = -0{\cdot}821$, $P < 0{\cdot}023$, $n = 7$).

TNF production/lymphocyte function

Since increased sensitivity to endotoxin *in vitro* was related to impaired lymphocyte function (Table 3, Fig. 2), and since sensitivity to endotoxin often parallels sensitivity to TNF [15,16], indices were derived incorporating basal TNF production/lymphocyte function, to determine whether increased TNF activity *in vivo* might relate to impairment of *in vitro* lymphocyte function. In patients, one or both of these indices (TNF/IL-2 or TNF/PHA) correlated significantly with increased CRP, increased CRP/albumin, endotoxin sensitivity, and survival (Table 4, Figs 3 and 4).

For all subjects, these indices correlated with parameters which had been associated with impaired survival, i.e. increased CRP, CRP/albumin, and leucocyte count, and decreased albumin (Table 4, Fig. 5).

IL-2 production failed to correlate with any of these parameters in patients, while TNF only correlated with leucocyte count ($r = 0{\cdot}676$, $P < 0{\cdot}011$) and survival ($r = 0{\cdot}534$, $P = 0{\cdot}049$). Indices (TNF/PHA and IL-2/PHA) were significantly higher in patients than in volunteers (Table 2).

DISCUSSION

The magnitude of the acute-phase protein response in patients with metastatic colorectal cancer correlated with reduced survival in the present study and in previous studies of patients with other tumours [31]. Patients and volunteers showed similar TNF production and cell concentrations, and neither variable correlated with the presence or magnitude of the acute-phase protein response, and failed to separate the diverse groups in terms of outcome.

These results do not necessarily exclude a role for TNF in the acute-phase protein response, since *in vivo* TNF production in the subjects may have been affected by levels of circulating endotoxin [7,8,12,26] and *in vivo* sensitivity to TNF may increase in the cancer state [15]. Sensitivity to endotoxin may also vary, and in cancer appears to parallel sensitivity to TNF [15]. In the present study, variations in endotoxin sensitivity (*in vitro* TNF

Table 3. Association between response to *in vitro* endotoxin and impaired *in vitro* lymphocyte function

	IL-2 production		PHA response	
All subjects ($n = 23$)	r value	P	r value	P
Change in TNF production				
TNF (pg/ml per million PBMC)	−0·458	0·032*	−0·45	0·036*
TNF (pg/ml per million monos)	−0·556	0·011*	−0·505	0·023*

n, Observations; (pg/ml per million) = pg of TNF per ml of supernatant per million cells in culture; r, Spearman correlation coefficient.

* Implies statistical significance by Spearman test.

PBMC, Peripheral blood mononuclear cells.

Table 4. Associations between *in vitro* basal peripheral blood mononuclear cell (PBMC) TNF production/lymphocyte function to parameters of cachexia and the acute-phase protein response

	TNF/IL-2		TNF/PHA	
	r value	*P*	*r* value	*P*
Patients (n = 13)				
CRP (mg/*l*)	0·569	0·042*	0·526	0·065
CRP/albumin (ratio)	0·617	0·025*	0·579	0·038*
All subjects (n = 22)				
CRP/albumin (ratio)	0·711	0·0005*	0·795	0·0005*
Albumin (g/*l*)	−0·689	0·0005*	−0·693	0·0005*
CRP (mg/*l*)	0·659	0·002*	0·738	0·0005*
Weight change (%)	0·588	0·004*	0·491	0·020*
In vitro *TNF production in response to endotoxin*				
Stimulation index	0·630	0·002*	0·491	0·020*
Change in production:				
pg/ml per million PBMC	0·473	0·026*	0·397	0·067
pg/ml per million monos	0·605	0·005*	0·485	0·030*

n, Observations; basal TNF production, unstimulated PBMC TNF production; PHA, phytohaemagglutinin response expressed as a percentage of the mean for volunteers; TNF/IL-2, basal PBMC TNF production divided by IL-2 production; TNF/PHA, basal PBMC TNF production divided by PHA response; *r*, Spearman correlation coefficient.

