Modulation of cytokine production *in vivo* by dietary essential fatty acids in patients with colorectal cancer

P. PURASIRI, A MURRAY, S. RICHARDSON, S. D. HEYS, D. HORROBIN* and O. EREMIN
*Department of Surgery, Medical School, Aberdeen University, Aberdeen, U.K., and *Efamol Research Institute, Nova Scotia, Canada*

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1. The effects of essential fatty acids (γ-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid), at a dose of 4.8 g/day, given in combination as dietary supplements, on cytokine production were investigated in patients with colorectal cancer.
2. Total serum cytokines – interleukin (interleukin-1β, 2, 4 and 6), tumour necrosis factor-α and interferon-γ – were analysed using the enzyme-linked immunosorbent assay technique at different time intervals during the course of essential fatty acid supplementation.
3. Fatty acid uptake and patient compliance were confirmed by a significant increase in serum levels of γ-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid in all three fractions: triacylglycerol, cholesterol and phospholipid.
4. There was no significant alteration in total serum cytokine concentration/levels in the first 2 months of essential fatty acid ingestion, but the levels of serum cytokines steadily declined thereafter, reaching minimum levels after 6 months of essential fatty acid supplementation.
5. Essential fatty acids, at the dose and duration (6 months) used in this study, reduced total serum interleukin-1β levels by 61% ($P=0.044$), interleukin-2 by 63% ($P=0.05$), interleukin-4 by 69% ($P=0.025$), interleukin-6 by 83% ($P=0.030$), tumour necrosis factor-α by 73% ($P=0.040$) and interferon-γ by 67% ($P=0.050$).
6. Three months after cessation of essential fatty acid intake, however, these cytokine levels returned to presupplementation values.
7. This present study has shown that long-term $n-3$ and $n-6$ EFA ingestion results in a significant reduction in circulating key cytokines. The precise mechanism of this reduction is unclear.

INTRODUCTION

The nutritional status of a host has a profound effect on an individual's immune responses and ability to deal with invading pathogens or aberrant/malignant cells. On the other hand, the metabolic changes associated with various diseases (e.g. infectious, neoplastic) can adversely affect the nutritional status of the host. Cytokines play an important role in this complex interrelationship [1, 2]. The production and biological function of cytokines are regulated by various factors, including eicosanoids, which are produced from essential fatty acids (EFAs) [1]. Alteration in dietary intake of EFAs (precursors of eicosanoids), therefore, may have important effects on the production and biological function of cytokines.

The cells of the immune system are the main source of cytokines, although other cell types such as fibroblasts, keratinocytes and endothelial cells are also capable of synthesizing and secreting these substances [3]. More recently, malignant cells themselves have been shown to both produce and/or have their growth modulated by cytokines [4, 5]. A notable feature of cytokines is that they act as modulators both of defence mechanisms and of pathological processes of various diseases. As modulators of host defences, they regulate non-specific immune processes, cell-mediated and humoral responses, haematopoiesis and the inflammatory response [1, 2, 6]. As modulators of disease, they induce pain, fever, inflammation and metabolic dysfunction [1, 2, 6]. When produced in an uncontrolled and prolonged manner, cytokines have been implicated in chronic inflammation, cachexia and fatal shock [1]. Recent studies of tumour cells *in vitro* suggest that inappropriate and/or selective activity by various cytokines may either inhibit or stimulate tumour cell growth [7].

Arachidonic acid and its metabolites play a key role in modulating the production and biological functions of various cytokines [6, 8–12]. It has been proposed, therefore, that selective alteration in diet would have differential effects on eicosanoid metabolism. These changes could significantly modify cytokine production and release and thus possibly modify disease processes, including cancer. Preliminary studies had documented beneficial clinical effects of EFAs in a small number of patients with

Key words: colorectal cancer, cytokines, essential fatty acids.
Abbreviations: AA, arachidonic acid; DGLA, dihomogamma-linolenic acid; DHA, docosahexaenoic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; IFN-γ, interferon; IL, interleukin; TNF-α, tumour necrosis factor.
Correspondence: Dr P. Purasiri, Department of Surgery, Medical School, Aberdeen University, Polwarth Buildings, Foresterhill, Aberdeen AB9 2ZD, U.K.

advanced malignancies. In the present study, serum levels of interleukins (IL-1β, IL-2, IL-4, IL-6), tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) were documented, before, during and after EFA supplementation in patients with colorectal cancer. These different cytokines are key regulatory molecules for various anti-cancer host defences (mediated by T cells, natural cytotoxic cells and macrophages) as well as being implicated in some of the metabolic disturbances (malaise, muscular atrophy, weight loss, etc.) associated with the malignant process.

