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Impact of fish oil enriched total parenteral nutrition on DNA synthesis, cytokine release and receptor expression by lymphocytes in the postoperative period

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A prospective randomized study on sixty patients was conducted to investigate the effects of a fish oil containing total parenteral nutrition (TPN) regimen in the postoperative period on lymphocyte subset distribution, proliferation, cytokine production and interleukin-2 receptor (IL-2R) expression. Patients who underwent large bowel surgery were divided into three groups. Nineteen patients received TPN with fish oil (0·2 g/kg body weight per day) plus soybean oil (1·0 g/kg per day), twenty patients received soybean oil (1·2 g/kg per day), and twenty-one patients who were on a fat-free regimen served as the control group. Natural killer (NK) cells, total, B-, T-, T_4-, T_8-lymphocytes, proliferation of lymphocytes, *in vitro* production of IL-2, IFN-γ, TNF-α, and IL-2R expression were measured. Fish oil administration did not affect subset distribution and proliferation of lymphocytes. Production of interleukin-2 (IL-2), interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) was augmented, and IL-2R expression less enhanced compared with the controls. It is concluded that administration of 0·2 g/kg per day fish oil after a moderate surgical stress is not immunosuppressive, but enhances the production of IFN-γ, TNF-α and possibly IL-2.

Parenteral nutrition: Fish oil: Cellular immunity

Introduction

Oral ingestion of long-chain polyunsaturated fatty acids from the *n*-3 family may exert metabolic effects of potential interest for the prevention and therapy of certain diseases. Some, but not all, epidemiological observations and human feeding trials have provided evidence that diets high in fatty fish may be beneficial in preventing cardiovascular disease (Sanders, 1985; Herold & Kinsella 1986; Ascherio *et al.* 1995). This has been attributed primarily to eicosapentaenic acid (EPA), a long-chain *n*-3 polyunsaturated fatty acid. EPA is one of the major fatty acids in fish oil, but other fatty acids with variable biological activities are also present. It has been suggested that *n*-3 fatty acids may confer protection through mechanisms such as affecting platelet function and platelet-endothelial interactions (Sanders & Roshanai 1983). In non-human primates dietary fish oil has been observed to interrupt vascular thrombus formation (Harker *et al.* 1993). This may be relevant in surgical patients.

To fully appreciate the potential therapeutic value of fish oil, its other effects have to be considered, including those on the immune system. A study on six normal volunteers revealed immunosuppresive properties of fish oil, including a decrease in the release of IL-2 after stimulation with mitogens (Virella *et al.* 1991). In an elaborate dietary trial, with an intervention period of 24 weeks, a 'high fish' diet was shown to diminish T-cell proliferation and cytokine synthesis in humans (Meydani *et al.* 1993). Similar observations were made in a cohort study (Endres *et al.* 1993). Recently a soybean emulsion enriched with EPA was shown to reduce in seven patients the stress response and stress-induced immunosuppression including lymphocyte proliferation after a severely stressful operation, i.e. after surgery for esophageal cancer (Furukawa *et al.* 1999).

In this study we investigated effects of a soybean oil and fish oil containing emulsion on lymphocyte subset distribution, proliferation, cytokine release and interleukin-2

Abbreviations: TPN, total parenteral nutrition; IL-2, interleukin-2; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; EPA, eicosapentanoic acid; PCA, perchloric acid.

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receptor (IL-2R) expression after a moderately stressful operation, i.e. after large bowel surgery.

