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Sir David Cuthbertson Medal Lecture

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

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BIOSYNTHESIS OF *N*-3 POLYUNSATURATED FATTY ACIDS

All mammals can synthesize fatty acids *de novo* from acetyl coenzyme A. The endproduct of the fatty acid synthetase (*EC* 2.3.1.85) 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 C-9 and C-10 is catalysed by the enzyme Δ^9-desaturase, which is universally present in both plants and animals. This enzyme results in the conversion 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 C-9 and the methyl terminus of the C 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 (ALA; 18:3 *n*-3). Many marine plants, especially the unicellular algae in phytoplankton, also carry out chain elongation and further desaturation of α-linolenic acid to yield C_{20} and C_{22} *n*-3 polyunsaturated fatty acids (PUFA) with five or six double bonds (Fig. 1). It is the formation of these long-chain *n*-3 PUFA by marine algae and their transfer through the food chain to fish, that accounts for the abundance of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (20:5*n*-3 and 22:6*n*-3 respectively) in some marine fish oils (FO). Animal cells can also convert ALA to EPA and DHA; by a similar series of reactions 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; Fig. 1). The *n*-9, *n*-6 and *n*-3 families of PUFA are not metabolically interconvertible in mammals.

IMMUNOMODULATORY AND ANTI-INFLAMMATORY EFFECTS OF *N*-3 POLYUNSATURATED FATTY ACIDS

In vitro *effects of* n-*3 polyunsaturated fatty acids on functions of cells of the immune system*

Lymphocyte functions. The *in vitro* effects of fatty acids on lymphocyte proliferation have been studied since the early 1970s and have been reviewed in detail elsewhere (Gurr, 1983;

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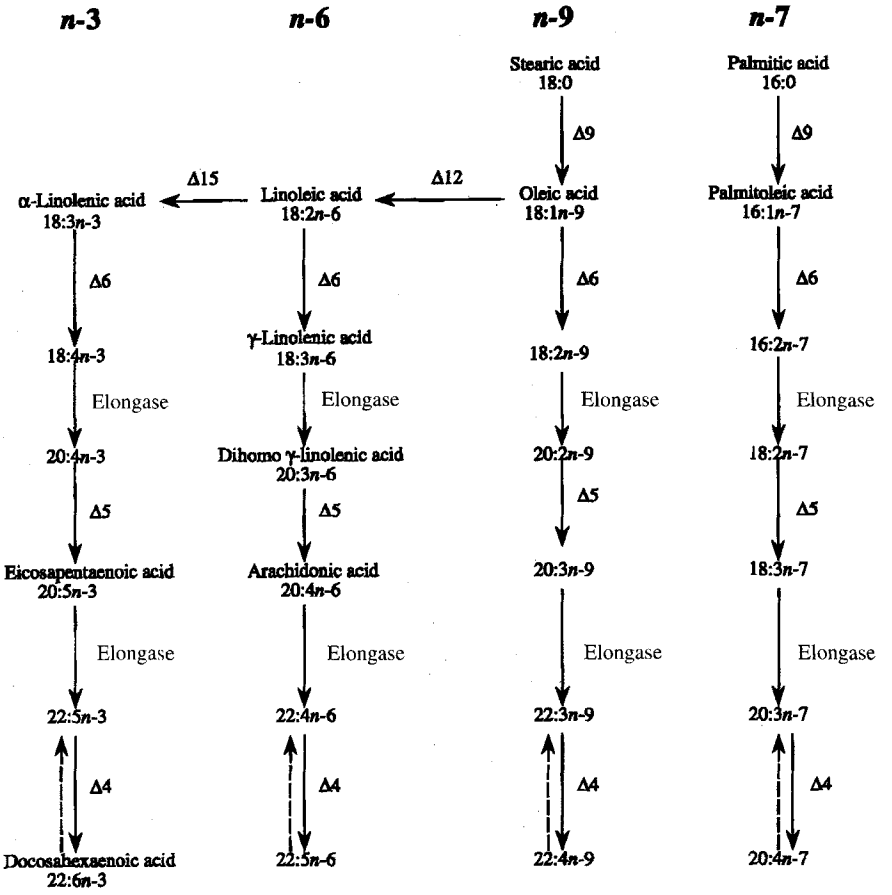

Fig. 1. Pathways of synthesis of unsaturated fatty acids. Δ4, Δ5, Δ6, Δ9, Δ12 and Δ15, respective desaturase enzymes.

Yaqoob & Calder, 1993; Calder, 1995, 1996*a*,*b*). Apart from an early study of the effect of ALA on the proliferation of human peripheral blood lymphocytes (PBL; Kelly & Parker, 1979), the effects of *n*-3 PUFA on lymphocyte functions were not investigated until relatively recently. These studies have been reviewed elsewhere (Calder, 1995, 1996*a*,*b*) and are summarized in Table 1. Several studies have shown that ALA, EPA and DHA inhibit the proliferation of lymphocytes isolated from rodent lymphoid tissues (lymph nodes, spleen, thymus) and from human peripheral blood (for references, see Table 1); in these studies proliferation has been stimulated by a variety of agents including T-cell mitogens (concanavalin A (Con A), phytohaemagglutinin (PHA)), a B-cell mitogen (bacterial lipopolysaccharide (LPS)), a monoclonal antibody directed against CD3 and cytokines (interleukin (IL)-1α, IL-2). Of all fatty acids tested, EPA appears to be the most inhibitory. The inhibitory effects of *n*-3 PUFA on lymphocyte proliferation *in vitro* are not mediated by eicosanoids (Santoli *et al.* 1990; Calder *et al.* 1992; Kumar *et al.* 1992; Soyland *et al.* 1993*b*; Rotondo *et al.* 1994). Not only do *n*-3 PUFA affect the response of lymphocytes to antigen (and to mitogens in experimental conditions), they may also affect the ability of antigen-presenting cells to present antigen (Fujikawa *et al.* 1992). The proliferation of lym-

Table 1. *Effects of* n-*3 polyunsaturated fatty acids (PUFA) on lymphocyte functions in vitro*