MATERIALS AND METHODS

Patients

Patients, male and female, aged 46–86 years (mean 63 ± 2.33), with clinically localized ($n=10$) and advanced ($n=20$) colorectal cancers were studied. Patients were staged by clinical examination, blood tests (full blood count, urea and electrolytes, liver function tests, serum carcinoembryonic antigen) and various imaging modalities (ultrasound, computerized tomographic scanning, barium studies, etc.). Where appropriate, tissue diagnosis was obtained prior to surgery. The patients were divided into three groups as outlined below.

Group 1. Patients with clinically localized colorectal cancer received EFA (EF6-Scotia) supplements orally for 15 days prior to surgical treatment. EFA supplements were discontinued following surgical excision of the tumour.

Group 2. Patients with advanced colorectal tumours (e.g. extensive hepatic metastases, irresectable intra-abdominal disease) received EFA (EF6-Scotia) supplements orally in increasing doses (four capsules twice daily for 15 days, then six capsules twice daily for the next 15 days and, thereafter, eight capsules twice daily) without surgical intervention for 6 months.

Group 3. Patients with advanced colorectal tumours received no EFA supplementation for 6 months and no surgical intervention (control group).

This was part of a phase II study evaluating the effect of EFAs on disseminated tumour growth. The compliance of the patients was excellent.

EFAs (EF6-Scotia) were provided by Scotia Pharmaceutical Ltd, as enteric-coated, oblong hard gel capsules containing 146 mg of γ-linolenic acid (GLA), 20 mg of docosahexaenoic acid (DHA), 132 mg of eicosapentaenoic acid (EPA), 34.8 mg of lithium and 0.25 mg of ascorbyl palmitate. Some of the GLA was in the form of the lithium salt, which was partially water soluble and may assist in the absorption and distribution of fatty acids. The total daily intake of EFAs was 2.384 g (days 0–15), 3.576 g (days 16–30) and 4.768 g (days 31–182).

Approval for the study was given by the Joint Ethics Committee of the University of Aberdeen and by Grampian Health Board. All subjects gave their informed consent.

Serum preparations

For the measurement of serum fatty acids and serum cytokines, 20 ml of venous blood was obtained and centrifuged at 800 *g* for 20 min. The serum was removed and frozen at −70°C until analysis. Samples were collected on days 0 and 15 and months 1, 2, 3, 4, 5 and 6.

Fatty acid analysis

Serum fatty acids were analysed by the Efamol Research Institute, Kentville, Nova Scotia, Canada. The analysis and transesterification of serum fatty acids was performed as outlined below.

To each tube in which a serum sample was to be analysed was added 100 μl of a solution containing 40 mg of heptadecanoic acid in 100 ml of iso-octane. The iso-octane was evaporated under a stream of nitrogen. To the tube was added 100 μl of serum followed by the addition of 2 ml of methanol–benzene (4:1, v/v). Subsequently, 200 μl of acetyl chloride was added while the solution was stirred. The tubes were capped and heated at 100°C for 1 h. The tubes were cooled to room temperature and 5 ml of 6% (w/v) K_2CO_3 was added and mixed thoroughly. The tubes were then centrifuged for 20 min (950 *g* at 4°C). The benzene layer was removed and added to a gas chromatograph vial for analysis by g.l.c. The values presented are calculated as percentages of the total area of the identified fatty acid peaks in the triglyceride, cholesterol and phospholipid fractions.

Cytokine analysis

Commercial e.l.i.s.a. kits for quantitative measurement of human interleukins (IL-1β, IL-2, IL-6), tumour necrosis factor (TNF-α) and interferon (IFN-γ) were obtained from Medgenix, High Wycombe, U.K., and IL-4 from Genzyme Corporation, Cambridge, U.K.

The assays were solid-phase enzyme-amplified sensitivity immunoassays performed in microtitre plates, and were based on the oligoclonal system. These assays employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody (MAb) specific for each individual cytokine had been coated onto the microtitre plate provided in the kit. Standards and serum samples were pipetted into the wells and any cytokine present was bound by the immobilized antibody. After washing away any unbound proteins, an enzyme-linked polyclonal antibody specific for the cytokine was added to the wells to sandwich the cytokine immobilized during the first incubation. Following another wash to remove any unbound antibody–enzyme reagent, a substrate solution was added to the wells, and colour developed in propor-

Table 1. Percentage of serum fatty acids during EFA supplementation in cholesterol, phospholipid and triacylglycerol fractions in patients with advanced colorectal cancer ($n=14$). Values are means ± SEM.