Patients, materials and methods

Patients and clinical protocol

This prospective, randomized, double-blind protocol was approved by the Ethics Committee of the University of Göttingen. Sixty consecutive patients were enrolled into the study. Informed written consent was obtained from all patients. They underwent large bowel surgery for malignant ($n = 49$) or benign ($n = 11$) conditions (Table 1). After giving informed consent, the patients were randomly assigned to one of three study groups. Exclusion criteria were diabetes mellitus, lipid disorders, serum creatinine >2·0 mg/dl, axillary temperature >38°C, impaired liver function and an increased risk for postoperative complications (Peter *et al.* 1986). Patients were fed exclusively by total parenteral nutrition (TPN) which provided 1·5 g amino acids/kg per day and 6·0 g glucose/kg per day on the preoperative day. Nineteen patients received TPN with soybean oil emulsion plus fish oil (Omegavenös 10 %) (group A), and twenty patients (group B) received soybean oil emulsion (Lipovenös 10 %). Twenty-one patients were on a regimen without fat (group C). All infusion regimens contained amino acids (Aminosteril KE 10 %) and Glucose (Glucosteril 40 %). The solutions were from Fresenius AG, Bad Homburg, Germany. Vitamins, electrolytes and trace elements were added as recommended (Schmitz, 1999). Administration of the solutions was by continuous infusion. Energy content in the regimens was 100·42 kJ/kg per day on day one after operation, and thereafter 125·52 kJ/kg per day (Table 2). Administration of EPA was 0·021 g/kg per day on day 1 and 0·042 g/kg per day on days 2–5 after surgery. Venous blood was obtained from the vena cava between 08·00 and 08·30 prior to operation (day – 1) and on days 3 and 6 after operation.

Table 1. Patient characteristics in the study groups

Patient characteristics	Group A	Group B	Group C
Women (*n*)	7	8	14
Men (*n*)	12	12	7
Age (years)*	68 (2)	66 (2)	65 (2)
Height (cm)*	169 (2)	167 (1)	166 (2)
Weight (kg)*	68 (3)	70 (2)	66 (3)
Diagnosis			
Colon carcinoma	7	7	8
Sigmoid carcinoma	2	3	–
Rectum carcinoma	7	6	9
Colon adenoma	1	1	–
Sigmoid diverticulitis	2	3	4
Procedures			
Right hemicolectomy	5	7	4
Left hemicolectomy	3	3	4
Sigmoid resection	3	3	4
Anterior rectum resection	6	3	7
Quenue's operation	1	3	2
Polypectomy	1	–	–

* Mean values (SD).

Reagents

Reagents and their sources were as follows: concanavalin A (ConA) and poke weed mitogen (PWM) (SIGMA Chemie GmbH, Deisenhofen, Germany); Ficoll^{R}, phosphate – buffered saline (PBS), medium RPMI 1640, heat inactivated dialysed fetal calf serum (FCS), glutamine, and phytohaemagglutinin (PHA) (Biochrom KG, Berlin, Germany); penicillin/streptomycin (Gibco Europe, Karlsruhe, Germany); fatty acid free bovine albumin (BSA), insulin, and transferrin (Boehringer, Mannheim, Germany); eosin red (Serva, Heidelberg, Germany); ^{3}H-thymidine, and Aquasol^{R} (DuPont de Nemours Dreieich, Germany); formaldehyde potassium hydroxide and perchloric acid (Merck, Darmstadt, Germany); Quantikine human IL-2-ELISA (Biermann, Bad Nauheim, Germany); IFN-γ (IRMA) system (Medgenix Ratingen, FRG); anti-human CD25-PE (phycoerythrin), immunoglobin G_{2a}-PE (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Laboratory analyses

Lymphocyte subsets were analysed in whole blood by flow cytometry (FACScan, Becton Dickinson) after specific staining with monoclonal antibodies by the APAAP-method (Cordell *et al.* 1984), using the SimulSet 2.3 software for calculations. The following antibodies were used (Becton Dickinson, San Jose, CA): anti CD3 for T-lymphocytes, anti CD19 for B-lymphocytes, anti CD4 for T-helper cells, anti CD8 for T-suppressor cells, anti CD16 and anti CD56 for NK cells.

