Brazilian Journal of Medical and Biological Research (1998) 31: 467-490
ISSN 0100-879X

Immunoregulatory and anti-inflammatory effects of *n*-3 polyunsaturated fatty acids

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Abstract

1. Fish oils are rich in the long-chain *n*-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic (20:5*n*-3) and docosahexaenoic (22:6*n*-3) acids. Linseed oil and green plant tissues are rich in the precursor fatty acid, α-linolenic acid (18:3*n*-3). Most vegetable oils are rich in the *n*-6 PUFA linoleic acid (18:2*n*-6), the precursor of arachidonic acid (20:4*n*-6).
2. Arachidonic acid-derived eicosanoids such as prostaglandin E_2 are pro-inflammatory and regulate the functions of cells of the immune system. Consumption of fish oils leads to replacement of arachidonic acid in cell membranes by eicosapentaenoic acid. This changes the amount and alters the balance of eicosanoids produced.
3. Consumption of fish oils diminishes lymphocyte proliferation, T-cell-mediated cytotoxicity, natural killer cell activity, macrophage-mediated cytotoxicity, monocyte and neutrophil chemotaxis, major histocompatibility class II expression and antigen presentation, production of pro-inflammatory cytokines (interleukins 1 and 6, tumour necrosis factor) and adhesion molecule expression.
4. Feeding laboratory animals fish oil reduces acute and chronic inflammatory responses, improves survival to endotoxin and in models of autoimmunity and prolongs the survival of grafted organs.
5. Feeding fish oil reduces cell-mediated immune responses.
6. Fish oil supplementation may be clinically useful in acute and chronic inflammatory conditions and following transplantation.
7. *n*-3 PUFAs may exert their effects by modulating signal transduction and/or gene expression within inflammatory and immune cells.

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Presented at the XII Annual Meeting of the Federação de Sociedades de Biologia Experimental, Caxambu, MG, Brasil, August 27-30, 1997.

Received August 21, 1997
Accepted August 25, 1997

Key words
- Lymphocyte
- Macrophage
- Immune system
- Inflammation
- Fish oil
- Polyunsaturated fatty acid
- Cytokine
- Eicosanoid

Biosynthesis and sources of different fatty acids

All mammals can synthesise fatty acids *de novo* from acetyl coenzyme A. The end product of the fatty acid synthetase enzyme is palmitic acid (16:0), which in turn can be elongated to stearic acid (18:0). There is little need for the synthesis of saturated fatty acids in Western man, since the diet normally supplies adequate amounts. However, cell membranes require unsaturated fatty acids to maintain their structure, fluidity and function. Therefore, a mechanism for the introduction of double bonds (i.e., desaturation) exists. The introduction of a single double bond between carbon atoms 9 and 10 is catalysed by the enzyme Δ^9-desaturase, which is universally present in both plants and animals. This enzyme catalyses the con-

version of stearic acid to oleic acid (18:1 *n*-9). Plants, unlike animals, can insert additional double bonds into oleic acid between the existing double bond at the 9 position and the methyl terminus of the carbon chain; a Δ^{12}-desaturase converts oleic acid into linoleic acid (18:2 *n*-6) while a Δ^{15}-desaturase converts linoleic acid into α-linolenic acid (18:3 *n*-3). Since animal tissues are unable to synthesise linoleic and α-linolenic acids, these fatty acids must be consumed in the diet and so are termed essential fatty acids. Using the pathway outlined in Figure 1, animal cells can convert dietary α-linolenic acid into eicosapentaenoic acid (20:5 *n*-3) and docosahexaenoic acid (22:6 *n*-3); by a similar series of reactions dietary linoleic acid is converted via γ-linolenic (18:3 *n*-6) and dihomo-γ-linolenic (20:3 *n*-6) acids to arachidonic acid (20:4 *n*-6). The *n*-9, *n*-6 and *n*-3 families of polyunsaturated fatty acids (PUFAs) are not metabolically interconvertible in mammals. Many marine plants, especially the unicellular algae in phytoplankton, also carry out chain elongation and further desaturation of α-linolenic acid to yield the long-chain *n*-3 PUFAs eicosapentaenoic and docosahexaenoic acids. It is the formation of these long chain *n*-3 PUFAs by marine algae and their transfer through the food chain to fish that accounts for their abundance in some marine fish oils.

Influence of dietary *n*-3 PUFAs on the functions of cells of the immune system

Many studies have investigated the effects of the amount and type of fat in the diet upon immune cell functions, particularly lymphocyte proliferation in response to mitogens. These studies have been reviewed several times in recent years (1-9); the effects of *n*-3 PUFAs are the most well documented and will be summarised here.

Lymphocyte proliferation

Lymphocyte proliferation is usually measured as the incorporation of radioactively labelled precursors (e.g., thymidine) into DNA. A suitable stimulus (termed a mitogen) to activate the lymphocytes is required; mitogens used most frequently are concanavalin A (Con A) and phytohaemagglutinin (PHA) which stimulate T lymphocytes specifically, bacterial lipopolysaccharide (LPS) which stimulates B lymphocytes and pokeweed mitogen (PWM) which stimulates a population of both T and B lymphocytes. Monoclonal antibodies to lymphocyte surface structures (e.g., CD3) can also be used to stimulate proliferation.

Feeding animals (rats, mice, chickens, rabbits) diets containing high levels (70 to 200 g/kg) of fish oil (rich in eiocosapentaenoic and docosahexaenoic acids) has been shown to result in suppressed proliferation of lymphocytes stimulated with T- or B-cell mitogens compared with feeding diets rich in other fats such as lard, coconut oil, corn oil, safflower oil or linseed oil (10-15). Recently, it was reported that eicosapentaenoic and docosahexaenoic acids are equipotent in reducing murine spleen lymphocyte proliferation (16). Diets containing large amounts of linseed oil (rich in α-linolenic acid) decrease lymphocyte proliferation compared with saturated fatty acid- or *n*-6 PUFA-rich diets (12,17,18). Recently, it was shown that increasing the amount of α-linolenic acid in the rat diet at the expense of linoleic acid decreases spleen lymphocyte proliferation (19).

Sometimes, the effects of *n*-3 PUFA-rich diets on lymphocyte proliferation were demonstrated when the cells were cultured in autologous serum, but were lost if the cells were cultured in foetal calf serum (11-13). Culture of cells in foetal calf serum may explain the lack of effect upon spleen lym-