Cell source	Stimulus	Fatty acid	Effect	Reference
Proliferation*:				
Human blood	PHA	ALA	↓†	Kelly & Parker (1979)
Human blood	IL-2	ALA, EPA	↓	Santoli *et al.* (1990)
Rat lymph node	LPS	ALA, EPA, DHA	↓	Calder *et al.* (1990*a*)
Rat lymph node	Con A	ALA, EPA, DHA	↓	Calder *et al.* (1990*b*, 1991, 1992), Calder & Newsholme (1992*a*, 1993)
Human blood	Anti-CD3	EPA	↓	Virella *et al.* (1991)
Human blood	Con A, PPD	ALA, EPA, DHA	↓	Calder & Newsholme (1992*b*)
Human blood	PHA	EPA, DHA	↓	Kumar *et al.* (1992)
Human blood	PHA	ALA, EPA, DHA	↓	Soyland *et al.* (1993*b*)
Human blood	PHA	EPA, DHA	↓	Brouard & Pascaud (1993)
Rat spleen	Con A	EPA, DHA	↓	Brouard & Pascaud (1993)
Rat lymph node	Con A	Tri-ALA‡	↓	Calder *et al.* (1994*c*)
Human blood	PHA	ALA, EPA DHA	↓	Devi & Das (1994), Das (1994)
Rat lymph node	Con A	*n*-3 PUFA-rich emulsion	↓	Calder *et al.* (1994*a*)
Human blood	Con A	*n*-3 PUFA-rich emulsion	↓	Calder *et al.* (1994*a*)
Human synovial fluid§	PHA+IL-2	EPA	↓	DeMarco *et al.* (1994)
Mouse thymus	PHA, IL-1α, IL-2	ALA, EPA	↓	Rotondo *et al.* (1994)
IL-2 production:				
Rat lymph node	Con A	ALA, EPA, DHA	↓	Calder & Newsholme (1992*a*)
Human blood	Con A	ALA, EPA, DHA	↓	Calder & Newsholme (1992*b*)
Human blood	PHA	ALA, EPA, DHA	↓	Devi & Das (1994), Das (1994)
Degranulation of CTL:				
Rat spleen	Allogenic cells	ALA	↓	Richieri *et al.* (1990)
NK cell-mediated cytolysis:				
Human blood	Allogenic cells	Tri-EPA‡	↓	Yamashita *et al.* (1986)
Human blood	Allogenic cells	Tri-EPA‡, Tri-DHA‡	↓	Yamashita *et al.* (1991)
Antigen presentation:				
Mouse spleen	KLH‖	Tri-EPA‡	↓	Fujikawa *et al.* (1992)

ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosohexaenoic acid; PHA, phytohaemagglutinin; IL, interleukin; LPS, bacterial lipopolysaccharide; Con A, concanavalin A; PPD, purified protein derivative of *Mycobacterium tuberculosis*; KLH, keyhole limpet haemacyanin; ↓, depression of lymphocyte function; CTL, cytotoxic T lymphocytes.

* In all cases proliferation was measured as thymidine incorporation.

† Stimulation was observed at low ALA concentrations (<5 μg/ml).

‡ Triacylglycerol with ALA, EPA or DHA at all three *sn*-positions.

§ Taken from patients with rheumatoid arthritis.

‖ Presented as antigen.

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phocytes and the regulation of the function of cytotoxic T lymphocytes (CTL), natural killer (NK) cells, B-cells and macrophages depend on the production of IL-2. The *n*-3 PUFA, ALA, EPA and DHA all suppress the production of IL-2 by mitogen-stimulated rat or human lymphocytes (for references, see Table 1). Triacylglycerols (TAG) containing *n*-3 PUFA have been reported to directly inhibit NK cell activity (Yamashita *et al.* 1986, 1991). In addition, ALA inhibits the degranulation of CTL (Richieri *et al.* 1990) and, therefore, presumably CTL activity, although this has not been directly tested. Other unsaturated fatty acids which inhibit CTL degranulation (Richieri *et al.* 1990) inhibit CTL activity (Taylor *et al.* 1985; Richieri & Kleinfeld, 1990).

Monocyte and phagocyte functions. Although it has been known for some time that fatty acids can directly affect the functioning of phagocytic cells (e.g. Badwey *et al.* 1984), there are few studies of the effects of *n*-3 PUFA on such functions. The proliferation of a monocytic cell line is inhibited by addition of FO to the cultures (Watson *et al.* 1990) and *n*-3 PUFA have been shown to influence the production of cytokines by monocytic cell lines and human peripheral blood monocytes *in vitro* (Baldie *et al.* 1993). Poulos *et al.* (1991) reported that both EPA and DHA are potent activators of superoxide production by human neutrophils; DHA also enhanced the responses to two known neutrophil agonists, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) and phorbol ester. In contrast to these observations, Chen *et al.* (1994) recently showed that EPA and DHA significantly suppressed phorbol ester-stimulated superoxide generation by human neutrophils. DHA inhibited the interferon (IFN)-γ-stimulated tumouricidal action of murine peritoneal macrophages and a macrophage cell line towards a TNF-α-resistant, NO-sensitive target cell line (Dustin *et al.* 1990). It appeared that this effect was due to inhibition of IFN-γ-dependent signals. Recently, it has been shown that DHA inhibits IL-4- or IFN-γ-induced cell surface expression of Ia (i.e. major histocompatibility class (MHC) II antigens) on mouse peritoneal macrophages; DHA was more inhibitory than EPA and other C_{20} fatty acids (Khair-El-Din *et al.* 1995). DHA acted by inhibiting the increase in Ia mRNA after stimulation of macrophages with IFN-γ (Khair-El-Din *et al.* 1995). These effects of DHA were not due to changes in the levels and types of eicosanoids produced (Dustin *et al.* 1990; Khair-El-Din *et al.* 1995).

Effects of dietary n-*3 polyunsaturated fatty acids on* ex vivo *functions of cells of the immune system*

Lymphocyte proliferation. Studies involving the feeding of diets rich in *n*-6 PUFA, such as maize, soyabean, safflower or sunflower oils, to laboratory animals have been reviewed several times (Gurr, 1983; Calder, 1995, 1996*a*). In recent years there has been increased interest in the effects of *n*-3 PUFA-containing oils (rapeseed, linseed (LO), FO) on lymphocyte proliferation. Many studies have reported that feeding such oils to laboratory animals (rats, mice, rabbits, chickens) suppresses the response of spleen lymphocytes to mitogenic stimuli, including Con A, PHA and pokeweed mitogen (for references, see Table 2). Recently, we reported that feeding rats for 10–12 weeks on a diet containing 200 g FO/kg results in markedly suppressed Con A- and PHA-stimulated spleen, thymus, lymph node and PBL proliferation *ex vivo* (Yaqoob *et al.* 1994*a*, 1995*a*; Sanderson *et al.* 1995*a*). Feeding the FO diet did not affect the proportions of T-, B-, $CD4^+$- or $CD8^+$-cells or macrophages in the spleen, thymus, lymph nodes or blood of these rats (Yaqoob *et al.* 1994*a*, 1995*a*; Sanderson *et al.* 1995*a*). However, FO feeding lowered the proportion of spleen lymphocytes bearing the IL-2 receptor (IL-2R), the proportion of thymic lympho-