Lymphocytes were isolated from heparinized venous blood by Ficoll^{R}-Hypaque gradient (Boyum 1968) as previously described (Koch *et al.* 1990; Schröder *et al.* 1990). To reduce the variability of monocytes/macrophages (10–20 %) in the preparation, cells were incubated for 15 min at 37°C in RPMI 1640 containing 5 % (v/v) dialysed FCS, glutamine (2 mM), penicillin (100 U/ml, and streptomycin (100 μg/ml). Under these conditions monocytes/macrophages preferentially adhered to the plastic of the tissue culture flasks. Nonadherent cells were harvested, washed in PBS, and suspended for culture. The composition of cells in the suspension was as follows: 76 % T-lymphocytes, 10 % NK cells, 7 % B-lymphocytes, and 5–8 % monocytes/macrophages. Lymphocytes (7×10^{6} cells/5 ml) were cultured for 24 or 48 h in a

Table 2. Macronutrients in the postoperative parenteral nutrition regimen

Nutrient	Study group	Day 1	Days 2–5
Glucose	A	3·0	3·0
	B	3·0	3·0
	C	4·5	6·0
Fat	A	0·5 (soybean oil)	1·0 (soybean oil)
		0·1 (fish oil)	0·2 (fish oil)
	B	0·6 (soybean oil)	1·2 (soybean oil)
	C	–	–
Amino acids	A	1·5	1·5
	B	1·5	1·5
	C	1·5	1·5

Values represent g/kg body weight per day.

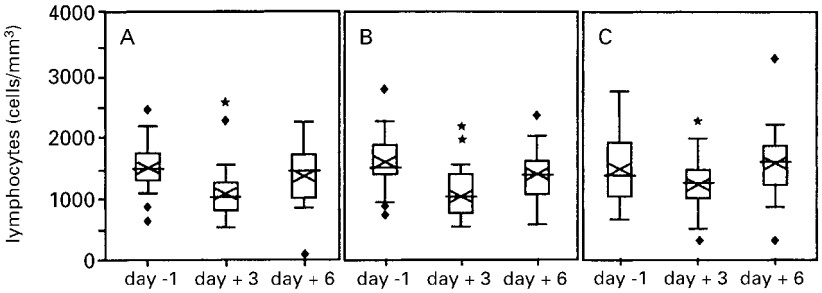

Fig. 1. The number of venous lymphocytes in patients receiving fat free, soybean oil–enriched or fish oil-enriched parenteral nutrition post-surgery. Mean values from group A, B and C are shown by the cross and the medians by the horizontal line in the box. Minimal, maximal and extreme cell numbers are indicated by a rhombus. *$P<0{\cdot}025$; **$P<0{\cdot}005$ *v.* pre-operative values (day − 1).

humidified atmosphere (5 % CO_2 in air) in medium RPMI 1640 supplemented with 1 % fatty acid free bovine albumin (w/v), glutamine (2 mM), insulin (10 μg/ml), transferrin (10 μg/ml), penicillin (100 U/ml), and streptomycin (100 μg/ml).

In the experiments designed to study DNA synthesis, ConA (10 μg/ml) was used as mitogen. Production of interferon γ (IFN-γ), interleukin-2 (IL-2) and IL-2R expression were analyzed in cells stimulated with 0·24 μg PHA/ml for 48 h. In experiments to study tumor necrosis factor α (TNF-α) production, 2·0 μg PWM/ml was added for 24 h. Before and after incubation cell viability was examined by eosin exclusion.

DNA synthesis was determined by measuring ^{3}H-thymidine incorporation into DNA. ^{3}H-thymidine (0·2 μCi/ml) was added to the cultures during the final 4 h of a 48 h incubation. The labelled cells were harvested by centrifugation (10 min at 250 *g*), washed twice in PBS, and precipitated with 1·5 ml cold 0·5 N perchloric acid (PCA). RNA was hydrolyzed by incubation with 0·3 N KOH (0·5 ml) at 37°C for 90 min. After reprecipitation with 0·5 ml 10 % PCA and washing three times with 1·5 ml 0·5 N PCA, the precipitate was finally resuspended in 1·5 ml 0·5 N PCA. To hydrolyze DNA, suspensions were heated for 20 min at 75°C. Aliquots of the solubilized material were transferred to scintillation vials, mixed with 5 ml Aquasol^R, and measured for radioactivity in a Mark III liquid scintillation spectrometer (Searle, Des Plaines, IL).