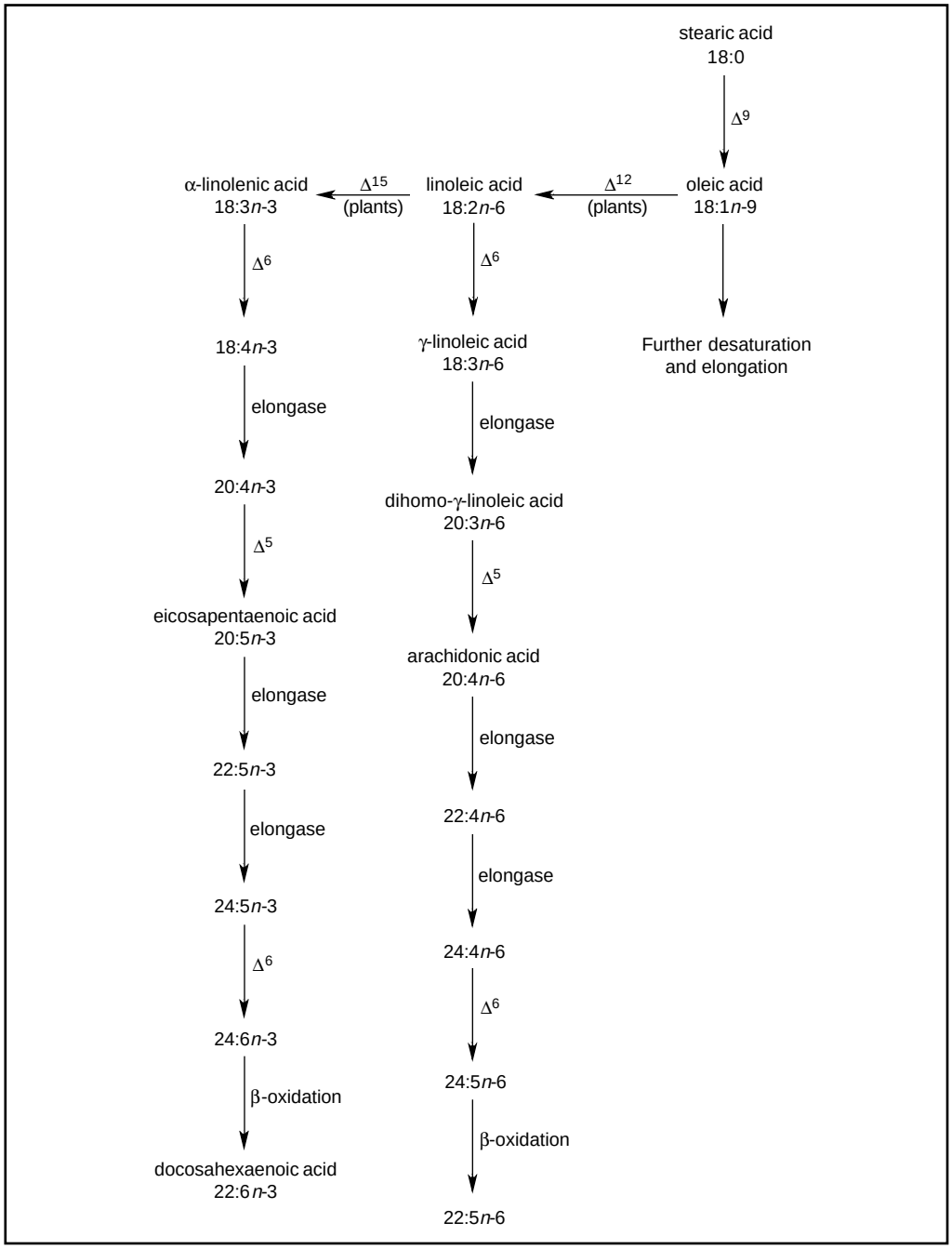

Figure 1 - PUFA metabolism. Δ^5, Δ^6, Δ^9, Δ^{12} and Δ^{15} indicate desaturase enzymes.

phocyte proliferation of feeding fish and linseed oils to mice reported in some studies (20,21). It has been shown that the changes in lymphocyte fatty acid composition induced by dietary manipulations are better maintained if the cells are cultured in autologous rather than foetal calf serum (22).

Twelve weeks of supplementation of the diets of healthy women (51 to 68 years of age) with encapsulated *n*-3 PUFAs (approximately 2.4 g/day) resulted in a lowered mitogenic response of peripheral blood mononuclear cells (PBMNCs) to PHA (23). More recently, a decreased response of PBMNCs to Con A and PHA following supplementation of the diet of volunteers on a low fat, low

cholesterol diet with 1.23 g *n*-3 PUFAs/day was reported (24), while 18 g of fish oil/day (approximately 6 g *n*-3 PUFAs/day) for 6 weeks resulted in lowered PHA-stimulated proliferation of PBMNCs 10 weeks after supplementation had ended (but not at the end of the supplementation period) (25).

Cytotoxic T lymphocyte-mediated cytolysis

The cytotoxic T lymphocyte activity of spleen lymphocytes was reported to be lower after feeding mice 100 g/kg fish oil for up to 10 weeks than after feeding 100 g/kg linseed oil (26). Feeding chickens diets containing 70 g/kg fish or linseed oil significantly reduced spleen cytotoxic T-cell activity compared with diets containing 70 g/kg lard or corn oil (27).

Natural killer cell-mediated cytolysis

Feeding mice diets containing 100 g/kg fish oil caused a decrease in spleen natural killer (NK) cell activity compared with feeding chow or 100 g/kg corn oil (28,29). In the study of Berger et al. (21) female mice were fed for 5 months on diets containing 100 g/kg olive, safflower, linseed or fish oil and the spleen NK cell activity of the pups was determined before they were weaned; the activity was lower in the fish oil group than in the safflower or olive oil groups. Yaqoob et al. (30) fed weanling rats for 10 weeks on a low fat diet or on diets containing 200 g/kg hydrogenated coconut, olive, safflower, evening primrose or fish oil before measuring spleen NK cell activity. It was found that feeding each of the high fat diets resulted in lower NK cell activity compared with feeding the low fat diet; feeding the fish oil diet resulted in the lowest activity. Similar results have been found in more mature rats fed on these diets for 12 weeks (15). It was reported that a 200 g/kg linseed oil diet decreased rat spleen lymphocyte NK cell activity compared with feeding a 200 g/kg sunflower oil diet (18). More recently, it was shown that increasing the amount of α-linolenic acid in the rat diet at the expense of linoleic acid decreases spleen NK cell activity (19).

No studies have investigated the effect of dietary lipids on human NK cell activity, although it was reported that intravenous injection of a triacylglycerol-containing eicosapentaenoic acid into healthy human volunteers results in suppression of peripheral blood NK cell activity 24 h later (31).

Macrophage-mediated cytotoxicity

Dietary fish oil (100 g/kg) has been reported to significantly suppress lysis of target tumour cells by mouse-elicited peritoneal macrophages (32-35). The target cell lines used in these studies are sensitive to killing by tumour necrosis factor (TNF)-α (L929 cells; 33,34) or nitric oxide (P815 cells; 32,35). Thus, the suppressed macrophage-mediated cytolysis observed after fish oil feeding suggests that fish oil reduces the production of nitric oxide and TNF (see later).

Neutrophil and monocyte chemotaxis

Chemotaxis of human peripheral blood neutrophils and monocytes towards a variety of chemoattractants including leukotriene (LT) B_4, platelet-activating factor, formyl-methionyl-leucyl-phenylalanine and autologous serum is suppressed following the supplementation of the human diet with *n*-3 PUFAs (36-40).

MHC expression and antigen presentation

Inclusion of *n*-3 PUFAs in the diet of mice or rats results in a diminished percentage of peritoneal exudate cells bearing the major histocompatibility class (MHC) II antigens on their surface (41-44). The level of MHC II expression on positive cells is also

suppressed by fish oil feeding (43). In accordance with these animal studies, supplementation of the diet of human volunteers with *n*-3 PUFAs (approximately 1.56 g/day) for 3 weeks resulted in a decreased level of MHC II (HLA-DP, -DQ and -DR) expression on the surface of peripheral blood monocytes (45). These observations suggest that diets rich in *n*-3 PUFAs will result in diminished antigen presentation. Indeed, feeding mice the ethyl ester of eicosapentaenoic acid for a period of 4 to 5 weeks resulted in diminished presentation of antigen (keyhole limpet haemocyanin; KLH) by spleen cells *ex vivo* (46). Dendritic cells are the key antigen-presenting cells *in vivo.* We have recently observed that, compared with a low fat diet or a diet containing 200 g/kg safflower oil, feeding rats a diet containing 200 g/kg fish oil significantly diminishes MHC II expression on dendritic cells and *ex vivo* antigen (KLH) presentation by dendritic cells (obtained by cannulation of the thoracic duct) to KLH-sensitised spleen lymphocytes (47).

Influence of dietary *n*-3 PUFAs on the interactions between cells of the immune system

Communication between cells of the immune system is achieved by virtue of the production of chemical mediators (e.g., eicosanoids, cytokines, nitric oxide (NO)) and by direct cell-to-cell contact mediated by adhesion molecules. *n*-3 PUFAs have been found to influence each of these communication links.

n-3 PUFAs and eicosanoid production

Eicosanoids are a family of oxygenated derivatives of dihomo-γ-linolenic, arachidonic and eicosapentaenoic acids. Eicosanoids include prostaglandins (PGs), thromboxanes (TXs), LTs, lipoxins (LXs), hydroperoxyeicosatetraenoic acids (HPETEs) and hydroxyeicosatetraenoic acids (HETEs). In most conditions the principal precursor for these compounds is arachidonic acid. The precursor PUFA is released from membrane phosphatidylcholine by the action of phospholipase A_2 or from membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) by the actions of phospholipase C (PLC) and a diacylglycerol (DAG) lipase. The pathways of eicosanoid synthesis begin with cyclooxygenase, which yields the PGs and TXs, or with the 5-, 12- or 15-lipoxygenases, which yield the LTs, HPETEs, HETEs and LXs (Figure 2). The amounts and types of eicosanoids synthesised are determined by the availability of arachidonic acid, by the activities of phospholipase A_2 and PLC, by the activities of cyclooxygenase and the lipoxygenases, by the cell type and by the nature of the stimulus.