Table 2. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on lymphocyte functions tested* ex vivo

Cell source	Details of diet used	Stimulus	Effect	Reference
Proliferation*:				
Rat spleen	100 g LO/kg, 3 weeks†	Con A, PHA	↓	Marshall & Johnston (1985)
Mouse spleen	200 g FO/kg, 8 weeks	Con A	↓	Alexander & Smythe (1988)
Rabbit spleen	76 g FO/kg, 20 weeks	Con A, PHA	↓	Kelley *et al.* (1988)
Rabbit blood	76 g FO/kg, 20 weeks	Con A, PHA, LPS	↓	Kelley *et al.* (1988)
Chicken spleen	70 g LO or FO/kg, 3 weeks	Con A, PWM	↓	Fritsche *et al.* (1991)
Human blood	Encapsulated *n*-3 PUFA (2·4 g/d), 12 weeks	Con A, PHA	↓‡	Meydani *et al.* (1991)
Human blood	Encapsulated FO (8 g/d), 6 weeks	Anti-CD3	↓	Virella *et al.* (1991)
Human blood	Encapsulated FO (4 g/d), 7 weeks	PHA	↓	Molvig *et al.* (1991)
Human blood	Low-fat, low-cholesterol + *n*-3 PUFA (1·23 g/d), 24 weeks	Con A, PHA	↓	Meydani *et al.* (1993)
Human blood	FO concentrate (18 g/d), 6 weeks	PHA	↓	Endres *et al.* (1993)
Rat spleen	200 g FO/kg, 10 weeks	Con A, PHA	↓	Yaqoob *et al.* (1994*a*)
Rat lymph node	200 g FO/kg, 10 weeks	Con A, PHA	↓	Yaqoob *et al.* (1994*a*)
Rat thymus	200 g FO/kg, 10 weeks	Con A	↓	Yaqoob *et al.* (1994*a*)
Human blood§	*n*-3 PUFA ethyl esters (6 g/d), 16 weeks	PHA	No effect	Soyland *et al.* (1994)
Rat blood	200 g FO/kg, 10 weeks	Sub-optimal Con A	↓	Yaqoob *et al.* (1995*a*)
Rat spleen	150 g rapeseed oil/kg, 8 weeks	Con A	↓	Calder *et al.* (1995)
Rat spleen	200 g FO/kg, 12 weeks	Con A	↓	Sanderson *et al.* (1995*a*)
Mouse spleen	200 g FO/kg, 8 weeks	Con A	↓	Yaqoob & Calder (1995*a*)
Rat spleen	200 g LO/kg, 10 weeks	Con A	↓	Jeffery *et al.* (1996)
NK cell-mediated cytolysis:				
Mouse spleen	100 g FO/kg, 6 weeks	Allogenic cells	↓	Meydani *et al.* (1988)
Mouse spleen	100 g FO/kg, 8–10 weeks	Allogenic cells	↓	Fritsche & Johnstone (1990)
Mouse spleen	100 or 186 g FO/kg, 6 weeks	Allogenic cells	↓	Lumpkin *et al.* (1993)
Mouse spleen	100 g LO or FO/kg, dams fed 20 weeks	Allogenic cells	↓	Berger *et al.* (1993)
Rat spleen	200 g FO/kg, 10 weeks	Allogenic cells	↓	Yaqoob *et al.* (1994*b*)
Rat spleen	200 g FO/kg, 12 weeks	Allogenic cells	↓	Sanderson *et al.* (1995*a*)
Human blood	Tri-EPA‖ (3 g intravenously)	Allogenic cells	↓	Yamashita *et al.* (1991)
LAK cell-mediated cytolysis:				
Mouse spleen	100 g LO or FO/kg, dams fed 20 weeks	Allogenic cells	↓	Berger *et al.* (1993)
Rat spleen	200 g FO/kg, 10 weeks	Allogenic cells	↓	Yaqoob *et al.* (1994*b*)

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Table 2. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on lymphocyte functions tested* ex vivo

Cell source	Details of diet used	Stimulus	Effect	Reference
CTL-mediated cytolysis:				
Mouse spleen	100 g FO/kg, 8–10 weeks	Vaccinia virus *in vivo*	↓	Fritsche & Johnstone (1990)
Chicken spleen	70 g LO or FO/kg, 9 weeks	Sheep erythrocytes *in vivo*	↓	Fritsche & Cassity (1992)
Antigen presentation:				
Mouse spleen	20 g EPA ethyl ester/kg, 4–5 weeks	KLH¶	↓	Fujikawa *et al.* (1992)

LO, linseed oil; FO, marine fish oil; NK, natural killer; LAK, lymphokine-activated killer; CTL, cytotoxic T lymphocyte; Con A, concanavalin A; PHA, phytohaemagglutinin; LPS, bacterial lipopolysaccharide; PWM, pokeweed mitogen; KLH, keyhole limpet haemacyanin; ↓ depression of lymphocyte function; EPA, eicosapentaenoic acid.

* In all cases proliferation was measured as thymidine incorporation.

† Dams fed on 100 g LO/kg throughout gestation and lactation.

‡ Effect more significant in older than younger women.

‖ Triacylglycerol with EPA at all three *sn*-positions.

§ Subjects were patients with psoriasis or atopic dermatitis.

¶ Presented as antigen.

cytes bearing the IL-2R and transferrin receptor and the proportion of lymph-node lymphocytes bearing the transferrin receptor following Con A stimulation (Yaqoob *et al.* 1994*a*). Spleen lymphocytes from animals fed on this diet also showed a 60% lower level of expression of the IL-2R following Con A stimulation (Sanderson *et al.* 1995*a*).

Meydani *et al.* (1991) reported the results of supplementing the diets of healthy young (22–33 years of age) or older (51–68 years of age) women with encapsulated *n*-3 PUFA (approximately 2·4 g/d); the mitogenic response of PBL to PHA was lowered after 12 weeks of supplementation in the older women. Recently, Meydani *et al.* (1993) reported a decreased response of PBL to Con A and PHA following supplementation of the diet of volunteers on a low-fat low-cholesterol diet with *n*-3 PUFA, while Endres *et al.* (1993) found that 18 g FO/d for 6 weeks resulted in lowered PHA-stimulated proliferation of PBL 10 weeks after supplementation had ended (but not at the end of the supplementation period). Meydani *et al.* (1993) observed that consumption of the low-fat low-cholesterol *n*-3 PUFA-rich diet resulted in a lower proportion of $CD4^+$ and a higher proportion of $CD8^+$ PBL; the proportion of $CD3^+$ cells (i.e. T-cells) was unaffected. Soyland *et al.* (1994) reported that supplementation of the diet of patients with psoriasis or atopic dermatitis with *n*-3 PUFA ethyl esters (6 g/d) caused a significant reduction in the percentage of IL-2^+ blood lymphocytes following PHA stimulation; the level of expression of the IL-2R on the positive cells was also significantly reduced.