Cytokine concentrations in culture supernatants were measured using commercially available assay kits based on the ELISA (IL-2, TNF-α) or the IRMA (IFN-γ) technique. None of the assays showed cross-reactivity with other cytokines. Minimum detectable doses were as follows: IL-2, 6·0 pg/ml; IFN-γ, 0·2 IU/ml; TNF-α 60–100 pg/ml. Coefficients of variations for intra-assay precision were as follows: TNF-α 5–8 %, IL-2 4–7 %, IFN-γ 2–3 %. Those for inter-assay precision were as follows: IL-2 6–9 %, IFN-γ 7–10 %, TNF-α 8–10 %. After incubations aliquots from supernatants were frozen at −20°C pending assay. Samples had to be diluted with culture medium 1:10 (v/v) when assaying IFN-γ. Readings of optical densities were made on a MR 5000 microplate reader (Dynatech Laboratories, Guernsey, Channel Islands, UK). ^{125}-Iodine was counted in a 1277 Automatic Gamma Counter (LKB Wallac, Turku, Finland).

IL-2 receptor expression was determined using the phycoerythrin (PE)-conjugated monoclonal antibody anti-human CD25. After a 48 h culture period, 5×10^5 lymphocytes of each culture flask were incubated for 30 min with 10 μl antibody solution. Thereafter, cells were washed twice using PBS containing 2 % (v/v) FCS and finally resuspended in 1 % formaldehyde in PBS. Cells incubated with aliquots of the Simultest^{TM} control mixture (containing IgG_{2a}-PE) were used to correct for nonspecific staining. Analyses were performed on a FACScan flow cytometer (Becton-Dickinson). Mean peak channels (MPC)

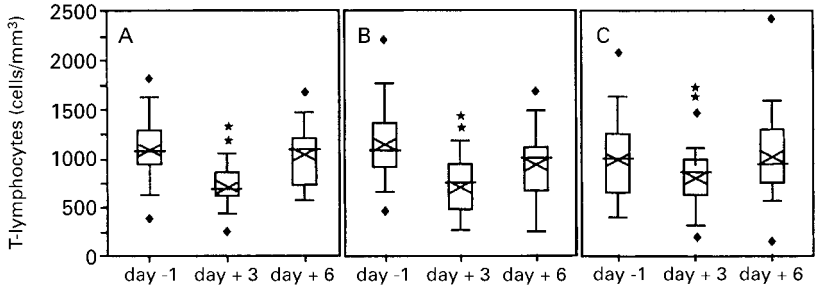

Fig. 2. The number of venous T-lymphocytes in patients receiving fat free, soybean oil–enriched or fish oil-enriched parenteral nutrition post-surgery. Mean values from group A, B and C are shown by the cross and the medians by the horizontal line in the box. Minimal, maximal and extreme cell numbers are indicated by a rhombus. *$P<0{\cdot}025$; **$P<0{\cdot}005$.

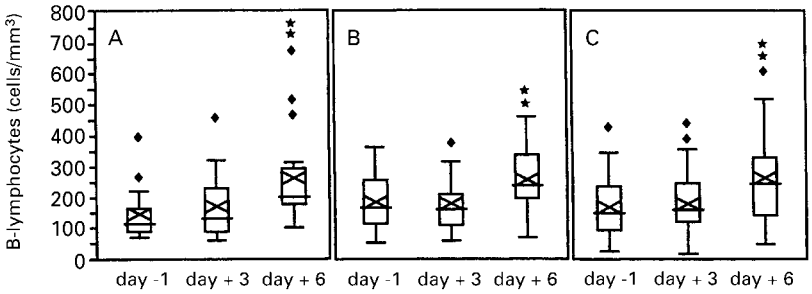

Fig. 3. The number of venous B-lymphocytes in patients receiving fat free, soybean oil–enriched or fish oil-enriched parenteral nutrition post-surgery.Mean values from group A, B and C are shown by the cross and the medians by the horizontal line in the box. Minimal, maximal and extreme cell numbers are indicated by a rhombus. *$P<0{\cdot}025$.