* Implies statistical significance.

CRP, C-reactive protein.

production in response to endotoxin) were related to lymphocyte function rather than the presence of tumour, or variability in monocyte concentrations in blood or culture. Increased sensitivity to endotoxin, and TNF, may therefore be related to impaired lymphocyte function.

Increased ratios of TNF/lymphocyte function correlated with clinical disturbances normally attributed to TNF activity [7–12], with reduced survival, and with *in vitro* sensitivity to endotoxin. Improvement of lymphocyte function can prolong survival in animal models of conditions associated with endotoxaemia and increased TNF activity, e.g. Gram-negative sepsis, in whom mortality is related to impaired lymphocyte function [32–34]. Addition of IL-2 reduces *in vitro* PBMC TNF production in response to endotoxin [26]. These reports suggest a role for lymphocyte function in determining sensitivity to endotoxins and/or TNF.

This hypothesis may explain the variations in metabolic status in patients with similar tumours [1–3,26,31]; why patients with certain cancers, e.g. breast cancer, who are seldom immunosuppressed [35], rarely develop cachexia [1]; the lack of an acute-phase response in healthy subjects with endotoxaemia; and the poor correlation between serum endotoxin and TNF levels with outcome in immuno- suppressed patients [13,14], in whom impaired lympho-cyte function may predict those at risk of death from sepsis [20,21].

While it is possible that tumour circulation may influence TNF production, tumour homogenates have not been shown to incite significant *in vitro* TNF production. Moreover, serum levels of TNF in humans rarely correlate with clinical abnormalities. Alternatively, other cytokines (IL-1 [5], IL-6 [6] and IL-8 [36]) known to influence inflammatory and metabolic processes [8,9] may be important mediators of the acute-phase response in cancer. However, since TNF stimulates IL-1, TNF, IL-6 and IL-8 production

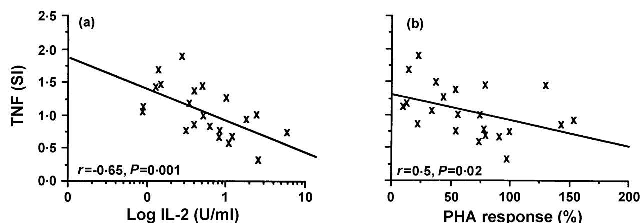

Fig. 2. Associations between *in vitro* peripheral blood mononuclear cell (PBMC) IL-2 production (a) and phytohaemagglutinin (PHA) response (expressed as percentage volunteer response) (b) and the stimulation index (SI) for TNF production in response to *in vitro* endotoxin (1·25 μg/ml). *r* = Spearman correlation coefficient between absolute values. (IL-2 production is indicated in log form for illustration purposes.)

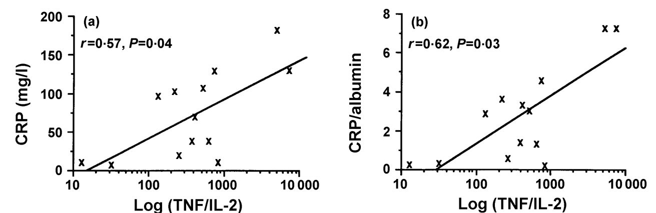

Fig. 3. Association between the ratio TNF/lymphocyte function and patient C-reactive protein (CRP) (*n* = 14) (a), and CRP/albumin ratio (*n* = 13) (b). TNF/IL-2 = basal peripheral blood mononuclear cell (PBMC) TNF production (pg/ml) divided by IL-2 production (U/ml), *r* = Spearman correlation coefficient between absolute values. (IL-2 is expressed in log form for illustration purposes.)