EFA	Day 0	Day 15	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Cholesterol fraction								
GLA	0.9 ± 0.24	1.84 ± 0.17	2.17 ± 0.2	2.02 ± 0.4	2.26 ± 0.23	2.369 ± 0.478	2.721 ± 0.355	2.817 ± 0.401
EPA	1.27 ± 0.36	3.73 ± 0.36	5.98 ± 0.94	6.26 ± 0.75	8.44 ± 1.53	5.662 ± 1.419	6.225 ± 1.22	7.452 ± 1.616
DHA	0.22 ± 0.17	0.82 ± 0.1	0.88 ± 0.29	0.79 ± 0.34	0.92 ± 0.28	0.913 ± 0.075	0.881 ± 0.131	1.086 ± 0.10
AA	7.46 ± 0.52	7.51 ± 0.48	8.62 ± 0.73	7.96 ± 0.54	6.76 ± 0.86	3.983 ± 1.20	2.443 ± 0.963	6.819 ± 0.457
Phospholipid fraction								
GLA	0.02 ± 0.02	0.17 ± 0.05	0.18 ± 0.04	0.19 ± 0.07	0.16 ± 0.05	0.258 ± 0.048	0.254 ± 0.021	0.171 ± 0.017
EPA	1.59 ± 0.24	3.05 ± 0.76	5.54 ± 0.69	5.49 ± 0.82	7.43 ± 0.69	8.572 ± 1.068	7.841 ± 0.693	6.54 ± 0.917
DHA	5.27 ± 0.56	5.36 ± 0.23	5.33 ± 0.23	5.44 ± 0.7	6.99 ± 1.15	6.508 ± 0.287	7.026 ± 0.520	6.627 ± 0.337
AA	11.24 ± 0.35	10.57 ± 0.6	10.98 ± 0.97	10.34 ± 0.38	9.2 ± 0.21	8.825 ± 0.664	8.66 ± 0.525	9.681 ± 0.680
Triacylglycerol fraction								
GLA	0.15 ± 0.09	0.69 ± 0.29	1.59 ± 0.25	0.99 ± 0.31	1.67 ± 0.13	2.083 ± 0.361	2.013 ± 0.164	2.096 ± 0.197
EPA	0.32 ± 0.11	1.03 ± 0.24	2.38 ± 0.34	1.75 ± 0.41	3.48 ± 0.36	3.974 ± 0.709	2.555 ± 0.523	2.328 ± 0.367
DHA	1.44 ± 0.11	1.32 ± 0.14	2.39 ± 0.35	1.55 ± 0.3	2.67 ± 1.09	2.142 ± 0.139	2.069 ± 0.216	2.189 ± 0.132
AA	1.42 ± 0.09	1.67 ± 0.12	1.99 ± 0.12	1.8 ± 0.14	1.67 ± 0.06	1.661 ± 0.312	1.883 ± 0.300	1.862 ± 0.362

tion to the amount of cytokine bound in the initial step. The colour development was stopped and the intensity (absorbance) of the colour was measured by reading the microtitre plate at 450 nm (reference filter 650 nm) and 490 nm (reference filter 650 nm) with a Dynatech MR 500 microplate reader.

The standard curve was constructed using all standard points for which absorbances were less than 1.5, plotting the absorbance against the standard concentrations of cytokine using linear–linear graph paper. The concentrations of the cytokines in the samples or controls for which absorbance was no greater than 1.5 were determined. If any control or sample had an absorbance greater than the absorbance of the standard reading at 450 nm, a second reading at 490 nm was needed and a second standard curve at 490 nm was constructed using all the standard points. The segment of the curve drawn between the last standard reading at 450 nm and the most concentrated standard would be considered at 490 nm. The concentration of samples and controls for which absorbance was included in this segment was read at 490 nm.

Statistical analysis

The results were expressed as means ± SEM. Student's paired t-tests were used for statistical analysis. P-values less than 0.05 were considered to be significant.

RESULTS

Serum fatty acids

Fatty acid uptake and patient compliance were confirmed by a significant increase in percentage of serum GLA, EPA and DHA in all three cholesterol fractions (triacylglycerol, cholesterol and phospholipid) following EFA supplementation (Table 1). However, the increase in GLA and EPA was more pronounced than the increase in DHA in all three fractions. The percentage of EFAs in serum started to rise 15 days after EFA supplementation and continued to increase with time and dose of EFA ingested. However, there appeared to be a plateau in the level of EPA and, in some cases, DHA at 3–4 months of supplementation. Serum arachidonic acid (AA) showed no significant and consistent alteration in all three fractions following EFA ingestion.

Serum cytokines

Localized colorectal cancer. There was no alteration in serum levels of IL-1β, IL-2, IL-4, IL-6, TNF-α and IFN-γ after 15 days of EFA supplementation in patients with localized cancer, just prior to undergoing elective and curative surgery (data not shown).

Advanced colorectal cancer. IL-1β. Serum IL-β levels began to decline (by 29%) at 3 months following EFA supplementation and continued to decrease at months 4, 5 and 6, by 30%, 60% and 61% ($P=0.044$) respectively (Table 2 and Fig. 1)..

IL-4. Table 2 shows a reduction (by 26%) in serum IL-4 at month 2 and at months 3, 4, 5 and 6 by 38%, 55%, 65% and 69% ($P=0.025$) respectively (Table 2 and Fig. 1).