Lymphocyte-mediated cytolysis. CTL activity has been reported to be significantly diminished following the feeding of *n*-3 PUFA-rich diets to mice or chickens (Fritsche & Johnstone, 1990; Fritsche & Cassity, 1992).

Meydani *et al.* (1988) found that feeding young mice on a diet containing 100 g FO/kg

for 6 weeks caused a decrease in spleen NK cell activity compared with feeding chow or maize oil; there were no differences in NK cell activity when these diets were fed to older mice. Feeding mice on a diet containing 100 g FO/kg suppressed spleen NK cell activity when compared with a 100 g LO/kg diet (Fritsche & Johnstone, 1990). In the study of Berger *et al.* (1993) female mice were fed for 5 months on diets containing 100 g olive oil, safflower oil, LO or FO/kg and the spleen NK cell activity of the pups was determined before they were weaned; the activity was lower in the FO group than in the safflower-oil or olive-oil groups. Recently, Yaqoob *et al.* (1994*b*) showed that feeding 200 g FO/kg to weanling rats for 10 weeks significantly decreased spleen NK cell activity compared with feeding a low-fat diet or high-fat diets containing coconut, safflower or evening primrose (*Oenothera biennis*) oils. No studies have investigated the effect of dietary lipids on human NK cell activity, although intravenous injection of a TAG containing EPA into healthy human volunteers resulted in suppression of peripheral blood NK cell activity 24 h later (Yamashita *et al.* 1991).

Lymphokine-activated killer cells are generated by the culture of mitogen-stimulated lymphocytes with exogenous IL-2. The resulting activity is diminished if animals have been previously fed on a FO-rich diet (Berger *et al.* 1993; Yaqoob *et al.* 1994*b*).

Production of reactive oxygen species and nitric oxide. The enzymes which result in the synthesis of superoxide, H_2O_2 and NO are regulated by eicosanoids, cytokines and protein kinase C (PKC). Since *n*-3 PUFA affect the production of eicosanoids and cytokines (see p. 748) and might modulate PKC activity (see p. 762), they might affect the production of reactive oxygen species and NO by macrophages and so regulate the cytotoxic activities of these cells. However, investigations of the effects of diets rich in *n*-3 PUFA on the production of H_2O_2, superoxide and NO have yielded contradictory results (for references, see Table 3). Studies have reported that production of these mediators is enhanced, diminished or not affected following FO feeding to laboratory animals (for references, see Table 3). The reasons for such significantly different experimental observations might include the different species of origin of the cells studied (mouse, rat, pig, guinea-pig, rabbit, man), the anatomical site of origin of the cells (liver, lung, peritoneal cavity, blood), the state of cellular differentiation (monocyte, macrophage), the state of activation of the cell (resident, inflammatory, activated) the stimulus used to elicit mediator production (for details, see Table 3), the nature of the culture conditions used (presence or absence of serum, serum source, duration of culture, etc.), the level of FO in the diet, the duration of feeding, the level of antioxidant in the diet, etc. In a recent study, thioglycollate-elicited peritoneal macrophages from mice fed on 200 g FO/kg for 8 weeks produced more superoxide and H_2O_2 in response to stimulation by phorbol-12-myristate-13-acetate than macrophages from mice fed on a low-fat diet (Yaqoob & Calder, 1995*b*). Furthermore, macrophages from the FO-fed mice produced more NO in response to stimulation with LPS than those from mice fed on the low-fat diet (Yaqoob & Calder, 1995*b*). Since superoxide, H_2O_2 and NO are important macrophage-derived cytotoxic agents these observations suggest that dietary FO could affect the killing of microbial or tumour cells by macrophages. NO appears to regulate lymphocyte functions and so FO-induced modulation of its generation from macrophages could affect lymphocyte activity.

Phagocytosis by macrophages. There are reports that dietary FO suppresses or does not affect phagocytosis of bacterial cells or particles by macrophages of various origins (for references, see Table 3).

Table 3. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on phagocytic cell functions tested* ex vivo

Cell type	Details of diet used	Stimulus	Effect	Reference
NO production:				
Mouse-elicited peritoneal Mϕ	100 g FO/kg, 6–15 weeks	IFN-γ	↑	Renier *et al.* (1993)
Pig alveolar Mϕ	105 g FO/kg, 4 weeks	LPS	↑	Turek *et al.* (1994)
Rat alveolar Mϕ	90 g FO/kg, 12 d	IFN-γ + LPS	↑	Chaet *et al.* (1994)
Rat resident peritoneal Mϕ	80 g FO/kg, 6 weeks	LPS	↓	Joe & Lokesh (1994)
Mouse elicited peritoneal Mϕ	100 g LO or FO/kg, 3 weeks	IFN-γ + LPS	No effect	Hubbard *et al.* (1994)
Rat resident peritoneal Mϕ	150 g FO/kg, 6 weeks	LPS	↓	Boutard *et al.* (1994)
Mouse elicited peritoneal Mϕ	200 g FO/kg, 8 weeks	LPS	↑	Yaqoob & Calder (1995*b*)
Superoxide production:				
Guinea-pig elicited peritoneal neutrophils	30 g FO/kg, 12 weeks	fMLP, PMA	↓	Fletcher & Ziboh (1990)
Human blood neutrophils	2·16 g EPA/d, 7 weeks	fMLP, PAF	↓	Thompson *et al.* (1991)
Human blood monocytes	3·6 g EPA + 2·4 g DHA/d, 6 weeks	Latex particles	↓	Fisher *et al.* (1990)
Rabbit alveolar Mϕ	5 g FO/kg body wt per d by gastric tube, 1 week	Zymosan	↓	D'Ambola *et al.* (1991)
Mouse elicited peritoneal Mϕ	100 g LO or FO/kg, dams fed 20 weeks	PMA	↑	Berger *et al.* (1993)
Rat resident peritoneal Mϕ	80 g FO/kg, 6 weeks	PMA	↓	Joe & Lokesh (1994)
Mouse Kupffer	170 g FO/kg, 4 weeks	Bacterial challenge *in vivo**	↓	Eicher & McVey (1995)
Mouse elicited peritoneal Mϕ	200 g FO/kg, 8 weeks	PMA	↑	Yaqoob & Calder (1995*b*)
H_2O_2 production:				
Mouse elicited peritoneal Mϕ	100 g FO/kg, 4 weeks	Zymosan	↓	Hubbard *et al.* (1991)
Mouse elicited peritoneal Mϕ	100 g FO/kg, 4 weeks	PMA	No effect	Hubbard *et al.* (1991)
Rat resident peritoneal Mϕ	80 g FO/kg, 6 weeks	PMA	↓	Joe & Lokesh (1994)
Mouse elicited peritoneal Mϕ	200 g FO/kg, 8 weeks	PMA	↑	Yaqoob & Calder (1995*b*)
Phagocytosis:				
Rabbit alveolar Mϕ	5 g/kg body wt per d FO by gastric tube, 1 week	*Staphylococcus aureus*	No effect	D'Ambola *et al.* (1991)
Mouse elicited peritoneal Mϕ	100 g FO/kg, 4 weeks	Sheep erythrocytes, yeast	No effect	Hubbard *et al.* (1991)
Pig alveolar Mϕ	105 g FO/kg, 4 weeks	Latex beads	No effect	Turek *et al.* (1994)
Mouse Kupffer	170 g FO/kg, 4 weeks	Bacterial challenge *in vivo**	↓	Eicher & McVey (1995)