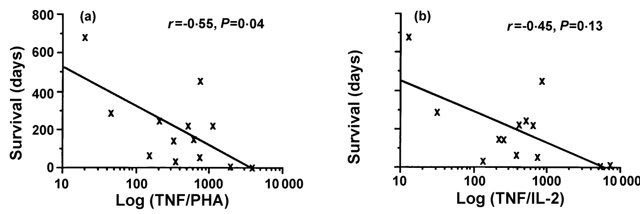

Fig. 4. Association between survival and TNF/IL-2 (a) and TNF/phytohaemagglutinin (PHA) ratios (b). TNF/IL-2 = basal peripheral blood mononuclear cell (PBMC) TNF production (pg/ml) divided by IL-2 production (U/ml), TNF/PHA = basal PBMC TNF production (pg/ml) divided by PHA response (expressed as a percentage of the mean for volunteers). r = Spearman correlation coefficient between absolute values. (TNF/IL-2 and TNF/PHA are presented in log form for illustration purposes.)

[37], lymphocyte function may also indirectly regulate production of these other metabolically active monokines.

The index TNF/lymphocyte function was chosen for the following reasons. TNF has been implicated in the induction of cancer cachexia, the acute-phase response and sepsis [8–12]. Impaired lymphocyte function is related to death from Gram-negative sepsis [20,21,33], a phenomenon related to increased TNF activity [7,10,12,16] and in this study was related to endotoxin sensitivity. In a large proportion of patients who suffer metabolic disturbance and in whom TNF activity is increased, depressed lymphocyte function is common [17–22,38]. Lymphocyte function may be impaired by age, diet, medication and the levels of circulating endotoxins [39], variables which were not strictly controlled in this study, but which might, in the light of our findings, have indirectly influenced monokine activity and metabolism.

Ficoll density centrifugation may not be optimal in separating pure mononuclear cells [40], and methods other than bioassays are available for cytokine assay. However, the methods employed in this study approximate those used in studies quoted, discussing links between impaired lymphocyte function, metabolic disturbance, TNF, and impaired survival in various conditions [7–9,13,14,20,21,33–35,39]. Correlations derived using TNF/lymphocyte activity involved different assessments of lymphocyte function, using different concentrations of stimulant (PHA) and assays of function (thymidine incorporation and bioassay). TNF production by PBMC was chosen, rather than serum levels or secretion by pure monocyte/macrophage cells, as the latter would have neglected the potential influence of lymphocytes on TNF production, while the former fails to consider the short half-life of, and variable sensitivity to, TNF [7,15,16].

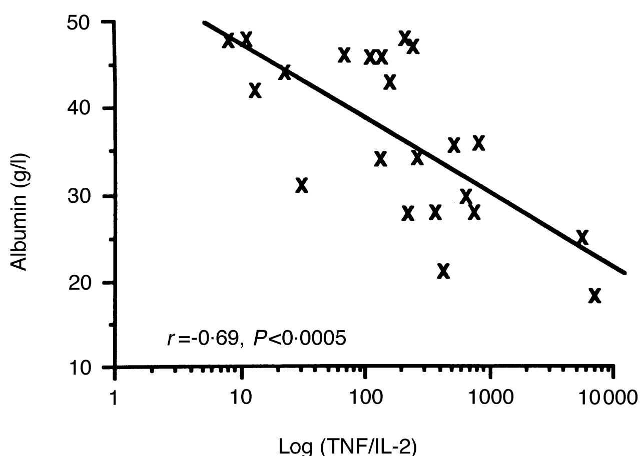

Fig. 5. Association between TNF/IL-2 ratio and albumin in all subjects. TNF/IL-2 = basal TNF production (pg/ml), divided by peripheral blood mononuclear cell (PBMC) IL-2 production (U/ml). r = Spearman correlation coefficient between absolute values. (TNF/IL-2 is presented in log form for illustration purposes.)