IL-6. Production of IL-6 was markedly reduced to a minimum at 6 months following EFA supplementation. The serum IL-6 level started to fall (by 33%) at month 2 and continued to decrease at months 3, 4, 5 and 6 by 64%, 79%, 85% and 83% ($P=0.030$) respectively (Table 2 and Fig. 1).

TNF-α. Serum levels of TNF-α began to fall at month 2 with slight fluctuations at month 3 after EFA ingestion, but steadily declined at months 4, 5

Table 2. Serum cytokine levels during EFA supplementation in patients with advanced colorectal cancer ($n=14$). *Refers to a significant alteration in level of cytokine. Values are means ± SEM.

Cytokine	Day 0	Day 15	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	P-value
IL-1β(pg/ml)	131.87 ± 22.5	133 ± 22.38	107.41 ± 18.54	131.04 ± 27.47	93.82 ± 20.85	92.3 ± 27.77*	53.15 ± 3.74*	51.63 ± 8.84*	0.044
IL-2(IU/ml)	3.52 ± 0.57	3.22 ± 0.57	3.41 ± 0.88	3.19 ± 0.83	2.78 ± 0.85	2.24 ± 0.69*	1.9 ± 0.6*	1.31 ± 0.34*	0.050
IL-4(pg/ml)	166.71 ± 37.87	169.29 ± 40.56	123.5 ± 20.78	104 ± 32.67*	92.67 ± 23.04*	75.5 ± 18.45*	58.8 ± 21.5*	51.75 ± 14.73*	0.025
IL-6(pg/ml)	146.14 ± 34.34	172.38 ± 44.58	141.04 ± 45.89	97.37 ± 30.8*	53.02 ± 18.21*	30.9 ± 1.47*	22.58 ± 5.23*	25.01 ± 1.8*	0.030
TNF-α(pg/ml)	44.58 ± 9.77	43.69 ± 9.98	34.92 ± 9.85	30.31 ± 9.84	36.66 ± 14.47	29.22 ± 11.63*	19.89 ± 12.82*	11.86 ± 1.5*	0.040
IFN-γ(IU/ml)	3.91 ± 0.74	2.82 ± 0.57	2.64 ± 0.6	3.17 ± 0.81	3.37 ± 1.17	1.94 ± 0.31*	1.23 ± 0.03*	1.29 ± 0.02*	0.050

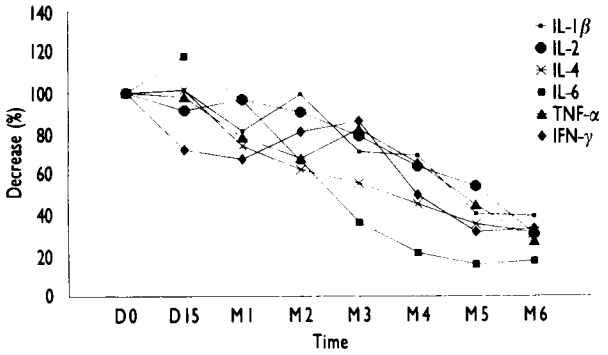

Fig. 1. Percentage decrease in total serum cytokines during EFA supplementation in patients with advanced colorectal cancer ($n=14$), measured at different time intervals [days (D) 0 and 15 and months (M) 1, 2, 3, 4, 5 and 6]

and 6 by 35%, 55% and 73% ($P=0.040$) respectively (Table 2 and Fig. 1).

IFN-γ. There was a fluctuation of serum levels of IFN-γ in the first 3 months after EFA supplementation. Nevertheless, serum levels of IFN-γ began to fall at months 4 (Table 2 and Fig. 1), 5 and 6 by 50%, 69% and 67% ($P=0.050$) respectively, (Table 2 and Fig. 1).

There was no alteration in serum cytokine levels in the group of patients with advanced colorectal cancer (control group) who did not receive EFA supplementation (Table 3).

Serum cytokines after cessation of EFA supplementation ($n=6$)

Six patients with advanced colorectal cancer were available for evaluation of serum cytokines following cessation of EFA intake. Serum cytokine levels in these patients returned to presupplementation values 3 months after cessation of EFA intake (Figs. 2–4).