Cells of the immune system are an important source of eicosanoids and they are subject to their regulatory effects (48-51); the most well-documented effects are those of PGE_2. *In vivo*, PGs are involved in modulating the intensity and duration of inflammatory and immune responses; PGE_2 has a

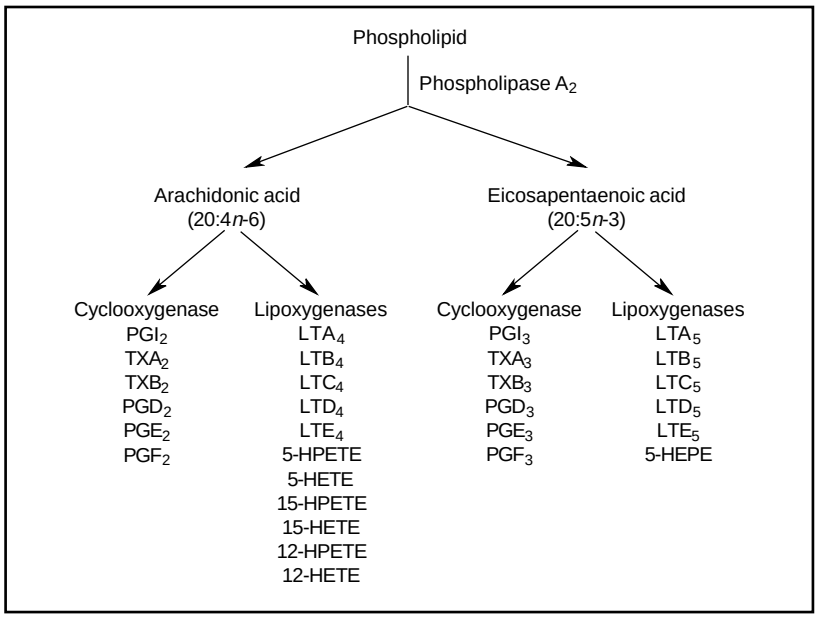

Figure 2 - Synthesis of eicosanoids from arachidonic and eicosapentaenoic acids. HEPE indicates hydroxyeicosapentaenoic acid; see text for explanation of other abbreviations.

number of pro-inflammatory effects including induction of fever and erythema, increased vascular permeability and vasodilation and enhancement of pain and oedema caused by other agents such as bradykinin and histamine. PGE_2 also regulates the production of both monocyte/macrophage- and lymphocyte-derived cytokines (see 52). In chronic inflammatory conditions increased rates of PGE_2 production are found, and elevated PGE_2 production has been observed in patients suffering from infections. LTs have chemotactic properties and are involved in the regulation of inflammatory and immune processes; 4-series LTs regulate cytokine production.

The *n-3* PUFAs, eicosapentaenoic and docosahexaenoic acids, competitively inhibit the oxygenation of arachidonic acid by cyclooxygenase. In addition, eicosapentaenoic acid (but not docosahexaenoic acid) is able to act as a substrate for both cyclooxygenase and 5-lipoxygenase. Thus, ingestion of fish oils which contain *n*-3 PUFAs will result in a decrease in membrane arachidonic acid levels and a concomitant decrease in the capacity to synthesise eicosanoids from arachidonic acid (e.g., 53); eicosapentaenoic acid gives rise to the 3-series PGs and TXs and the 5-series LTs (Figure 2). The eicosanoids produced from eicosapentaenoic acid do not always have the same biological properties as the analogues produced from arachidonic acid.

n-3 PUFAs and cytokine production

Since cytokine production is regulated by eicosanoids (see 52) and since dietary lipids affect eicosanoid production, it might be expected that dietary lipids, especially those containing *n*-3 PUFAs, will affect cytokine production. The effects of *n*-3 PUFAs on cytokine production have been reviewed several times in recent years (7,54-59).

Animal studies. All studies which have used murine resident peritoneal macrophages (60-65), one study using rat resident alveolar macrophages (66) and one study using rat resident peritoneal macrophages (67) report an enhancing effect of *n*-3 PUFAs upon TNF production; only one study which used rat resident peritoneal macrophages has reported reduced TNF production following fish oil feeding (68). Three studies report that *n*-3 PUFA-rich diets do not affect TNF production by CFA-elicited peritoneal macrophages from rats or mice (62,63,67). The effect of dietary *n*-3 PUFAs upon TNF production by thioglycollate-elicited peritoneal macrophages is unclear, with studies reporting no effect (35,63,69,70), reduction (34, 53,71) or enhancement (35,69). Comparison of the outcome of these studies is complicated by the different procedures used for *ex vivo* culture of the cells. That such details are important in determining outcome is shown by the studies of Erickson's group (35,69) in which there was no effect of diet upon TNF production when thioglycollate-elicited macrophages were stimulated with LPS for 1 or 8 h but if they were stimulated for 24 h there was increased TNF production by cells from fish oil-fed mice. The only animal study which has investigated TNF production by PBMNCs showed decreased production following the infusion of a 10% (v/v) fish oil emulsion (72); this is an interesting observation since it agrees with the findings of a number of studies using human PBMNCs (see below). In addition to studies measuring TNF production *ex vivo*, Black and Kinsella (33) and Renier et al. (34) showed that feeding mice *n*-3 PUFA-rich diets resulted in reduced ability of elicited peritoneal macrophages to kill L929 cells; L929 cells are killed by TNF and so the reduced cytotoxicity of macrophages towards these cells suggests a reduced ability to produce TNF. Two animal studies have investigated the effects of dietary lipids upon circulating TNF levels which would perhaps reflect *in vivo* production of the cytokine. Watanabe et al. (63)

found that TNF levels were significantly higher in the plasma of LPS-injected mice fed a diet containing perilla oil than in the plasma of those fed a safflower oil-rich diet. Similarly, Chang et al. (65) reported higher serum TNF levels 1 and 1.5 h following intraperitoneal injection of LPS into fish oil-fed mice compared with those fed coconut or corn oil.

All studies which have used thioglycollate-elicited peritoneal macrophages and the only study to use Kupffer cells report that dietary fish oil results in decreased *ex vivo* production of IL-1 (34,53,70,71,73). In contrast, two studies have reported that fish oil enhances IL-1 production by murine resident macrophages (60,64). In addition, Ertel et al. (74) showed that the reduction in *ex vivo* IL-1 production by resident peritoneal macrophages which accompanies haemorrhagic shock in corn or safflower oil-fed mice was prevented by fish oil feeding. This study also showed no difference in IL-1 production by resident peritoneal macrophages taken from sham-operated mice fed these three diets (74).

There are no studies reporting the effect of dietary fatty acids upon IL-6 production by resident peritoneal macrophages. One study using murine thioglycollate-elicited peritoneal macrophages showed a significant reduction in LPS-stimulated IL-6 production following fish oil feeding (53); production following stimulation of the cells with TNF was also significantly reduced (Yaqoob P and Calder PC, unpublished observations). Rat PBMNCs showed reduced IL-6 production following fish oil infusion for 4 days (72).

In contrast to the large number of studies in animals of the effects of dietary lipids, especially fish oil, upon the *ex vivo* production of macrophage-derived cytokines (see above), there have been relatively few studies on lymphocyte-derived cytokines. Decreased IL-2 production by alveolar lymphocytes from pigs fed diets containing fish or linseed oil was reported (75). Recently, it was observed that feeding mice a diet containing 10 g/kg of ethyl esters of eicosapentaenoic or docosahexaenoic acid for 10 days significantly decreased *ex vivo* IL-2 production by spleen lymphocytes stimulated with Con A; both *n*-3 PUFAs were equally effective (16). In another study weanling mice were fed for 8 weeks on a low fat diet or on diets containing 200 g/kg hydrogenated coconut, olive, safflower or fish oil; the spleen lymphocytes were subsequently stimulated with Con A (14). The concentration of IL-2 was higher in the medium of spleen lymphocytes from mice fed olive or safflower oil than in the medium of cells from mice fed the low fat diet or hydrogenated coconut oil; fish oil feeding had no effect upon the IL-2 concentration in the medium. In contrast, higher IL-2 production by Con A-stimulated spleen lymphocytes taken from autoimmune disease-prone mice fed 100 g/kg fish oil compared with those fed 100 g/kg corn oil has been reported (76); the intracellular levels of mRNA for IL-2 were also elevated in the fish oil-fed mice although these levels could not be quantified. A recent study reported that inclusion of α-linolenic acid or eicosapentaenoic plus docosahexaenoic acid in the diet of monkeys resulted in enhanced *ex vivo* production of IL-2 (77). The latter study related the discrepancies in the literature to the levels of vitamin E included in the diets used.