Endotoxin is known to stimulate TNF production *in vivo* and *in vitro* [29,39]. In a further study, the index TNF/lymphocyte function was multiplied by the levels of circulating endotoxins. Highly significant correlations were observed between this new index (endotoxin × TNF/lymphocyte function) and factors which reflect altered metabolic state, i.e. CRP, albumin, CRP/albumin, weight loss, body mass index and lean body mass in nine of 12 subjects in this study in whom plasma endotoxin values were significant (unpublished data).

Tolerance to TNF and endotoxins in animals with tumour [15,41,42] renders them less sensitive to cachexia and sepsis, and allows them to survive far longer than those who exhibit normal sensitivity patterns to TNF and endotoxins. The induction of tolerance to TNF or endotoxins in humans might also result in a reduction of cachexia and inflammatory processes. Anti-endotoxin and anti-TNF antibodies have so far been relatively unsuccessful in dealing with endotoxin and TNF [43,44], perhaps because this technology disregards individual differences in sensitivity to endotoxins and TNF, and the fact that healthy subjects secrete TNF [26], and may occasionally have transient endotoxaemias.

Perhaps a study administering low concentrations of IL-2 to improve lymphocyte function might be appropriate. Such an approach improves lymphocyte function in patients with hepatitis B and AIDS [45,46], and in addition, a single dose of 36 000 units of IL-2 reduces TNF mRNA, IL-6 mRNA, and serum CRP in hepatitis B patients for 72 h [45]. Low dose IL-2 (< 250 U/mouse) reduces sensitivity to endotoxins in models of thermal injury, cancer cachexia and endotoxin hypersensitivity [34,35,47], and improves lymphocyte function in animals with burns in whom mortality following septic challenge is related to depressed lymphocyte function [32]. In contrast, high dose IL-2, which is usually toxic [48], is administered in tumour-targeted protocols in concentrations based on surface area, not immune function, with little regard for potential differences in pharmacological and physiological effects of cytokines.

In conclusion, these data suggest that a combination of factors, which include lymphocyte function and PBMC TNF production, may account for increased sensitivity to sepsis and metabolic disturbance in humans with cancer. Administration of physiological concentrations of IL-2 to immunosuppressed patients, in an attempt to improve lymphocyte function and reduce abnormalities associated with TNF and endotoxins, may be worthwhile. Current strategies seem unrewarding in these patients who are subject to an ongoing acute-phase protein response, a phenomenon which was associated with the ultimate demise of the patient group in this study.