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Table 3. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on phagocytic cell functions tested* ex vivo

Cell type	Details of diet used	Stimulus	Effect	Reference
Macrophage-mediated cytolysis:				
Mouse elicited peritoneal Mϕ	100 g FO/kg, 4 weeks	Allogenic cells + IFN-γ + LPS	↓	Somers *et al.* (1989)
Mouse elicited peritoneal Mϕ	15 g *n*-3 PUFA/kg, 2 weeks	Allogenic cells + LPS	↓	Black & Kinsella (1993)
Mouse elicited peritoneal Mϕ	100 g FO/kg, 6–15 weeks	Allogenic cells + LPS	↓	Renier *et al.* (1993)
Mouse elicited peritoneal Mϕ	100 g FO/kg, 3 weeks	Allogenic cells + IFN-γ + LPS	↓	Hubbard *et al.* (1994)
Chemotaxis:				
Human blood neutrophils	Encapsulated *n*-3 PUFA (3·2 g EPA + 2·2 g DHA/d), 6 weeks	LTB_4	↓	Lee *et al.* (1985)
Human blood monocytes	Encapsulated *n*-3 PUFA (2·7 g EPA + 1·8 g DHA/d), 6 weeks	LTB_4	↓	Endres *et al.* (1989)
Human blood monocytes	5·3 g *n*-3 PUFA/d, 6 weeks or 9 months	fMLP	↓	Schmidt *et al.* (1989, 1992)
Human blood neutrophils	5·3 g *n*-3 PUFA/d, 6 weeks or 9 months	fMLP	↓	Schmidt *et al.* (1989, 1992)
Human blood neutrophils	9·4 g EPA + 5 g DHA/d, 3 or 10 weeks	LTB_4, PAF	↓	Sperling *et al.* (1993)
MHC II expression:				
Mouse peritoneal exudate†	200 g FO/kg, 12 weeks	None	↓§	Kelley *et al.* (1985)
Rat peritoneal exudate	18 mg EPA + 12 mg DHA/d, 4 weeks	Bacterial challenge *in vivo*‡	↓§	Mosquera *et al.* (1990)
Mouse peritoneal exudate	9 mg EPA + 6 mg DHA/d, 4 weeks	Bacterial challenge *in vivo*‡	↓§	Mosquera *et al.* (1990)
Mouse peritoneal exudate	170 g FO/kg, 6 weeks	Bacterial challenge *in vivo*‡	↓§‖	Huang *et al.* (1992)
Human blood monocytes	930 mg EPA + 630 mg DHA/d, 3 weeks	None	↓‖	Hughes *et al.* (1995)
Rat elicited peritoneal Mϕ	200 g FO/kg, 12 weeks	None	↓‖	Sherrington *et al.* (1995*b*)

FO, marine fish oil; LO, linseed oil; EPA, eicosapentaenoic acid; IFN-γ, interferon-γ; LPS, bacterial lipopolysaccharide; fMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; PMA, phorbol-12-myristate-13-acetate; PAF, platelet-activating factor; LTB_4, leukotriene B_4; DHA, docosahexaenoic acid; ↓, ↑, depression and promotion of phagocytic cell function respectively; Mϕ, macrophage.

* Oral *Salmonella typhimurium*.

† Mice used were prone to autoimmune disease.

‡ Intraperitoneal *Listeria monocytogenes*.

§ Decreased proportion of positive cells.

‖ Decreased density of expression on positive cells.

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Macrophage-mediated cytolysis. Dietary FO has been reported to significantly suppress lysis of target tumour cells by mouse elicited peritoneal macrophages (for references, see Table 3). The target cell lines used in these studies are sensitive to killing by tumour necrosis factor (TNF)-α (L929 cells; Black & Kinsella, 1993) or NO (P815 cells; Somers *et al.* 1989; Hubbard *et al.* 1994). Thus, the suppressed macrophage-mediated cytolysis observed after FO feeding is consistent with the decreased production of NO (see Table 3) and TNF (see Tables 4 and 5) reported from some studies.

Major histocompatibility class II expression and antigen presentation. Kelley *et al.* (1985), Mosquera *et al.* (1990) and Huang *et al.* (1992) showed that inclusion of *n*-3 PUFA in the diet of mice or rats results in a diminished percentage of peritoneal exudate cells bearing the MHC II antigens on their surface (see Table 3). Huang *et al.* (1992) also reported that the level of MHC II expression on positive cells was suppressed by FO feeding. Recently, it was found that feeding weanling rats on a diet containing 200 g FO/kg for 12 weeks results in a decreased level of MHC II expression on thioglycollate-elicited peritoneal macrophages (Sherrington *et al.* 1995*b*). In accordance with these animal studies, Hughes *et al.* (1995) reported that supplementation of the diet of human volunteers with *n*-3 PUFA for 3 weeks results in a decreased level of MHC II (human leukocyte-associated antigen DR) expression on the surface of peripheral blood monocytes. These observations suggest that diets rich in *n*-3 PUFA will result in diminished antigen presentation. Indeed Fujikawa *et al.* (1992) found that feeding mice on the ethyl ester of EPA for a period of 4–5 weeks resulted in diminished presentation of antigen (keyhole limpet haemacyanin) by spleen cells *ex vivo*.