There have been few animal studies of the effects of dietary lipids on lymphocyte-derived cytokines other than IL-2. The studies which have been reported suggest minimal effects of dietary fat upon production of IL-4, IL-10 and interferon (IFN)-γ (14,76), but more studies need to be performed to confirm this.

Human studies. Endres et al. (37) were the first to show that supplementation of the diet of healthy volunteers with *n*-3 PUFAs diminishes *ex vivo* production of IL-1α, IL-1ß and TNF by human PBMNCs. Interest-

ingly, the production of these cytokines remained suppressed for 10 weeks after the supplementation had ended, indicating the long period of "washout" required to reverse the effects of fish oil supplementation. It is also worth noting that this study showed that the precise effects of dietary *n*-3 PUFAs vary according to the stimulus used to induce cytokine production. A later study by Endres et al. (25) using the same supplementation regime reported a decrease in *ex vivo* IL-2 production in response to Con A; the reduction was greater 10 weeks after supplementation had ended than at the end of supplementation. Virella et al. (78) reported that 6 weeks of supplementation of the diet with 2.4 g eicosapentaenoic acid per day resulted in lowered production of IL-2 by PBMNCs stimulated with PHA or PWM; in agreement with the long "washout" period suggested by Endres et al. (25,37), IL-2 production remained low 8 weeks after the supplementation had ended but returned to pre-supplementation levels 22 weeks post-supplementation. Meydani et al. (23) supplemented the diet of healthy young (20-33 years of age) and older (51-68 years of age) women with 2.4 g *n*-3 PUFAs per day and examined *ex vivo* production of a range of cytokines after 4, 8 and 12 weeks. There were time-dependent decreases in the production of IL-1ß, IL-2, TNF and IL-6 by PBMNCs from the older women; production of TNF and IL-6 by cells from the young women was also significantly decreased and there were trends towards decreased production of IL-1ß and IL-2 by these cells. The same workers have studied the effect of including fish (providing 1.23 g *n*-3 PUFAs/day) in a low fat, low cholesterol diet for 24 weeks; the *ex vivo* production of IL-1ß, IL-6 and TNF by PBMNCs was significantly reduced and there was a nonsignificant reduction in IL-2 production (24). The production of IL-1 and IL-6 in whole blood cultures was decreased if the subjects had consumed 1.1 to 1.6 g *n*-3 PUFAs per day for 6 to 8 weeks but only if the cultures were stimulated with "low" concentrations (≤0.001 µg/ml) of LPS (79). Interestingly, if higher LPS concentrations were used there was no effect of the supplementation upon production of these two cytokines; TNF production was unaffected by *n*-3 PUFAs in this study. Recently, the effects of dietary α-linolenic acid upon IL-1ß and TNF-α production by human cells have been reported; subjects consumed a sunflower oil-rich diet (which was very similar to their typical diet) or a diet rich in α-linolenic acid which was provided by linseed oil capsules and linseed oil-based spreads and cooking oils (80). In this way the linseed oil consumption increased to a mean of 13.7 g/day. *Ex vivo* production of both IL-1ß and TNF-α by PBMNCs was decreased by the linseed oil diet. If the subjects then supplemented their diet with encapsulated eicosapentaenoic plus docosahexaenoic acids (2.7 g/day) production of both cytokines was further decreased. These authors showed a correlation between mononuclear cell eicosapentaenoic acid content and production of IL-1ß and TNF-α.

Supplementation of the diet of healthy volunteers or multiple sclerosis patients with encapsulated fish oil providing approximately 5 g *n*-3 PUFAs per day for 24 weeks resulted in lower *ex vivo* production of IL-1ß, TNF-α, IL-2 and IFN-γ by PBMNCs (81). An earlier study reported lower production of IL-1 by PBMNCs taken from rheumatoid arthritis patients who had consumed *n*-3 PUFAs for 24 weeks (82); interestingly, cells from the patients who consumed an olive oil placebo (9 g/day) in this trial also showed diminished IL-1 production. There was increased production of IL-2 by PBMNCs from rheumatoid arthritis patients who supplemented their diet with olive oil or a "low" level of *n*-3 PUFAs for 24 weeks; this increase did not occur in patients consuming a "high" level of *n*-3 PUFAs (82).

Recently, circulating cytokine levels in patients receiving intravenous infusions of

lipid emulsions post-surgery were reported (83); patients received either a medium chain/long chain triacylglycerol mix (50:50 v/v) or this mix also containing fish oil (50:30:20 v/v/v). Patients received 50 g fat per day on days 1 and 2 post-surgery and 100 g fat per day on days 3, 4 and 5; thus patients in the group receiving the fish oil-containing emulsion received approximately 3 (days 1 and 2) or 6 (days 3, 4 and 5) g *n*-3 PUFAs per day. Plasma TNF-α levels were significantly lower in the fish oil group at 6 days post-surgery; plasma IL-6 levels were lower (but not significantly) 10 days post-surgery and the post-surgery increase in plasma IL-10 levels was reduced in this group.

n-3 PUFAs and cytokine receptor expression

Feeding rats a diet containing 200 g/kg fish oil lowered the proportion of spleen and thymic lymphocytes bearing the IL-2 receptor (IL-2R; CD25) following Con A stimulation (13). Spleen lymphocytes from rats fed fish oil also showed a lower level of expression of the IL-2R following mitogenic stimulation (15). In accordance with these animal studies, supplementation of the diet of patients with psoriasis or atopic dermatitis with *n*-3 PUFA ethyl esters (6 g/day) caused a significant reduction in the percentage of IL-2R$^+$ blood lymphocytes following PHA stimulation (84); the level of expression of the IL-2R on the positive cells was also significantly reduced.

n-3 PUFAs and nitric oxide production by macrophages

Two studies using rat resident peritoneal macrophages reported that NO production is significantly diminished by fish oil feeding (68,85). In contrast, Hubbard et al. (35) found no effect of feeding mice 100 g/kg linseed or fish oil upon ex *vivo* NO production by thioglycollate-elicited macrophages; nevertheless these workers, as well as Somers et al. (32), reported that *n*-3 PUFA feeding diminishes macrophage cytotoxicity towards NO-sensitive P815 cells, suggestive of reduced NO production. Yaqoob and Calder (53) found that several high fat diets, including 200 g/kg fish oil, enhanced NO production by murine thioglycollate-elicited macrophages compared with a low fat diet; there were no significant differences in NO production by macrophages from mice fed different high fat diets. Fish oil has been reported to enhance NO production by murine peritoneal (34), rat lung (66) and pig lung (75) macrophages.

Recently, Harris et al. (86) reported increased appearance of NO metabolites in the urine of healthy human volunteers who supplemented their diet with 5 g fish oil concentrate per day (providing 3 g eicosapentaenoic acid plus docosahexaenoic acid) for 3 weeks; interestingly, 3 g of eicosapentaenoic acid alone did not alter urinary NO metabolite levels. The levels measured by Harris et al. (86) were assumed to indicate whole body NO production; as such, a variety of sources of NO are likely to exist and the authors concluded that much of it originated from endothelial cells. There is much direct and circumstantial evidence that *n*-3 PUFAs enhance NO production by endothelial cells (see 86 for references).

n-3 PUFAs and adhesion molecule expression

Adhesion molecules are involved in many cell-to-cell interactions. For example, interaction between T lymphocytes and antigen-presenting cells is in part mediated by the ligand-receptor pairs CD11a/CD18:CD54, CD11a/CD18:CD102 and CD2:CD58 (for reviews, see 87-89). Thus, an efficient cell-mediated immune response requires appropriate levels of expression of these molecules on T lymphocytes. In addition, leucocyte adhesion to the endothelium involves a number of ligand-receptor pairs including CD11a/CD18:CD54, CD54:CD11a/CD18,

CD49d/CD29:CD106, CD2:CD58, CD62L: MAdCAM-1 and CD44: hyaluraonate (for reviews, see 87-89). Thus, the movement of leucocytes between body compartments, into and out of lymphoid organs and into sites of immune or inflammatory reactivity requires adhesion molecule expression. Adhesion molecule expression appears to be involved in several acute and chronic inflammatory disease processes (for a review, see 90), and antibodies against certain adhesion molecules can reduce chronic inflammatory disease (90).