DISCUSSION

This study has shown that prolonged $n-3$ and $n-6$ EFA (GLA, EPA, DHA) supplementation, in the doses used in this study, in patients with advanced colorectal cancer, suppressed production of total IL-1β, IL-2, IL-4, IL-6, TNF-α and IFN-γ. There was no significant alteration in the serum levels of these cytokines in patients with localized cancer after a short course (15 days) of EFA supplementation. Serum IL-1β and IL-2 began to decline significantly 4 months after EFA supplementation, as did serum TNF-α and IFN-γ (Table 2). The reduction in serum IL-4 and IL-6, on the other hand, occurred much sooner (2 months; Table 2). The reduction in each of the cytokines tested showed a similar pattern, with a time-dependent response (Fig. 1). There was no alteration in serum cytokine levels in the control group (Table 3). Furthermore, all these cytokine levels returned to the presupplementation values 3 months after discontinuation of EFA intake (Figs. 2–4). This rebound phenomenon suggested that the reduction in cytokine release was probably due to direct inhibition of cytokine synthesis by EFAs. Our study, however, does show that the low level of total serum cytokines documented sequentially with time was not the result of the progressive malignant process but a consequence of EFA ingestion.

The production and biological function of cytokines are under the control of products of AA metabolism [6, 8–12]. Our results show that serum AA levels remained unchanged throughout the course of ingestion of the EFAs (Table 1). However, we did not investigate alterations in AA products, either produced locally or released systemically. It has been proposed that selective dietary changes could have differential effects on eicosanoid metabolism and thus could modify cytokine production and/or release. Billar et al. [13] showed that IL-1 production by Kupffer cells from rats fed fish oil for 6 weeks is significantly lower than IL-1 production by Kupffer cells from rats fed corn oil. Endres et al. [14] demonstated that $n-3$ fatty acid (EPA and DHA) supplementation (4.69 g/day) in healthy men for 6 weeks significantly reduces IL-1β and TNF synthesis. The reduced level (and production) of IL-β continued for 10 weeks following cessation of EFA supplements, but returned to normal 20 weeks after cessation of ingestion of supplements. Meydani et al. [15] showed that oral $n-3$ EFA (2.4 g/day) for 3 months reduced serum levels of IL-1β, IL-2, IL-6 and TNF in both young and older healthy women. Virella et al. [16] and Santoli and Zurier [17] showed that *in vitro* addition of EPA to

Table 3. Serum cytokine levels in patients with advanced colorectal cancer ($n=6$) without EFA supplementation (controls). Values are means ± SEM.

Cytokine	Day 0	Day 15	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
IL-1β(pg/ml)	125.27 ± 6.5	130.2 ± 5.3	120.4 ± 12.4	123.4 ± 7.4	120.2 ± 7.5	115.3 ± 8.1	120.1 ± 10.2	124.3 ± 7.4
IL-2(IU/ml)	4.2 ± 0.3	4.1 ± 0.2	3.9 ± 0.2	4.1 ± 0.3	4.2 ± 0.1	3.9 ± 0.2	3.9 ± 0.2	4.1 ± 0.1
IL-4(pg/ml)	170.1 ± 9.4	168.2 ± 10.5	171.4 ± 9.7	174 ± 8.7	169.7 ± 12.4	174.5 ± 7.4	168.3 ± 8.5	169.5 ± 6.3
IL-6(pg/ml)	150.1 ± 6.3	153.3 ± 8.8	149.4 ± 7.7	147.7 ± 6.8	152.2 ± 5.1	154.9 ± 6.7	152.8 ± 5.3	149.1 ± 6.3
TNF-α(pg/ml)	48.5 ± 2.7	45.3 ± 3.8	48.2 ± 2.7	50.1 ± 1.8	52.5 ± 3.2	49.1 ± 1.3	51.5 ± 3.2	50.3 ± 2.2
IFN-γ(IU/ml)	4.2 ± 0.5	4.3 ± 0.4	3.8 ± 0.3	3.7 ± 0.4	4.4 ± 0.2	4.2 ± 0.3	4.1 ± 0.5	3.9 ± 0.3