Chemotaxis. Chemotaxis of blood neutrophils and monocytes towards a variety of chemoattractants including leukotriene (LT) B_4, platelet-activating factor, fMLP and autologous serum is suppressed following the supplementation of the human diet with *n*-3 PUFA (for references, see Table 3).

Effects of n-*3 polyunsaturated fatty acids on cellular adhesion*

In vitro *studies with* n-*3 polyunsaturated fatty acids.* That *n*-3 PUFA can affect the expression of adhesion molecules on the cell surface was shown by the recent study of De Caterina *et al.* (1995) who reported that culture of human adult saphenous vein endothelial cells with DHA (but not EPA) significantly decreased the cytokine-induced expression of vascular cell adhesion molecule 1 (VCAM-1), E-selectin (also known as endothelial leukocyte adhesion molecule 1 or ELAM-1) and CD54 (also known as intercellular adhesion molecule 1 or ICAM-1) in a dose-dependent manner. The effects of DHA were independent of eicosanoid production. The adhesion of U937 monocytes and human peripheral blood monocytes to endothelial cells was diminished following incubation of the latter with DHA (De Caterina *et al.* 1995). The binding between monocytes and endothelial cells partially depends on VCAM-1 expression on the endothelial cells; thus the reduced expression of VCAM-1 caused by DHA appears to have a functional effect. Recently, Kim *et al.* (1995) reported that incubation of LPS-stimulated pig aortic endothelial cells with EPA resulted in diminished binding between these cells and U937 monocytes (they did not investigate the effects of DHA). EPA was shown to reduce the expression of VCAM-1, ELAM-1 and ICAM-1 on the surface of LPS-stimulated human umbilical vein endothelial cells (Kim *et al.* 1995). Calder *et al.* (1990*c*) reported that murine thioglycollate-elicited peritoneal macrophages cultured in the presence of EPA or DHA were less adherent to artificial surfaces (the adhesion to one of these surfaces is mediated by CD11b/CD18) than those

Table 4. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on* ex vivo *cytokine production by rodent and pig macrophages and lymphocytes*

Species	Cell type	Details of diet used	Cytokine	Effect	Reference
Rat	Kupffer	150 g FO/kg, 6 weeks	IL-1	↓	Billiar *et al.* (1988)
			TNF	↓	
Mouse	Resident peritoneal Mϕ	100 g FO/kg, 4 weeks	IL-1	↑	Lokesh *et al.* (1990)
			TNF	↑	
Mouse	Resident peritoneal Mϕ	100 g fat rich in ALA/kg, 5 weeks	TNF	↑	Watanabe *et al.* (1991)
Mouse	Elicited peritoneal Mϕ	100 g fat rich in ALA/kg, 5 weeks	TNF	No effect	Watanabe *et al.* (1991)
Mouse	Resident peritoneal Mϕ	100 g fat rich in *n*-3 PUFA/kg, 5 weeks	TNF	↑	Hardardottir & Kinsella (1991)
Rat	Resident peritoneal Mϕ	125 g LO/kg, 4 weeks	TNF	↑	Turek *et al.* (1991)
Rat	Elicited peritoneal Mϕ	125 g LO/kg, 4 weeks	TNF	No effect	Turek *et al.* (1991)
Mouse	Resident peritoneal Mϕ	100 g fat with increasing *n*-3 PUFA content/kg, 6 weeks	TNF	↑	Hardardottir & Kinsella (1992)
Mouse	Elicited peritoneal Mϕ	200 g FO/kg, 4 weeks	TNF	↑	Chang *et al.* (1992*a*)
Mouse	Elicited peritoneal Mϕ	100 g FO/kg, 6–15 weeks	TNF-α	↓	Renier *et al.* (1993)
Mouse	Elicited peritoneal Mϕ	100 g FO/kg, 4 weeks	TNF-α	↑	Somers & Erickson (1994)
Pig	Alveolar Mϕ	105 g LO or FO/kg, 4 weeks	TNF-α	↓	Turek *et al.* (1994)
Pig	Alveolar lymphocytes	105 g LO or FO/kg, 4 weeks	IL-2	↓	Turek *et al.* (1994)
Rat	Elicited peritoneal Mϕ	100 g FO/kg, 8 weeks	IL-1	↓	Tappia & Grimble (1994)
			IL-6	↑	
			TNF	No effect	
Mouse	Elicited peritoneal Mϕ	100 g FO or LO/kg, 3 weeks	TNF-α	No effect	Hubbard *et al.* (1994)
Mouse	Elicited peritoneal Mϕ	100 g FO/kg, 3 weeks	TNF-α	↑	Somers & Erickson (1994)
Mouse	Elicited peritoneal Mϕ	200 g FO/kg, 8 weeks	IL-1α	No effect	Yaqoob & Calder (1995*b*)
			IL-6	↓	
			TNF-α	↓	
Rat	Alveolar Mϕ	90 g FO/kg, 12 d	TNF	↑	Chaet *et al.* (1994)
Rat	Resident peritoneal Mϕ	150 g FO/kg, 6 weeks	TNF	↓	Boutard *et al.* (1994)
Mouse*	Spleen lymphocytes	100 g FO/kg, 6·5 months	IL-2	↑	Fernandes *et al.* (1994)
			IL-4	No effect	
Mouse	Spleen lymphocytes	200 g FO/kg, 8 weeks	IL-2	No effect	Yaqoob & Calder (1995*a*)
			IL-4	No effect	
			IL-10	No effect	
			IFN-γ	No effect	

FO, marine fish oil; LO, linseed oil; Mϕ, macrophage; ALA, α-linolenic acid; IL, interleukin; TNF, tumour necrosis factor; IFN-γ, interferon-γ; ↓, ↑, depression and promotion of cytokine production respectively.

* Mice used were autoimmune-disease prone.

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cultured with some other fatty acids; ALA was without effect. Twisk *et al.* (1991) found that culture of murine spleen lymphocytes with ALA did not affect their ability to bind to high endothelial venules of either lymph nodes or Peyer's patches; the effects of EPA and DHA were not examined. The expression of leukocyte-function-associated molecule (LFA)-1 on the surface of spleen T and B lymphocytes was unaffected by culture of the cells in the presence of ALA (Twisk *et al.* 1991).