In vitro studies of n-3 PUFAs and adhesion molecule expression. It has become apparent that *n*-3 PUFAs can affect adhesion molecule expression by some cell types, at least *in vitro*. Calder et al. (91) observed that murine thioglycollate-elicited peritoneal macrophages cultured in the presence of eicosapentaenoic or docosahexaenoic acid were less adherent to artificial surfaces (the adhesion to one of these surfaces is mediated by CD11a/CD18) than those cultured with some other fatty acids. This observation suggests that *n*-3 PUFAs decrease expression of either CD11a or CD18 or both proteins. More recently, De Caterina et al. (92) reported that culture of human adult saphenous vein endothelial cells with docosahexaenoic acid significantly decreased the cytokine-induced expression of CD106, CD62E and CD54 in a dose-dependent manner. The adhesion of U937 monocytes or human peripheral blood monocytes to the endothelial cells was diminished following incubation of the latter with docosahexaenoic acid (92). Since the binding between monocytes and endothelial cells partially depends upon CD106 expression on the endothelial cells, the reduced expression of CD106 caused by docosahexaenoic acid appears to have a functional effect. Kim et al. (93) reported that incubation of LPS-stimulated pig aortic endothelial cells with eicosapentaenoic acid resulted in diminished binding between these cells and U937 monocytes. Eicosapentaenoic acid was shown to reduce the expression of CD106, CD62E and CD54 on the surface of LPS-stimulated human umbilical vein endothelial cells (93). Recently, it was shown that inclusion of eicosapentaenoic or docosahexaenoic acid in the medium of cultured resting or LPS- or cytokine-stimulated human umbilical vein endothelial cells decreased the ability of peripheral blood lymphocytes to bind (94); both *n*-3 PUFAs were shown to decrease the level of expression of CD106, but not of CD54 or CD62E, on the surface of cytokine-stimulated endothelial cells. This latter study also reported, for the first time, the *in vitro* effect of *n*-3 PUFAs on adhesion molecule expression on lymphocytes: incubation of lymphocytes with either eicosapentaenoic or docosahexaenoic acid reduced the level of expression of CD11a and CD62L but did not affect CD49d expression (94). In parallel with this reduction, the binding of lymphocytes to untreated or cytokine-stimulated endothelial cells was diminished. In another recent study, incubation with eicosapentaenoic acid was shown to reduce the level of expression of CD54, but not CD11a, on the surface of resting or IFN-γ-stimulated human monocytes (95); docosahexaenoic acid did not alter expression of these molecules. Thus, it appears that culture of macrophages, monocytes, lymphocytes or endothelial cells with *n*-3 PUFAs can decrease adhesion molecule expression resulting in diminished ability to bind to other cell types.

Effects of dietary n-3 PUFAs on adhesion molecule expression. There are few studies of the effects of inclusion of *n*-3 PUFAs in the diet upon adhesion molecule expression, although it was recently shown that supplementation of the human diet with *n*-3 PUFAs results in significantly lower levels of expression of CD11a and CD54 on peripheral blood monocytes (45). Feeding rats a diet containing 200 g fish oil/kg significantly reduced (by 20 to 35%) the levels of expression of CD2 and CD11a on freshly

prepared lymphocytes and of CD2, CD11a and CD54 on Con A-stimulated lymphocytes (15). Furthermore, the levels of CD2, CD11a and CD54 were reduced on popliteal lymph node lymphocytes following localised graft *vs* host or host *vs* graft responses *in vivo* (96). Reduced adhesion molecule expression suggests that cells will be less able to interact with receptor-bearing cells. In accordance with this, we have recently observed that lymph node lymphocytes obtained from fish oil-fed rats adhere less well to macrophage and endothelial cell monolayers (Sanderson P and Calder PC, unpublished observations). These observations suggest that *n*-3 PUFA feeding will affect the movement of lymphocytes and monocytes between body compartments and perhaps into sites of inflammatory or autoimmune activity.

Dietary fatty acids and *in vivo* measures of cell-mediated immunity and inflammation

The studies outlined above have investigated the effects of dietary manipulations upon *ex vivo* functions of isolated cell populations. Although a number of consistent patterns have emerged from these studies, there are also contradictory reports and it is evident that the outcome of such *ex vivo* measures is strongly influenced by the experimental conditions used. Furthermore, *in vivo* cells exist as part of a network being influenced by other cell types; often such interactions are disturbed by the purification of the particular cell types to be studied. Therefore, it is important to investigate the effect of dietary fats on the intact, fully functioning system in which all normal cellular interactions are in place. The ability to make *in vivo* measures of inflammation and cell-mediated immunity offers the prospect of investigating the effects of dietary manipulations upon the overall responses of these systems.

Acute inflammatory responses

Arachidonic acid-derived eicosanoids are involved in mediating inflammatory responses. Since *n*-3 PUFAs diminish the production of these mediators, they should exert anti-inflammatory activities. Indeed, *n*-3 PUFAs decrease the production of pro-inflammatory mediators such as PGE_2 and LTB_4 during antigen-induced inflammation of the air pounch (97), carrageenan-induced inflammation of the footpad (97) and zymosan-induced peritoneal inflammation (98). The latter study also reported that dietary *n*-3 PUFAs inhibited the influx of neutrophils into the peritoneal cavity which accompanies such treatment. Feeding rats 100 g/kg cod liver oil for 10 weeks significantly lowered (by 40%) the inflammatory response to carrageenan injection into the footpad compared with feeding coconut oil or groundnut oil (99). In accordance with that observation, feeding rats high fat diets containing ethyl esters of eicosapentaenoic or docosahexaenoic acid resulted in a 50% reduction in footpad swelling in response to carrageenan injection compared with feeding safflower oil (100); both *n*-3 PUFAs were equally effective.

In vivo responses to endotoxin and cytokines

Intravenous infusions of a 10% (v/v) lipid emulsion rich in fish oil into guinea pigs significantly enhanced survival to intraperitoneally injected LPS compared with infusion of a 10% (v/v) safflower oil emulsion (101); the total amount of lipid infused was 13 g/animal. The same workers later showed that feeding a fish oil-rich diet to guinea pigs for 6 weeks significantly increased survival to an intraperitoneal injection of LPS compared with animals fed a safflower oil-rich diet (102).

In accordance with the diminished susceptibility to the lethal effects of endotoxin in experimental animals, feeding weanling

rats a 100 g/kg fish oil diet for 8 weeks significantly decreased a number of responses to intraperitoneal TNF-α: the rises in liver zinc and plasma C3 concentrations, the fall in plasma albumin concentration and the increases in liver, kidney and lung protein synthesis rates were all prevented by the fish oil diet (103). Fish oil feeding to rats or guinea pigs also diminishes the pyrogenic (104,105) and anorexic effects (103, 106) of IL-1 and TNF-α compared with feeding *n*-6 PUFA-containing oils.

Delayed-type hypersensitivity

The delayed-type hypersensitivity (DTH) reaction is the result of a cell-mediated response to challenge with an antigen to which the individual has already been primed. There are several reports that the DTH response in rodents is significantly reduced by *n*-3 PUFAs. For example, intragastric administration of a fish oil concentrate to rats for 50 days lowered the DTH response to bovine serum albumin compared with administering safflower oil, olive oil or water (97) while addition of ethyl esters of either eicosapentaenoic or docosahexaenoic acid to the diet of mice consuming a safflower oil diet reduced the DTH response to tuberculin (107); both *n*-3 PUFAs were equally effective. The DTH response to sheep red blood cells in mice was diminished following tail-vein injections of emulsions of triglycerides rich in eicosapentaenoic or docosahexaenoic acid (108); a soybean oil emulsion was injected in the control animals. Most recently, it was reported that feeding aged beagle dogs a long-chain *n*-3 PUFA-rich diet significantly diminished the DTH response to intradermal KLH compared with feeding diets containing smaller amounts of long-chain *n*-3 PUFAs (109).