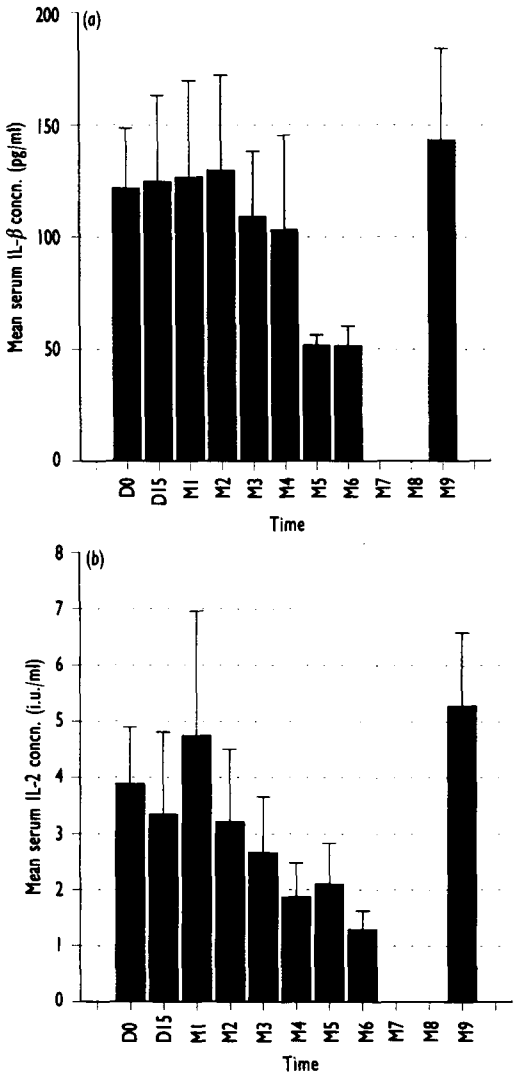

Fig. 2. Serum IL-1β (*a*) and IL-2 (*b*) levels in patients with advanced colorectal cancer before, during and 3 months after cessation of EFA intake ($n=6$). Values are means ± SEM.

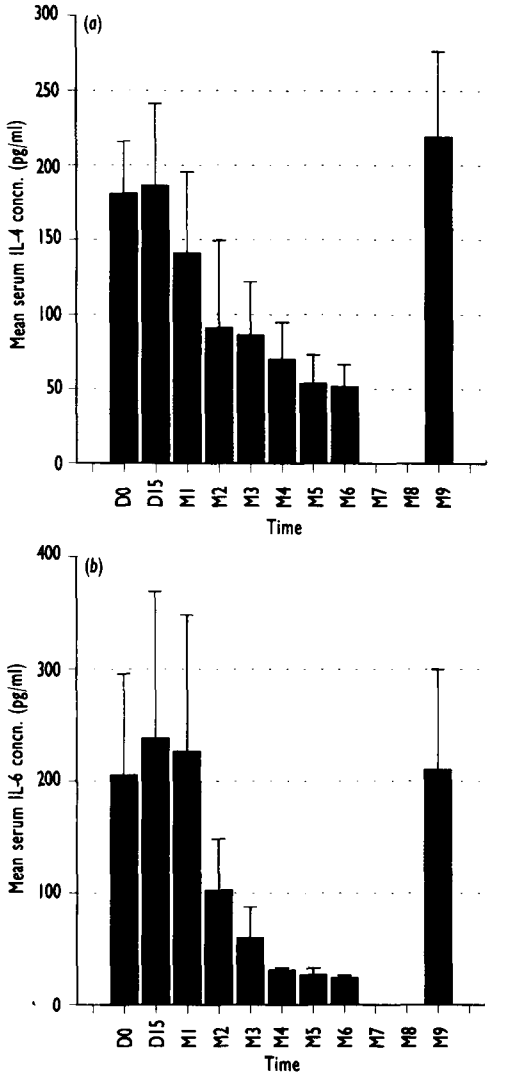

Fig. 3. Serum IL-4 (*a*) and IL-6 (*b*) levels in patients with advanced colorectal cancer before, during and 3 months after cessation of EFA intake ($n=6$). Values are means ± SEM.

human peripheral blood mononuclear cells inhibits the production of IL-2 and expression of the IL-2 receptor. In the study by Santoli and Zurier [17], dihomogamma-linolenic acid (DGLA) also reduced IL-2 production. Calder and Newsholme [18] have shown that *in vitro* $n-3$ and $n-6$ EFAs suppress IL-2 production and mitogen-stimulated lymphocyte proliferation of human lymphocytes. A recent study of ours [19] also showed that *in vivo* EFA (GLA, EPA, DHA) supplementation inhibits *in vitro* mitogen-stimulated lymphocyte proliferation. Supplementation *in vivo* with $n-3$ EFAs, in patients with non-malignant diseases (e.g. rheumatoid arthritis), has been shown to decrease both T- and B-cell