Effects of dietary n-*3 polyunsaturated fatty acids.* Feeding rats a diet containing 200 g FO/kg for 10–12 weeks caused a 30% reduction in the level of expression of CD18 (the β-chain of LFA-1) on thioglycollate-elicited peritoneal macrophages (Sherrington *et al.* 1995*b*). Feeding rats on this diet caused a 20–30% decrease in the level of expression of the adhesion molecules CD2 and CD11a (the α-chain of LFA-1) on resting spleen lymphocytes; similar effects were observed on both $CD4^+$ and $CD8^+$ cells (Sanderson *et al.* 1995*a*). The level of expression of the adhesion molecules CD2, ICAM-1, LFA-1 on Con A-stimulated spleen lymphocytes was reduced by 25, 33 and 20% respectively if the animals were fed on a FO-rich diet (Sanderson *et al.* 1995*a*). In addition, FO feeding reduced the level of expression of CD2, ICAM-1 and LFA-1 on the surface of lymphocytes obtained from the popliteal lymph nodes (PLN) of rats undergoing a localized graft (G) *v.* host (H) response (see p. 751) by 20, 12 and 10% respectively (Sanderson *et al.* 1995*b*). Finally, following a localized H*v*.G response the level of expression of CD2 and LFA-1 on PLN lymphocytes was reduced by 12 and 10% respectively if the animals had been fed on FO (Sanderson *et al.* 1995*b*). In accordance with these observations we have recently observed that lymph-node lymphocytes obtained from FO-fed rats adhere less well to macrophage monolayers (P. Sanderson and P. C. Calder, unpublished results). These observations suggest that FO feeding will affect the movement of lymphocytes between body compartments and perhaps into sites of inflammatory or autoimmune activity.

Effects of dietary n-*3 polyunsaturated fatty acids on* ex vivo *cytokine production*

Macrophage-derived cytokines. Since cytokine production by macrophages is regulated by eicosanoids and since dietary lipids affect eicosanoid production (see p. 759), it might be expected that dietary lipids, especially those containing *n*-3 PUFA, will affect cytokine production. A number of studies have reported that feeding rodents *n*-3 PUFA-containing oils results in enhanced production of TNF *ex vivo* (for references, see Table 4), although there are reports of decreased production or no effect following FO feeding (for references, see Table 4). A recent study showed that dietary FO increases IL-6 production by rat peritoneal macrophages (Tappia & Grimble, 1994), while an earlier study reported increased IL-1 production (see Table 4). Again, however, there are contradictory reports (see Table 4). The most likely reason for the variations in experimental observations is the differing protocols used: studies have differed with respect to species of origin of the cells studied (mouse, rat, pig), the anatomical site of origin of the cells (liver, lung, peritoneal cavity), the state of activation of the cell (resident, inflammatory, activated), the stimulus used to elicit cytokine production (LPS, Ca ionophore, another cytokine), the nature of the culture conditions used (presence or absence of serum, serum source, duration of culture etc.), the level of FO in the diet, the duration of feeding and the method used to quantify cytokine concentrations. In a recent study in which all experimental procedures were standardized, it was found that feeding weanling mice on a 200 g FO/kg diet for 8 weeks resulted in diminished ability of thioglycollate-elicited peritoneal macrophages to produce TNF-α and IL-6 in response to

Table 5. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on* ex vivo *cytokine production by human peripheral blood mononuclear cells*

Details of diet used	Cytokine	Effect	Reference
Encapsulated *n*-3 PUFA (2·7 g EPA + 1·8 g DHA/d), 6 weeks	IL-1α	↓	Endres *et al.* (1989)
	IL-1β	↓	
	TNF	↓	
Encapsulated *n*-3 PUFA (2·4 g/d), 12 weeks	IL-1β	↓	Meydani *et al.* (1991)
	IL-2	↓	
	IL-6	↓	
	TNF	↓	
Encapsulated FO (4 g/d), 7 weeks	IL-1β	No effect	Molvig *et al.* (1991)
	TNF-α	No effect	
Encapsulated FO (4·5 g/d), 6–8 weeks	IL-1	↓	Cooper *et al.* (1993)
	IL-6	↓	
	TNF-α	No effect	
Low-fat, low-cholesterol + *n*-3 PUFA (1·23 g/d), 24 weeks	IL-1β	↓	Meydani *et al.* (1993)
	IL-6	↓	
	TNF	↓	
FO (6 g/d), 24 weeks	IL-1β	↓	Gallai *et al.* (1993)
	IL-2	↓	
	IFN-γ	↓	
	TNF-α	↓	
FO concentrate (18 g/d), 6 weeks	IL-Iβ	↓	Endres *et al.* (1993)
	IL-2	↓	
	TNF	↓	
n-3 PUFA ethyl esters (6 g/d), 16 weeks	IL-6	No effect	Soyland *et al.* (1994)*
	IL-2	No effect	
	TNF	No effect	

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FO, marine fish oil; IL, interleukin; TNF, tumour necrosis factor; IFN-γ, interferon-γ; ↓, depression of cytokine production.

* Subjects were patients with psoriasis or atopic dermatitis.

LPS *ex vivo*; IL-1α production was also decreased by FO feeding, but not significantly (Yaqoob & Calder, 1995*b*).

In agreement with some of the animal experiments (but in contrast to others) Endres *et al.* (1989, 1993), Meydani *et al.* (1991, 1993) and others have found that supplementation of the human diet with *n*-3 PUFA results in a significantly diminished ability of peripheral blood monocytes to produce TNF, IL-1α, IL-1β and IL-6 *ex vivo* (for references, see Table 5). These studies have been reviewed in detail elsewhere (Meydani, 1992; Endres *et al.* 1995).

Lymphocyte-derived cytokines. In contrast to the large number of studies of the effects of FO feeding on the *ex vivo* production of macrophage-derived cytokines (Tables 4 and 5), there have been few studies on lymphocyte-derived cytokines. Three studies have reported that supplementation of the diet of healthy human volunteers with *n*-3 PUFA significantly lowers *ex vivo* IL-2 production by PBL (for references, see Table 5; for a review, see also Meydani, 1992). Gallai *et al.* (1993) also reported diminished *ex vivo* IFN-γ production. One animal study has reported diminished *ex vivo* production of IL-2 (by pig alveolar lymphocytes) following LO and FO feeding. Fernandes *et al.* (1994) found that feeding autoimmune-disease-prone mice on a 100 g FO/kg diet resulted in enhanced *ex vivo* IL-2

production by spleen lymphocytes in response to Con A; IL-4 production was unaffected. In contrast, a recent study showed no significant effect of feeding 200 g FO/kg to weanling mice on *ex vivo* production of IL-2 or IFN-γ by Con A-stimulated spleen lymphocytes (Yaqoob & Calder, 1995*a*). Mitogen-stimulated spleen lymphocytes from mice fed on FO produced less IL-4 and IL-10 than those from mice fed on a low-fat diet, although this was not statistically significant (Yaqoob & Calder, 1995*a*).