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REFERENCES

1 Kern KA, Norton JA. Cancer cachexia. JPEN 1988; **12**:286–98.
2 Warren S. The immediate cause of death in cancer. Am J Med Sci 1932; **185**:610–5.
3 Inagaki J, Rodriguez V, Bodey GP. Causes of death in cancer patients. Cancer 1974; **33**:568–73.
4 Fearon KCH, Carter DC. Cancer cachexia. Ann Surg 1988; **208**:1–5.
5 Dinarello CA. Interleukin-1 and the pathogenesis of the acute phase response. N Eng J Med 1984; **311**:1413–8.
6 Hirano T. Interleukin-6 and its relationship to inflammation and disease. Clin Immunol Immunopathol 1992; **62**:S60–S65.
7 Beutler B, Cerami A. Cachectin and tumor necrosis factor as two sides of the same biological coin. Nature 1986; **320**:584–8.
8 Michie HR, Spriggs DR, Dinarello CA *et al.* Tumor necrosis factor and endotoxin induce similar metabolic responses in human beings. Surgery 1988; **104**:280–6.
9 Moldawer LL, Gelin J, Scherstein T, Lundholm KG. Circulating interleukin-1 and tumor necrosis factor during inflammation. Am J Physiol 1987; **253**:R922–R928.
10 Tracey KJ, Fong Y, Hesse DG *et al.* Anti-cachectin/TNF monoclonal antibodies prevent septic chock during lethal bacteraemia. Nature 1987; **330**:662.
11 Balkwill F, Burke F, Talbot D *et al.* Evidence for tumor necrosis factor production in cancer. Lancet 1987; **2**:1229–32.
12 Michie HR, Guillou P, Wilmore DW. Tumor necrosis factor and bacterial sepsis. Br J Surg 1989; **76**:670–1.
13 Selby P, Hobbs S, Viner C *et al.* Tumor necrosis factor in man: clinical and biological observations. Br J Cancer 1987; **56**:803–8.
14 Calandra T, Baumgartner JD, Grau GE *et al.* Prognosis values of tumor necrosis factor/cachectin, interleukin-1, alpha interferon, gamma interferon, in the serum of patients with septic shock. J Infect Dis 1990; **161**:982–7.
15 Bartholeyns J, Freudenberg M, Galanos C. Growing tumors induce hypersensitivity to endotoxin and tumor necrosis factor. Infect Immun 1987; **55**:2230–3.
16 Tracey KJ, Lowry SF, Cerami A. Cachectin/TNF mediates the pathophysiological effects of bacterial endotoxin/lipopolysaccharide. In: Levin J, ed. Bacterial endotoxins: pathophysiological effects, clinical significance, and pharmacological control. New York: Alan R Liss, 1988:77–78.
17 Lahdevirta J, Maury CPJ, Teppo A, Repo H. Elevated levels of circulating TNF/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am J Med 1988; **85**:289–91.
18 Tracey KJ, Beutler B, Lowry SF *et al.* Shock and tissue injury induced by recombinant human cachectin. Science 1986; **234**:470.
19 Marano MA, Moldawer LL, Fong Y *et al.* Cachectin/TNF in experimental burns and pseudomonas infection. Arch Surg 1988; **125**:1383–8.
20 Wood JJ, Rodrick ML, Palder SB *et al.* Inadequate interleukin-2 production: a fundamental immunological deficiency in patients with major burns. Ann Surg 1984; **200**:311–9.
21 Rodrick ML, Wood JJ, O'Mahony JB *et al.* Mechanisms of immunosuppression associated with severe non-thermal injuries in man: production of interleukin-1 and 2. Clin Immunol 1986; **6**:310–8.
22 Monson JRT, Ramsden C, Guillou PJ. Decreased interleukin-2 production in patients with gastrointestinal cancer. Br J Surg 1986; **73**:483–6.
23 Herrmann F, Cannistra SA, Lindemann A *et al.* Functional consequences of monocyte IL-2 receptor expression. Induction of IL-1 beta secretion by IFN gamma and IL-2. J Immunol 1989; **142**:139–43.
24 Oswald IP, Gazzinelli RT, Sher A. IL-10 synergizes with IL-4 and transforming growth factor-beta to inhibit macrophage cytotoxic activity. J Immunol 1992; **148**:3578–82.
25 Ingenbleck Y, Carpentier YA. A prognostic inflammatory and nutritional index scoring critically ill patients. Int J Vit Nutrit Res 1985; **55**:91–101.
26 Gough DB, Winstanley FP, Fearon KCH, Carter DC. Regulation of tumor necrosis factor production in healthy humans and in patients with cancer. Arch Surg 1992; **127**:713–6.