Feeding a linseed oil-rich diet to healthy human volunteers for 8 weeks lowered the DTH response to 7 recall antigens, although this reduction was not statistically significant (110). Supplementation of the diet of volunteers consuming a low fat, low cholesterol diet with 1.25 g *n*-3 PUFA/day diminished the DTH responses to 7 recall antigens (24).

Graft *vs* host and host *vs* graft responses

The so-called popliteal lymph node (PLN) assay provides a useful experimental model in rodents for measuring graft *vs* host (GvH) and host *vs* graft (HvG) responses elicited by injection of allogeneic cells into the footpad of the host. The GvH response primarily involves the polyclonal activation, and subsequent proliferation, of host B-cells, although NK cells may also be involved in the host defence. In contrast, the HvG reaction is a T-cell-mediated response, in which CTLs of the host recognise MHC antigens on the injected cells. In both cases the enlargement in PLN size (more than 15-fold in the GvH response and 4-fold in the HvG response) is due largely to proliferation of activated host cells; most of these originate within the PLN although there is also some recruitment of cells from the bloodstream. A suppressed HvG response was observed in mice fed a 160 g/kg fish oil diet compared with those fed a standard chow diet (111); lower levels of fish oil (25, 50, 100 g/kg) did not significantly affect the response. Significantly diminished GvH and HvG responses (by 34% and 20%, respectively) were observed in rats fed 200 g/kg fish oil compared with those fed a low fat diet or diets containing 200 g/kg coconut, olive, safflower or evening primrose oils (96). Such observations accord with the demonstrations of significantly diminished *ex vivo* T lymphocyte proliferation, NK cell activity and CTL activity following fish oil feeding. Feeding rats a 200 g/kg fish oil diet resulted in fewer $IL\text{-}2R^+$ cells and $CD16^+/CD3^-$ cells in the popliteal lymph node following the GvH

response, indicating an inhibition of lymphocyte activation and a decrease in the proportion of NK cells, respectively (96). Recently, a dose-dependent effect of linseed oil compared with sunflower oil upon the GvH response in rats was reported (18).

Animal models of organ transplantation

Graft rejection in transplantation surgery is caused by an immune reaction to the foreign material introduced into the body; T-cells have been implicated in accelerated graft rejection, but antibodies with specificity for the graft donor have also been observed following rejection, implying that both cell-mediated immunity and humoral immunity play a part in the rejection process. Studies investigating the effects of eicosanoids on organ transplantation pre-date those investigating the effects of fatty acids. Some eicosanoids promote graft rejection while others promote graft survival (for a review, see 112). Therefore, since *n*-6 and *n*-3 PUFAs will affect the levels and types of eicosanoids formed, they would be expected to influence graft survival. In addition, PUFAs exert immunomodulatory effects which might be independent of eicosanoids and so these effects might play a role in enhancing graft survival. The effect of feeding *n*-3 PUFAs to recipient or donor or both upon subsequent cardiac allograft survival was investigated in rats (113). It was found that survival was prolonged if the recipients were fed fish oil or ethyl esters of eicosapentaenoic or docosahexaenoic acid regardless of the diet of the donor, or if the donors were fed fish oil or ethyl esters of eicosapentaenoic or docosahexaenoic acid or fish oil regardless of the diet of the recipient or if both donor and recipient were fed fish oil. Greater prolongation of cardiac survival in rats receiving an infusion of fish oil post-transplantation compared with those receiving soybean oil infusion was reported (114); in turn soybean oil enhanced survival compared with saline infusion. Oral fish oil (4.5 g/day) has also been shown to prolong the survival of islets of Langerhans grafts in mice (115).

Effects of *n*-3 PUFAs in animal models of inflammatory and autoimmune diseases

Dietary fish oil has been shown to have significantly beneficial clinical, immunological and biochemical effects in a number of animal disease models. These effects include increased survival and decreased proteinuria and anti-DNA antibodies in mice with autoimmune glomerulonephritis, a model for systemic lupus erythematosus (41,116-119), decreased joint inflammation in rodents with collagen-induced arthritis (120,121) and less inflammation in rats with various models of colitis (122-125). These observations suggest that diets enriched in *n*-3 PUFAs might be of some therapeutic benefit in these diseases in man.

Clinical studies of the effects of dietary fatty acids in inflammatory and autoimmune diseases and in transplantation

Inflammatory and autoimmune diseases

The low incidence of cardiovascular disorders amongst populations consuming large quantities of oily fish has been well documented (126-128), but the intense interest in fish oil and heart disease has overshadowed the unusual pattern of the incidence of some other diseases in native Greenland Eskimos. Kromann and Green (127) described a very low incidence of autoimmune and inflammatory disorders, such as psoriasis, asthma and type-I diabetes, and the complete absence of multiple sclerosis in a population of Greenland Es-

kimos compared with sex- and age-matched groups living in Denmark. Thus, the *n*-3 PUFA-containing fish oil in the Eskimo diet could have a protective role towards these types of diseases. Most of these diseases are characterised by inappropriate activation of T-cells resulting in attack on, and ultimately destruction of, host tissues. Typically, the sites of tissue destruction (e.g., joints in rheumatoid arthritis, neural tissue in multiple sclerosis) contain activated T-cells and macrophages and mediators produced by these cells, such as cytokines, eicosanoids and reactive oxygen species. The favourable outcome resulting from dietary fish oil in animal models of inflammatory and autoimmune diseases (see above) indicates that there may be some benefit from supplementation of the diet of suitable patients with *n*-3 PUFAs. There have been a number of clinical trials assessing the benefits of dietary supplementation with fish and other oils in several inflammatory and autoimmune diseases; these studies have been reviewed elsewhere (7,129-134).

Transplantation

The animal studies described above indicate that PUFAs, particularly *n*-3 PUFAs, could be used to prolong the survival of organ transplants. Recipients of kidney transplants who received 9 g fish oil/day for one year post-transplant (in conjunction with cyclosporin A and prednisolone) had significantly improved glomerular filtration rate and significantly diminished cyclosporin A nephrotoxicity although there was no effect upon graft survival (135). A similar finding was made by Homan van der Heide et al. (136) who reported that renal transplant patients who received fish oil (6 g/day for the first post-operative year) in combination with cyclosporin A had better kidney function and less rejections over one year compared with patients who received coconut oil and cyclosporin A. Better kidney function in kidney graft recipients who consumed fish oil (8 g/day for one year post-transplant) was reported, although there was no reduction in rejection episodes compared with controls (137). Bennett et al. (138) reported no rejection incidents in a group of kidney transplant recipients who received 9 or 18 g eicosapentaenoic acid/day for 16 weeks post-transplantation; cyclosporin nephrotoxicity did not occur in this group but did occur in the control group who supplemented their diet with corn oil.

Mechanisms by which *n*-3 PUFAs might exert their effects

Clearly *n*-3 PUFAs do influence the functional activities of cells of the immune system, although a number of conflicting observations have been made. These fatty acids appear to alter the production of mediators involved in communication between cells of the immune system (eicosanoids, cytokines, NO) and to alter the expression of key cell surface molecules involved in direct cell-to-cell contact (adhesion molecules). The production of cytokines and NO is regulated by eicosanoids and so an *n*-3 PUFA-induced change in the amount and types of eicosanoids formed could, at least partially, explain the effects of *n*-3 PUFAs. However, many of the effects of *n*-3 PUFAs appear to be exerted in an eicosanoid-independent manner. Thus, other mechanisms of action of *n*-3 PUFAs upon immune cell function must be considered. One such mechanism could be through regulating expression of key genes involved in immune cell functioning and in the production of immune cell-derived mediators.