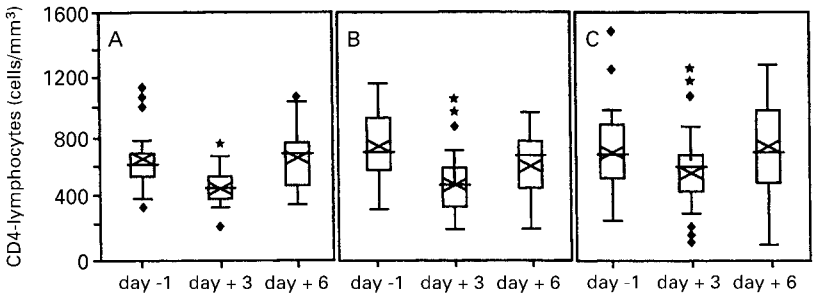

Fig. 4. The number of venous CD_4-lymphocytes in patients receiving fat free, soybean oil–enriched or fish oil-enriched parenteral nutrition post-surgery. Mean values from group A, B and C are shown by the cross and the medians by the horizontal line in the box. Minimal, maximal and extreme cell numbers are indicated by a rhombus. *$P<0{\cdot}025$; **$P<0{\cdot}005$.

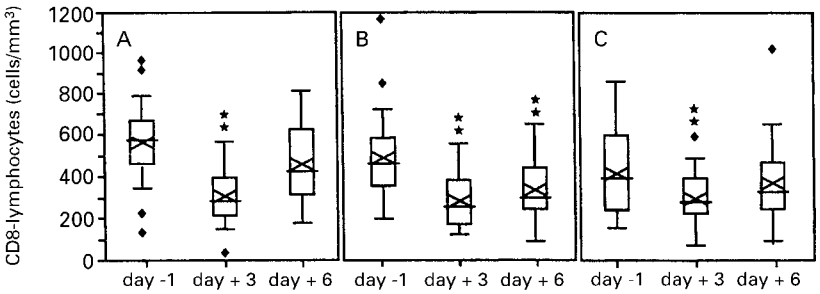

Fig. 5. The number of venous CD_8-lymphocytes in patients receiving fat free, soybean oil–enriched or fish oil-enriched parenteral nutrition post-surgery. Mean values from group A, B and C are shown by the cross and the medians by the horizontal line in the box. Minimal, maximal and extreme cell numbers are indicated by a rhombus. *$P<0{\cdot}025$; **$P<0{\cdot}005$.

found with IgG_{2a}-PE were subtracted from those found with anti-CD25-PE.

Statistics

Logarithmic transformation of the DNA-synthesis data rendered them parametric, and statistical analysis used the PSC 3.03 program (Top-Soft, Hannover, Germany). Data obtained before and after TPN with treatment groups were analysed using the paired *t*-test. A relatively small change in the logarithmic data in Table 4 represents a considerable change in the untransformed data. Results were considered statistically significant, if $P<0{\cdot}025$. Data are expressed as mean values (SD) in the tables and as box plots in the figures.

Results

The clinical details of all the patients studied are shown in Table 1. There were no statistical differences among the

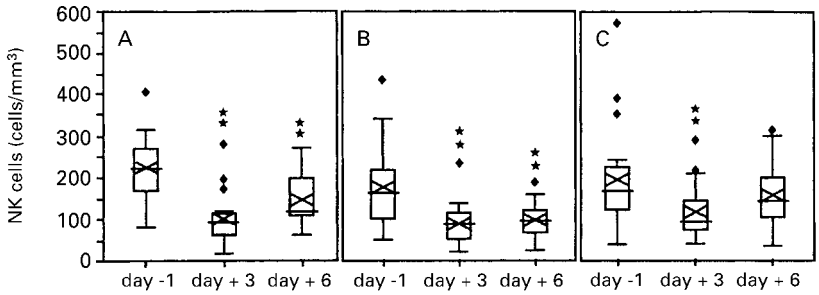

Fig. 6. The number of venous NK-cells in patients receiving fat free, soybean oil–enriched or fish oil-enriched parenteral nutrition post-surgery. Mean values from group A, B and C are shown by the cross, and the medians by the horizontal line in the box. Minimal, maximal and extreme cell numbers are indicated by a rhombus. *$P<0.025$.