27 Gillis S, Ferm MM, Ou W, Smith KA. T-cell growth factor: parameters of production and a quantitative microassay for activity. J Immunol 1978; **120**:2027–32.
28 Mosmann TR. A rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J Immunol Methods 1983; **53**:55–62.
29 Espevik T, Nissen-Meyer J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumour necrosis factor from human monocytes. J Immunol Methods 1986; **95**:99.
30 De-Lean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoid curves: application to bioassay, radioligand assay and physiological dose response curves. Am J Physiol 1978; **235**:E97–E101.
31 Falconer JS, Fearon KCH, Ross JA *et al.* Acute-phase protein response and survival duration of patients with pancreatic cancer. Cancer 1995; **75**:2077–82.
32 Moss NM, Gough DB, Jordan A *et al.* Temporal correlation of impaired immune response after thermal injury with susceptibility to infection in a murine model. Surgery 1988; **104**:882–7.
33 Gough DB, Moss NM, Jordan A, Grbic JT, Rodrick ML, Mannick JA. Recombinant interleukin-2 (rIL-2) improves immune response and host resistance to septic challenge in thermally injured mice. Surgery 1988; **104**:292–9.
34 Horgan PG, Mannick JA, Dubravec DB, Rodrick ML. Effect of low dose recombinant interleukin-2 plus indomethacin on mortality after sepsis in a murine burn model. Br J Surg 1990; **77**:401–4.
35 Stein JA, Adler A, Efraim SB, Maor M. Immunocompetence, immunosuppression and human breast cancer. An analysis of their relationship by known parameters of cell mediated immunity in well defined clinical stages of disease. Cancer 1976; **38**:1171–87.
36 Zwahlen R, Walz A, Rot A. *In vitro* and *in vivo* activity and pathophysiology of human interleukin-8 and related peptides. Int Rev Exp Pathol 1993; **34**:27–42.
37 Neta R, Sayers TJ, Oppenheim JJ. Relationship of TNF to interleukins. Immunol Series 1992; **56**:499–566.
38 Brenner BG, Gryllis C, Wainburg MA. Role of antibody dependent cell cytotoxicity and lymphokine activated killer cells in AIDS and related diseases. J Leukocyte Biol 1991; **50**:628–40.
39 Rodrick ML, Moss NM, Grbic JT *et al.* Effects of *in vivo* endotoxin infusions on *in vitro* cellular immune responses in humans. J Clin Immunol 1992; **12**:440–9.
40 Calvano SE, Greenlee PG, Reid AM, de Riesthal HF, Shires GT, Antonacci AC. Granulocyte contamination of Ficoll–Hypaque preparations of mononuclear cells following thermal injury may lead to overstimulation of lymphocyte recovery. J Trauma 1988; **28**:353–61.
41 Oliff A, Defeo-Jones D, Boyer M *et al.* Tumors secreting human TNF/cachectin induce cachexia in mice. Cell 1987; **50**:555–63.
42 Galanos C, Freudenburg MA, Matsuura M, Coumbos A. Hypersensitivity to endotoxin and mechanisms of host response. In: Levin J, ed. Bacterial endotoxins: pathophysiological effects, clinical significance, and pharmacological control. New York: Alan R Liss, 1988;295–308.
43 Dunn DL. Gram-negative bacterial sepsis and sepsis syndrome. Surg Clin N Am 1994; **72**:621–35.
44 Fisher CJ Jr, Opal SM, Dhainaut JF *et al.* Influence of an anti-tumor necrosis factor monoclonal antibody on cytokine levels in patients with sepsis. The CB0006 Sepsis Syndrome Study Group. Crit Care Med 1993; **21**:318–27.

45 Hladik F, Tratkiewicz JA, Tilg H *et al.* Biologic activity of low dosage IL-2 treatment *in vivo*. J Immunol 1994; **153**:1449–54.
46 Teppler H, Kaplan G, Smith KA, Montana AL, Meyn P, Cohn ZA. Prolonged immunostimulatory effect of low-dose polyethylene glycol interleukin-2 in patients with human immundeficiency virus type 1 infection. J Exp Med 1993; **177**:483–92.
47 Gough DB, Winstanley P, Tanner WA, Carter DC. Reduction of monocyte TNF production *in vitro* and tumour induced endotoxin sensitivity *in vitro* with IL-2. Br J Surg 1995; **82**:688–9.
48 Michie HR, Eberlein TJ, Spriggs DR, Manogue KR, Cerami A, Wilmore DW. Interleukin-2 initiates metabolic responses associated with critical illness in humans. Ann Surg 1988; **208**:493–503.
49 Gough DB. Educate the phagocyte! Br J Surg 1991; **78**:1–2.