Evidence that *n*-3 PUFAs affect gene expression in cells of the immune system

It is now well documented that fatty acids affect the expression of genes involved in hepatic fatty acid and lipoprotein metabo-

lism (for reviews, see 139-141) and the genes involved in adipocyte differentiation and development (for reviews, see 140,142). Dietary *n*-3 PUFAs have particularly potent effects upon the expression of genes for proteins involved in hepatic peroxisomal proliferation, fatty acid oxidation and lipoprotein assembly (e.g., 143). Studies of the effects of fatty acids upon the expression of genes important in immune cell functioning are few and relatively recent.

n-3 PUFAs and cytokine gene expression. Inclusion of fish oil in the diet of autoimmune disease-prone mice resulted in elevated levels of mRNA for IL-2, IL-4 and TGF-ß and reduced levels of mRNA for c-myc and c-ras in the spleen (76). The same authors also showed that dietary fish oil completely abolished mRNA production for IL-1ß, IL-6 and TNF-α in the kidneys of these animals (144). It has been reported that feeding mice a fish oil-rich diet significantly diminished the level of IL-1ß mRNA in LPS- or phorbol ester-stimulated spleen lymphocytes (145); the lower IL-1ß mRNA level was not due to accelerated degradation but to impaired synthesis. Fish oil feeding to mice lowered basal and LPS-stimulated TNF-α mRNA levels in peritoneal macrophages (34).

n-3 PUFAs and adhesion molecule gene expression. De Caterina et al. (92) showed that incubation of human saphenous vein endothelial cells with docosahexaenoic acid results in reduced levels of mRNA for CD106.

n-3 PUFAs and NO synthase gene expression. Khair-El-Din et al. (146) reported that incubation of murine thioglycollate-elicited peritoneal macrophages with docosahexaenoic acid decreased NO production in response to LPS plus IFN-γ (or IFN-γ plus TNF-α) and that the inhibition was concentration dependent. Neither arachidonic nor eicosapentaenoic acids at concentrations of up to 100 µM affected NO production. This study found that incubation of the cells with docosahexaenoic acid resulted in a lower level of mRNA for inducible NO synthase (iNOS), the enzyme responsible for NO production in macrophages stimulated in this way; the lower level of iNOS mRNA was due to an inhibition of transcription (146).

Mechanisms by which *n*-3 PUFAs might affect gene expression

Thus, it is now apparent that *n*-3 PUFAs might affect immune cell function partly by regulating the expression of genes encoding for proteins involved in cellular responses and in communication between cells. One mechanism by which *n*-3 PUFAs could affect gene expression is through changes in the signal transduction pathways which link cell surface receptors to the activation of nuclear transcription factors. Alternatively, *n*-3 PUFAs (indeed PUFAs in general) or their derivatives might bind directly to nuclear transcription factors thereby altering their activity.

Fatty acids and signal transduction. Many lipids are involved directly in intracellular signalling pathways; for example, hydrolysis of membrane phospholipids such as PIP_2 and phosphatidylcholine by phospholipases generates second messengers such as DAG and inositol-1,4,5-trisphosphate (IP_3). Other phospholipids play a role in activating or stabilising enzymes involved in intracellular signalling; for example, phosphatidylserine is required for protein kinase C (PKC) activation. DAG activates some isoforms of PKC and also activates sphingomyelinase to release the second messenger ceramide. Ceramide in turn may be converted to sphingosine and sphingosine-1-phosphate; sphingosine inhibits PKC and activates some phospholipases. Since PIP_2, phosphatidylcholine, phosphatidylserine and DAG all contain fatty acyl chains attached to the *sn*-1 and -2 positions of the glycerol moiety, it is conceivable that changing the type of fatty acid present may alter the precise properties of these compounds with regard to their functions in

signal transduction. Certainly, changing the fatty acid composition of the diet (e.g., by feeding fish oil) markedly alters the phospholipid and DAG molecular species compositions of lymphocytes (147) and macrophages (148). That such changes might directly influence signal transduction pathways is shown by the observations that PKC is more active in the presence of dioleoylglycerol or diarachidonoylglycerol than in the presence of DAGs containing two saturated fatty acids or one saturated and one unsaturated fatty acid (149) and that rat spleen PKC is less active in the presence of an *n*-3 PUFA-rich phosphatidylserine compared with a PUFA-poor phosphatidylserine (150).

In support of the idea that *n*-3 PUFAs influence intracellular signalling pathways, DAG generation was reduced in Con A-stimulated lymphocytes taken from mice fed the ethyl ester of docosahexaenoic acid compared with those taken from safflower oil-fed mice (151). More recently, it was shown that feeding either eicosapentaenoic or docosahexaenoic acid to mice results in reduced DAG generation by Con A-stimulated spleen lymphocytes (16); this study also showed, for the first time, that fish oil-derived *n*-3 PUFAs suppress ceramide generation in Con A-stimulated lymphocytes. The DAG in these studies could have been generated by the activity of phosphatidylcholine PLC, phospholipase D or phosphatidylinositol PLC or a combination of these. A recent study which showed reduced calcium ionophore-stimulated DAG production in macrophages from eicosapentaenoic acid-fed mice compared with those fed an *n*-6 PUFA-rich diet suggested that phosphatidylcholine hydrolysis contributed significantly to the total DAG formed (148), implicating phosphatidylcholine PLC and/or phospholipase D in DAG formation. These data indicate that *n*-3 PUFAs in some way affect the activity of one or more phospholipases responsible for the generation of key second messengers; this may be via changes in the fatty acid composition of the substrate phospholipids. Addition of 14.4 g per day of fish oil-derived *n*-3 PUFAs to the diet for 10 weeks resulted in lower platelet activating factor- or LTB_4-stimulated IP_3 generation in peripheral blood neutrophils (40). The platelet activating factor and LTB_4 receptors are coupled via G-proteins to PLCß. In a recent study, it was observed that calcium ionophore- or Con A-stimulated IP_3 generation in rat lymphocytes was significantly reduced if the lymphocytes came from animals fed fish oil (Sanderson P and Calder PC, unpublished observations). Furthermore, it was found that, although the amount of PLCγ-1 in rat lymphocytes was unaffected by diet, the ability to phosphorylate, and so to activate, the enzyme in response to suitable stimuli appeared to be significantly impaired by fish oil feeding (Sanderson P and Calder PC, unpublished observations). Lymphocyte PLCγ-1 is activated by one or more tyrosine kinases (lck, fyn, ZAP-70); thus, these observations are suggestive of lowered activity of certain tyrosine kinases following fish oil feeding. How *n*-3 PUFAs inhibit tyrosine kinase activity is not clear but these tyrosine kinases are associated with specific regions of the plasma membrane. This association might require certain phospholipid fatty acid compositions or membrane physical properties which could be markedly altered by *n*-3 PUFA incorporation, thus resulting in an inability of the tyrosine kinase to maintain its optimal activity.

It has been proposed that unsaturated fatty acids themselves may have a direct effect upon intracellular signalling pathways (for a review, see 152). This direct modulatory effect of fatty acids has been most extensively documented in relation to PKC activity which was shown to be enhanced by docosahexaenoic acid (153). In contrast, although it was reported that eicosapentaenoic and docosahexaenoic acids increased brain PKC activity in the absence of phosphatidylserine and DAG, in the presence of phospha-

tidylserine and DAG both *n*-3 PUFAs caused up to 60% inhibition of PKC activity (154). Other studies have shown that eicosapentaenoic and docosahexaenoic acids inhibit rat lymphocyte or macrophage PKC activity in the presence of calcium, phosphatidylserine and DAG (155,156), whereas protein kinase A activity was unaffected by these fatty acids. In accordance with both the direct effects of *n*-3 PUFAs *in vitro* (155,156) and the effects of enriching phosphatidylserine with *n*-3 PUFAs (150), feeding mice fish oil resulted in diminished spleen lymphocyte PKC activity (157).

A change in the concentration of intracellular free calcium is often a key component in the intracellular signalling pathway which follows the stimulation of lymphocytes, macrophages and other cells by growth factors, cytokines and antigens. There is now considerable evidence that free fatty acids influence these changes. For example, it was reported that oleic acid, but not stearic acid, inhibited the target cell- or Con A-stimulated rise in intracellular free calcium concentration in a cytotoxic T-cell line (158). Several unsaturated fatty acids including α-linolenic, eicosapentaenoic and docosahexaenoic acids inhibit the anti-CD3-induced increase in intracellular free calcium concentration in the Jurkat T-cell line (159,160); the fatty acids appeared to act by blocking calcium entry into the cells and it was concluded that they act directly upon receptor-operated calcium channels. Reduced IP_3 levels as a result of diminished phospholipase activity (see above) would also contribute to decreased intracellular free calcium concentrations, which in turn would reduce the activity of some isoforms of PKC.