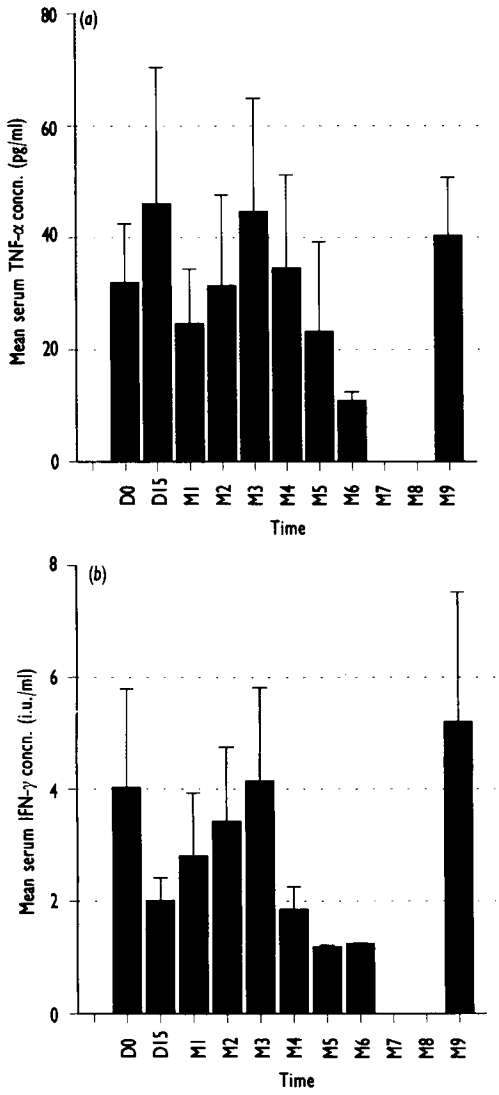

Fig. 4. Serum TNF-α (*a*) and IFN-γ (*b*) levels in patients with advanced colorectal cancer before, during and after cessation of EFA intake ($n=6$). Values are means ± SEM.

proliferation and delayed-type hypersensitivity skin responses [16, 20].

The mechanisms underlying the reduced serum levels of these cytokines following dietary supplementation of $n-3$ and $n-6$ EFAs remain unknown. However, alteration in the type of AA metabolites produced during prolonged EFA ingestion and subsequent differential effects on the stimulation of mononuclear cells may explain in part the decreased production of these cytokines [8, 21]. The $n-3$ and $n-6$ fatty acids induce changes in both cyclo-oxygenase and lipoxygenase products. One possible mechanism may be decreased 5-lipoxygenase metabolites such as leukotriene B_4 [22, 23]. Adding exogenous leukotriene B_4 to human mononuclear cells enhances the production of IL-1, TNF and IFN [24–26]. Conversely, inhibitors of 5-lipoxygenase reduce the production of IL-1 and TNF [14, 27, 28]. Also metabolites of GLA are precursors of the monoenoic prostaglandins, which have been documented to have suppressive effects of T cell and monocyte function and release of IL-2 [29]. Santoli and Zurier [17] showed that EFAs can reduce IL-2 production by mononuclear cells directly and independently of changes in cyclo-oxygenase products. An increased production of lipid peroxides following EFA supplementation might also contribute to the immunosuppressive effect of these fatty acids, since products of lipid peroxidation such as hydrogen peroxide have been shown to have a suppressive effect on lymphocyte proliferation [30]. Meydani et al. [15] demonstrated that supplementation of young and older women with $n-3$ EFAs increases plasma malondialdehyde levels. The decrease in serum cytokine levels observed in this study could be the result of an increase in the formation of products of lipid peroxidation following EFA supplementation. Furthermore, it is not clear whether the decrease in IL-2 production is the result of a direct effect of EFAs on IL-2 production and/or is mediated by the concurrent decrease in IL-1β and IL-6 production.

This study has clearly demonstrated that long-term $n-3$ and $n-6$ EFA ingestion reduces serum levels of a variety of cytokines in patients with advanced malignant disease. To the best of our knowledge, this is the first documentation of such cytokine changes *in vivo* in patients with cancer. Although the induced reduction in certain cytokines (e.g. IL-1β, TNF-α and IFN-γ) may have beneficial anti-inflammatory and anti-metabolic effects, in particular in eliminating fever, malaise, muscle wasting and weight loss in patients with malignancies, the suppression of IL-1β, IL-2 and IL-6 may inhibit T-cell cytotoxicity and natural cytotoxicity and may not be a desirable effect in malignant disease. However, cytokine receptors (e.g. IL-2) are found on many human solid tumours [31], and various tumour cell lines show a differential response (inhibition/stimulation) to specific cytokines (e.g. IL-2, IL-4, IL-6 and TNF-α) [4, 5, 7, 32]. There is, therefore, a complex interaction between the malignant process and cytokines, and the modulation of cytokine production and activity by EFAs merits further urgent and careful study.