Table 3. The CD_4/CD_8 ratio in patients receving fat free, soybean oil - enriched or fish oil-enriched parenteral nutrition post-surgery

	CD_4-CD_8 ratio		
Day	Group A (*n* 18)	Group B (*n* 20)	Group C (*n* 21)
−1	1·4 (0·2)	1·7 (0·1)	1·9 (0·2)
+3	1·6 (0·1)	1·8 (0·1)	2·2 (0·3)
+6	1·8 (0·3)**	2·0 (0·1)*	2·2 (0·3)

Mean values (SD); *$P<0.025$; **$P<0.005$ *v.* pre-operative value (day − 1).

Table 4. DNA-synthesis by lymphocytes in patients receiving fat free, soybean Oil-enriched or fish oil-enriched parenteral nutrition post-surgery

DNA synthesis	Day	Group A (*n* 15)	Group B (*n* 17)	Group C (*n* 16)
Unstimulated	−1	2·15 (0·16)	2·21 (0·11)	2·20 (0·14)
	+3	2·53 (0·14)**	2·73 (0·14)**	2·59 (0·13)*
	+6	3·21 (0·15)**	3·06 (0·11)**	2·92 (0·10)**
Stimulated	−1	2·29 (0·15)	2·38 (0·10)	2·37 (0·11)
	+3	2·67 (0·12)**	2·81 (0·10)**	2·74 (0·12)**
	+6	3·14 (0·13)**	2·99 (0·12)**	2·91 (0·10)**

Values represent log cpm/l × 10^6 cells per 4 h [mean (SD)]. Stimulation was with ConA. *$P<0.025$; **$P<0.005$ *v.* pre-operative values (day-1).

Table 5. Cytokine production by lymphocytes in patients receving fat free, soybean oil-enriched or fish oil-enriched parenteral nutrition post-surgery

Cytokine	Day	Group A (*n* 18)	Group B (*n* 18)	Group C (*n* 20)
IL-2	−1	495 (70)	959 (138)[a]	816 (76)[a]
	+3	689 (134)	803 (98)	800 (76)
	+6	847 (140)*	895 (112)	726 (83)
IFN-γ	−1	127 (18)	170 (19)	147 (14)
	+3	123 (16)	130 (20)*	101 (12)**
	+6	155 (37)	144 (26)	82 (10)**
TNF-α	−1	9·1 (1·4)	10·8 (1·4)	11 (1·5)
	+3	14·1 (3·4)	14·6 (1·8)	12·7 (1·6)
	+6	14·4 (2·1)*	17·0 (3·0)	13·4 (1·5)

Values represent pg, IU, or ng/l × 10^6 cells for IL-2, IFN-γ or TNF-α, respectively [mean (SD)].
*$P<0.025$; **$P<0.005$ *v.* pre-operative values (day -1). [a]$P<0.025$ compared with group A.

three groups in age, height and body weight. The ratios between men and women in groups A, B and C were 12:7, 12:8 and 7:14, and between malignant and benign disease 17:2, 17:3 and 17:4, respectively. The profiles of lymphocyte subset distributions are shown in Figs 1–6, and in Table 3. Total lymphocytes significantly declined in all groups on day 3, and returned towards baseline on day 6 after surgery (Fig. 1). This decline was due to a reduction in the number of T-lymphocytes (Fig. 2), whereas B-lymphocyte numbers increased in the post-operative period (Fig. 3). In all groups the number of CD_4- and CD_8-lymphocytes declined significantly, but this decrease was more pronounced for CD_8-lymphocytes (Figs 4 and 5). This resulted in an increase of the CD_4/CD_8 ratio, which reached statistical significance in groups A and B (Table 3). The postoperative numbers of NK cells declined significantly in all groups, compared with baseline values (Fig. 6).