Fatty acids and transcription factors within cells of the immune system. Lymphocytes and other immune and inflammatory cells contain many transcription factors including nuclear transcription factor-κB (NF-κB), nuclear transcription factor of activated T-cells (NFAT), AP-1, various oncogene products (e.g., myc, fos, jun), steroid hormone receptors and specific transcription factors such as NF-IL-2, NF-IL-6 and NF-ICAM-1. NF-κB plays a key role in inducing the production of many key mediators within the immune system: NF-κB regulates the synthesis of cytokines including IL-1, IL-2, IL-6, TNF-α and IFN-γ, of cytokine receptors including IL-2R, of adhesion molecules including CD54, CD62E and CD106, of enzymes involved in mediator generation such an iNOS and of a range of acute phase proteins (for a review, see 161). NF-κB is activated by the phosphorylation and subsequent dissociation of one of its three subunits, the so-called inhibitory-κB (IκB); this leaves the remaining dimer free to translocate to the nucleus and bind to appropriate response elements on target genes. It appears that, at least in response to some stimuli, the phosphorylation of IκB is performed by PKC. Given the effects of *n*-3 PUFAs outlined previously (e.g., reduced PLC activity, resulting in reduced DAG and IP_3 generation, resulting in turn in a reduced intracellular free calcium rise and a reduced activation of PKC isoforms), it is evident how these fatty acids could prevent activation of NF-κB and so suppress expression of a range of genes including those for cytokines, cytokine receptors, adhesion molecules and iNOS. Ceramide can also activate NF-κB independently of PKC activity; thus, the observation of Jolly et al. (16) of reduced ceramide production within lymphocytes from *n*-3 PUFA-fed mice could also partly account for the reduced functional responses (e.g., IL-2 production and proliferation) of these cells.

Some transcription factors are receptors for lipophilic molecules; these include steroid hormone receptors and the family of receptors known as peroxisomal proliferator activated receptors (PPARs). Eicosanoid derivatives of arachidonic acid including PGA_2, PGB_2, PGD_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2, but not PGE_2, $PGF_{2\alpha}$ or PGI_2, are activators of PPARα, γ and δ (162); LTB_4, 8-HETE

and 12-HETE appear to activate PPARα only (162). In contrast to their role as PPAR activators, most eicosanoids are poor PPAR ligands (162), although 8-HETE is a high affinity ligand for PPARα (162). The claim that LTB_4 is a PPARα ligand (163) is now disputed (162). It has been claimed that 15-deoxy-$\Delta^{12,14}$-PGJ_2 is a ligand for PPARγ (164-166) and PPARα (166), although Forman et al. (162) showed it does not bind PPARα or δ. Interestingly, the eicosapentaenoic acid derivative 8-HEPE is a potent activator of, and ligand for, PPARα (162). The various roles of arachidonic and eicosapentaenoic acid derivatives as activators and ligands of PPAR isoforms provide a mechanism by which these fatty acids can affect the activity of these transcription factors. It has been known for some time that PUFAs themselves are activators of PPARs (for reviews, see 140,141). Very recently, and in contrast to earlier reports, it has been found that unsaturated fatty acids (linoleic, α-linolenic, γ-linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids) bind directly to PPARα (162,166), δ (162) and γ (166). Thus, it appears that PUFAs are both activators of and ligands for some forms of PPARs. The mechanism of action and functional effects of PPARs have been reviewed in detail recently (140,141). PPAR isoforms have been identified in lymphoid tissues. Using *in situ* DNA hybridisation and immunohistochemical staining Braissant et al. (167) identified moderate to very strong expression of PPARα, ß and γ in rat spleen (both red and white pulp) and lymph nodes; Kliewer et al. (168) had earlier reported the presence of

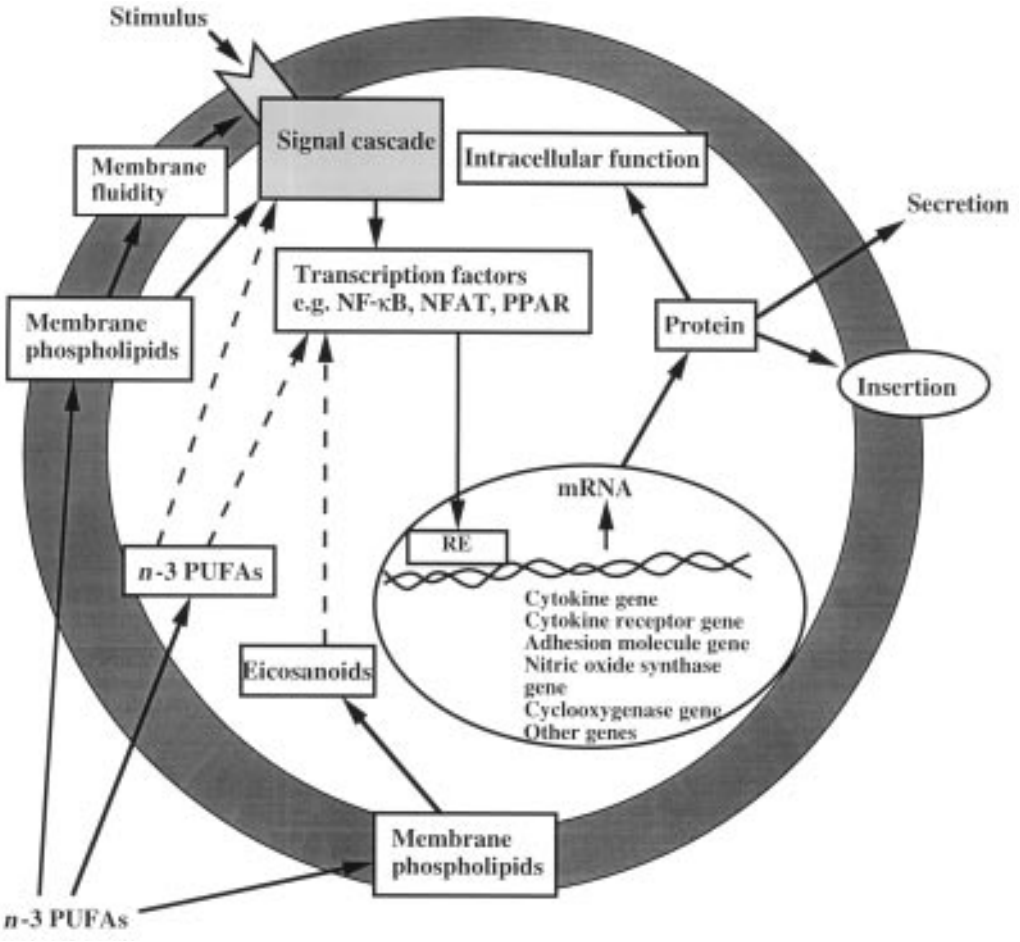

Figure 3 - Schematic representation of the mechanisms by which *n*-3 PUFAs might influence immune cell function.

PPARγ and δ mRNA in murine spleen. These observations suggest that genes within cells of the immune system will be subject to regulation by ligands for PPAR isoforms; these include PUFAs and PUFA derivatives. Cells of the immune system also possess steroid hormone, vitamin D and retinoic acid receptors making them subject to the effects of ligands of these transcription factors. Incidentally, it is proposed that PUFAs regulate the pathway of activation of steroid hormone receptors (for reviews, see 169-171), which may account for the observations that PUFAs, particularly *n*-3 PUFAs, sensitise immune cells to the effects of steroid hormones (e.g., 172).

In addition to the effects which influence the activity of transcription factors, *n*-3 PUFAs might also regulate the synthesis of transcription factors. Fernandes et al. (76) reported markedly decreased c-myc mRNA in spleens from fish oil-fed mice; c-myc mRNA encodes a transcription factor.

Figure 3 attempts to give an overview of the sites at which *n*-3 PUFAs might influence the functional activities of cells of the immune system.

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