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REFERENCES

1. Mizel SB. The interleukins. FASEB J 1989; **3:** 2379–88.
2. Poulam MP, Collins MKL. Cytokine modulation of cell growth and role in tumour therapy. Eur J Cancer 1992; **28:** 298–302.
3. Kennedy RL, Jones TH. Cytokines in endocrinology: their roles in health and in disease. J Endocrinol 1991; **129:** 167–78.
4. Wu S, Boyer CM, Whitaker RS, et al. Tumour necrosis factor-α as an autocrine and paracrine growth factor for ovarian cancer: monocrine induction of tumour cell proliferation and tumour necrosis factor-α expression. Cancer Res 1993; **53:** 1939–44.
5. Van Meir E, Sawamura Y, Diserens A-C, Hamou M-F, de Tribolet, N. Human gliobastoma cells release interleukin 6 in vivo and in vitro. Cancer Res 1990; **50:** 6683–88.
6. Dinarello CA. Biology of interleukin 1. FASEB J 1989; **2:** 108–15.
7. Giulio FP, Lawrence T. Interleukin-2 differentially affects the proliferation of a hormone-dependent and a hormone-independent human breast cancer cell line in vitro. Anticancer Res 1988; **8:** 1233–40.
8. Knudsen PJ, Dinarello CA, Strom TB. Prostaglandins post transcriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol 1986; **137:** 3189–94.
9. Gordon D, Bray MA, Morely J. Control of lymphokine secretion by prostaglandins. Nature (London) 1976; **262:** 401–2.
10. Rappaport RS, Dodge GR. Prostaglandin E inhibits the production of interleukin 2. J Exp Med 1982; **155:** 943–8.
11. Okusawa S, Gelfand JA, Ikejima T, Connolly RJ, Dinarello CA. Interleukin 1 induces a shock-like state in rabbits. Synergism with tumour necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest 1988; **81:** 1162–72.
12. Rola-Pleszczynski M. Immunoregulation by leukotrienes and other lipoxygenase metabolites. Immunol Today 1985; **6:** 302–7.
13. Billar TR, Bankey PE, Svingen BA, et al. Fatty acid intake and Kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. Surgery 1988; **104:** 343–9.
14. Endres S, Ghorbani R, Kelly VE, et al. The effect of dietary supplementation with $n-3$ polyunsaturated fatty acids on the synthesis of interleukin 1 and tumour necrosis factor by mononuclear cells. N Engl J Med 1989; **320:** 265–71.
15. Meydani SN, Endres S, Woods MM, et al. Oral ($n-3$) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J Nutr 1991; **121:** 547–55.
16. Virella G, Kilpatrick JM, Rugeles MT, Hyman B, Russel R. Depression of humoral responses and phagocytic functions in vivo and in vitro by fish oil and eicosapentaenoic acid. Clin Immunol Immunopathol 1989; **52:** 257–70.
17. Santoli D, Zurier RB. Prostaglandin E precursor fatty acids inhibit human IL-2 production by a prostaglandin E-dependent mechanism. J Immunol 1989; **143:** 1303–9.
18. Calder PC, Newsholme EA. Polyunsaturated fatty acids suppress human peripheral blood lymphocyte proliferation and interleukin-2. Clin Sci 1992; **82:** 695–700.
19. Purasiri P, Ashby J, Heys SD, Eremin O. Effect of essential fatty acids on circulating T-cell subsets in patients with colorectal cancer [Abstract]. Eur J Surg Oncol 1993.
20. Kremer JM, Jubiz W, Michalek A, et al. Fish oil fatty acid supplementation in active rheumatoid arthritis. A double blinded, controlled, crossover study. Ann Intern Med 1987; **106:** 497–02.
21. Kunkel SL, Chensue SW, Phan SW. Prostaglandins as endogenous mediators of interleukin 1 production. J Immunol 1986; **136:** 186–92.
22. Strasser T, Fisher S, Weber PC. Leukotriene B_5 is formed in human neutrophils after dietary supplementation with eicosapentaenoic acid. Proc Natl Acad Sci USA 1985; **82:** 1540–3.
23. Lee TH, Hoover RL, Williams JD, et al. Effect of dietary enrichment with eicosapentaenoic acid and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 1985; **312:** 1217–24.
24. Rola-Pleszczynski M, Lemaire I. Leukotrienes augment interleukin 1 production by human monocytes. J Immunol 1985; **135:** 3958–61.
25. Rola-Pleszczynski M, Gagnon L, Chavaellaz P-A. Innume regulation by leukotriene B4. Am NY Acad Sci 1988; **524:** 218–26.
26. Billingham MEJ. Cytokines as inflammatory mediators. Br Med Bull 1987; **43:** 350–70.
27. Dinarello CA, Bishai I, Rosenwasser LJ, Coceani F. The influence of lipoxygenase inhibitors on the in vitro production of human leukocytic pyrogen and lymphocyte activating factor (interleukin 1). Int J Immunopharmacol 1984; **6:** 43–50.
28. Kunkel SL, Chensue SW. Arachidonic acid metabolites regulate interleukin 1 production. Biochem Biophys Res Commun 1985; **128:** 892–7.
29. Zurier RB. Fatty acids, inflammation and the immune response. Prostaglandins Leukotrienes Essential Fatty Acids 1993; **48:** 57–62.
30. Zoschke DC, Messner RP. Suppression of human lymphocyte mitogenesis mediated by phagocyte-released reactive oxygen species, comparative activities in normal and in chronic granulomatous disease. Clin Immunol Immunopathol 1984; **49:** 670–92.
31. Weldmann E, Sacchi M, Plaisance S, et al. Receptor for interleukin 2 on human squamous cell carcinoma cell lines and tumour in situ. Cancer Res 1992; **52:** 5963–70.
32. Masakazu Toi, Bicknell R, Harris AL. Inhibition of colon and breast carcinoma cell growth by interleukin-4. Cancer Res 1992; **52:** 275–9.