Stimulated and unstimulated ^{3}H-thymidine uptake before and after surgery was not significantly different in the three treatment groups. Spontaneous (basal) ^{3}H-thymidine uptake by unstimulated lymphocytes was augmented significantly on days 3 and 6 after surgery. Mitogen-stimulated ^{3}H-thymidine uptake was low on day 3 and absent on day 6 after surgery. None of the TPN regimens had any effect on these profiles (Table 4).

There was no significant change in supernatant IL-2 content before and after TPN with the regimens based on glucose as energy source or on glucose plus soybean oil. The IL-2 content of supernatants from PHA-activated lymphocytes was significantly augmented following 5 days of fish oil containing TPN. However, IL-2 production in the fish oil group prior to the operation was significantly lower than in the two other groups (Table 5). Production of IFN-γ significantly declined in patients on the fat-free TPN regimen on days 3 and 6 after surgery. This was not observed in the group receiving fish oil, and only on day 3 in patients given soybean oil emulsion (Table 5). Production of TNF-α was significantly enhanced on day 6 after surgery in patients receiving fish oil (Table 5).

In lymphocytes from the controls (group C) an increase in IL-2R expression occurred on days 3 and 6 after operation. This was not observed in patients on the TPN regimen with soybean oil, whereas IL-2 R expression in patients receiving

Table 6. IL-2 receptor expression by lymphocytes in patients receving fat free, soybean oil–enriched or fish oil-enriched parenteral nutrition post-surgery

	IL-2 receptor positive cells (%)		
Day	Group A (*n* 8)	Group B (*n* 9)	Group C (*n* 7)
−1	27·6 (2·3)	32·3 (2·7)	30·4 (2·0)
+3	35·0 (2·5)*	33·9 (2·6)	36·9 (2·2)*
+6	34·5 (2·3)	34·7 (2·7)	39·2 (2·7)*

Mean values (SD); *$P<0{\cdot}025$ *v.* pre-operative values (day − 1).

the fish oil enriched emulsion (group A) was augmented on the third postoperative day only (Table 6).

Discussion

This investigation shows that the administration of a TPN regimen containing fish oil had few effects on aspects of cellular immunity which were not observed with regimens based on glucose only, or on glucose plus soybean oil as an energy source. As shown in Fig. 1, the total number of lymphocytes declined significantly during the postoperative period in patients receiving fish oil enriched fat emulsions, and in the two other groups. Lymphocytopenia is well established after major surgery. Stress induced by surgery enhances the release of steroids. This appears to cause redistribution of lymphocytes from the vascular compartment into lymph nodes, spleen and bone marrow. It has been shown that T-lymphocytes mainly contribute to these changes (Spey, 1972; Cox & Ford 1982; Lennard *et al.* 1985; Hisatomi *et al.* 1989; Lindh *et al.* 1992). Our report is in keeping with this.

As shown in Figs 2 and 3 total lymphocytes declined because T-lymphocyte numbers decreased while B-lymphocyte numbers increased. Previous investigators described no changes or a decrease in B-lymphocyte numbers following surgery (Lennard *et al.* 1985; Tönnesen & Wahlgreen 1988). The reason for these discrepancies is unclear.

There was a significant decline in CD_4- and CD_8-lymphocyte numbers in groups A, B and C (Figs 4 and 5). A decrease in CD_4-cell numbers after minor and major surgery, as well as in CD_8-cells after major surgery, has been previously observed (Lennard *et al.* 1985; Tönnesen & Wahlgreen 1988). Because of a more pronounced decrease in CD_8-lymphocyte numbers compared with CD_4-cell numbers, there was a significant augmentation in the CD_4/CD_8-ratio (Table 3). NK-cell numbers declined significantly in all groups (Fig. 6). A decrease in NK-cells was previously observed after major surgery. (Tönnesen *et al.* 1984, 1987, 1993; Ryhänen *et al.* 1984; Parillo & Fauci 1978). Thus, intravenous fish oil administration did not affect the lymphocyte subset distribution seen after a moderately stressful operation.

Data on DNA-synthesis by lymphocytes in response to lipids are conflicting (Guillou, 1993). Lipids reportedly augment (Kelly and Parker, 1979; Spieker-Polet & Polet 1981, diminish (Mertin & Hughes 1975; Calder *et al.* 1989; Calder & Newsholme 1992) or have no effect on lymphocyte proliferation (Tizard & Sheppard 1982). Similar effects can be exerted by single free fatty acids. Several free fatty acids are known to increase, to not affect or to decrease proliferation of lymphocytes from human subjects, depending upon their concentration in the incubation medium (Karsten *et al.* 1994).

Postoperative stress has been shown to impair immune functions (Slade *et al.* 1975; Walton, 1979; Eskola *et al.* 1984). It seems possible to modulate this stress response by a lipid-containing TPN regimen. Lectin-induced lymphocyte proliferation was reportedly increased in surgical patients by TPN that contained an emulsion with long-chain and medium–chain triglycerides (Sedman *et al.* 1991). EPA also appears to affect the immune system in postoperative patients. Administration of soybean oil supplemented with EPA reportedly partly prevented the postoperative decline of ConA and PHA-stimulated ^{3}H-thymidine uptake by lymphocytes in seven patients after esophageal surgery (Furukawa *et al.* 1999). This is in contrast to the data shown in Table 4, which indicates that none of the fat-containing TPN regimens affected the postoperative patterns of ^{3}H-thymidine uptake, and also reports that fish oil reduced lymphocyte proliferation in non-stressed (i.e. not operated) subjects (Calder, 1993; Endres *et al.* 1993). To explain the differences with the data from non-stressed subjects, it was suggested that the effect of EPA on immune functions in a stressed state is different from that in a non-stressed state (Furukawa *et al.* 1999). This can also serve as an explanation for the discrepancies with our results. Esophageal surgery is one of the most severe surgical procedures (Furukawa *et al.* 1999), while stress due to large bowel surgery, as in our patients, is considered to be comparatively modest. A further possible reason for differences in the results may be the amount of EPA administered per day. In the study of Furukawa *et al.* 1·8 g/day EPA was given as compared with 2·94 g/day EPA in this investigation, based on a body weight of 70 kg.

Even though there was a statistically significant postoperative increase in IL-2 production in the group receiving fish oil, this augmentation may not be of biological significance because it occurred against a background of a significantly lower preoperative production rate compared with the two other groups (Table 5). Expression of the IL-2 receptor was augmented in patients on the fat-free TPN regimen 3 and 6 days after surgery. This also occurred in patients receiving fish oil, but only on day 3. IL-2R expression in patients on soybean oil remained unaffected. Less augmentation of IL-2R expression in the fish oil group compared with controls may indicate interference with some effects mediated via IL-2. However, this does not seem to pertain to lymphocyte proliferation, as suggested by data in Table 4.

In the patients on the fat free-TPN regimen postoperative IFN-γ production was significantly diminished compared with the preoperative values. This was not observed in patients given fish oil, and only on day 3 after surgery in the patients receiving soybean oil. A postoperative increase in the production of TNF-α was observed with the fish oil regimen, unlike in patients on TPN without fat (Table 5).

Thus, the post-surgery pattern of lymphocyte subset distribution and proliferation of lymphocytes in patients on

TPN containing fish oil was the same as that seen in the controls. Therefore, in moderately stressed surgical patients, fish oil at a dosage of 0·1 g/kg per day given for 1 day, and 0·2 g/kg per day for 4 days, seems not to be immunosuppressive. The possible biological significance of the apparent increase in the production rates of IL-2, IFN-γ and TNF-α remains to be established.

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