Effects of dietary n-*3 polyunsaturated fatty acids on* in vivo *measures of inflammation and cell-mediated immunity*

Acute inflammatory responses. Arachidonic acid-derived eicosanoids are involved in mediating inflammatory responses. Since *n*-3 PUFA diminish the production of these mediators (see p. 759), they should exert anti-inflammatory activities. In fact, Yoshino & Ellis (1987) found that administering rats EPA (500 mg/kg per d) and DHA (333 mg/kg per d) by a gastric tube for 50 d did not affect either antigen-induced inflammation of the air pouch or carrageenan-induced inflammation of the footpad. This was despite a significant reduction in the production of pro-inflammatory eicosanoids (prostaglandin (PG) E_2 and LTB_4). Similar dietary *n*-3 PUFA-induced changes in the pattern of inflammatory eicosanoid production were also reported in a different model of acute inflammation (intraperitoneal injection of zymosan) in rodents (Lefkowith *et al.* 1990). The latter study also reported that dietary *n*-3 PUFA inhibited the influx of neutrophils into the peritoneal cavity which accompanies such treatment. In contrast to the findings of Yoshino & Ellis (1987), Reddy & Lokesh (1994) reported that feeding rats 100 g FO/kg for 10 weeks significantly lowered (by 40%) the inflammatory response to carrageenan injection into the footpad. In accordance with that observation, Nakamura *et al.* (1994) showed that feeding rats on high-fat diets containing 20 g EPA or DHA as their ethyl esters/kg resulted in a 50% reduction in footpad swelling in response to carrageenan injection; both *n*-3 PUFA were equally effective.

In vivo *response to endotoxin.* Mascoli *et al.* (1988) showed that two 24 h intravenous infusions of a 100 g lipid/l emulsion rich in FO into guinea-pigs significantly enhanced survival to intraperitoneally-injected LPS compared with infusion of a 100 g safflower oil/l emulsion; the total amount of lipid infused was 13 g per animal. Guinea-pig survival in the FO group was ten from eleven at 9 h post-injection and seven from eleven at 96 h post-injection. In comparison the survival rate at these time intervals post-injection in the safflower oil group was two from eleven. The same workers later showed that feeding a 145 g FO/kg diet to guinea-pigs for 6 weeks significantly increased survival to an intraperitoneal injection of LPS (3·5 mg/kg body weight) compared with animals fed on a 150 g safflower oil/kg diet (Mascoli *et al.* 1989). Guinea-pig survival 18 h post-injection was twenty-six from thirty in the FO-fed group (compared with sixteen from thirty in the safflower-oil-fed group) while at 96 h post-injection it was nineteen from thirty (compared with nine from thirty). In accordance with the diminished susceptibility to the lethal effects of endotoxin in experimental animals, Mulrooney & Grimble (1993) reported that feeding weanling rats a 100 g FO/kg diet for 8 weeks significantly decreased a number of responses to intraperitoneal TNF-α: the rises in liver Zn 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 FO diet. FO feeding also diminishes the pyrogenic (Pomposelli *et al.* 1990) and anorexic effects (Hellerstein *et al.* 1989; Mulrooney & Grimble, 1993) of IL-1 and TNF-α.

Delayed-type hypersensitivity (*DTH*). The 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 reports that the DTH response is significantly reduced following the feeding of diets rich in FO to rodents (Yoshino & Ellis, 1987; Kelley *et al.* 1989). Recently, Taki *et al.* (1992) reported suppression of the DTH response to sheep erythrocytes in mice following tail-vein injections of emulsions of TAG rich in EPA or DHA. Both Kelley *et al.* (1991) and Meydani *et al.* (1993) reported that supplementation of the human diet with *n*-3 PUFA diminished the DTH response to seven recall antigens.

Antibody production. Studies which have investigated the effects of diets rich in *n*-3 PUFA on antibody production in response to antigen challenge have produced contradictory results (for references, see Table 6). Prickett *et al.* (1982) reported enhanced production of immunoglobulin (Ig)G and IgE to ovalbumin in rats fed on 250 g FO/kg compared with those fed on 250 g tallow/kg, while Fritsche *et al.* (1991) showed that chickens fed on FO produced a higher level of antibodies to sheep erythrocytes than those fed on lard or maize oil. In contrast, Kelley *et al.* (1988) found no effect of feeding rabbits LO or FO on production of antibodies to albumin, while Prickett *et al.* (1984) reported that dietary FO decreased antibody production in rats. Recently, Atkinson & Maisey (1995) reported that the production of anti-rat erythrocyte antibodies by mice injected with rat erythrocytes was significantly reduced if the mice were fed on a 200 g FO/kg diet for 8 or 9 weeks. Virella *et al.* (1989) reported that supplementation of the diet of healthy human volunteers with 6 g FO/d for 6 weeks decreased the circulating levels of IgM and IgG following tetanus toxin challenge, but they used only a single subject and a later study with more subjects (Virella *et al.* 1991) found no significant effect of FO.

Animal models of inflammatory and autoimmune diseases. Dietary FO has been shown to have significantly beneficial clinical, immunological and biochemical effects in a number of animal disease models including autoimmune glomerulonephritis, a model for systemic lupus erythematosus (Prickett *et al.* 1983; Kelley *et al.* 1985; Robinson *et al.* 1985, 1986, 1993*b*; for a review, see Robinson *et al.* 1995), amyloidosis (Cathcart *et al.* 1987), collagen-induced arthritis (Prickett *et al.* 1984; Cathcart & Gonnerman, 1991) and ulcerative colitis (Wallace *et al.* 1989; Vilaseca *et al.* 1990). These observations suggest that diets enriched in *n*-3 PUFA might be of some therapeutic benefit in these diseases in man (see p. 754).

Graft v. *host and host* v. *graft responses.* The so-called PLN assay provides a useful experimental model in rodents for measuring G*v*.H and H*v*.G responses elicited by injection of allogenic cells into the footpad of the host. The G*v*.H 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 H*v*.G reaction is a T-cell-mediated response, in which CTL of the host recognize MHC antigens on the injected cells. In both cases, the enlargement in PLN size (more than 15-fold in the G*v*.H response and 4-fold in the H*v*.G 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. Using this assay, Mertin *et al.* (1985) reported that both the G*v*.H and H*v*.G responses were suppressed following a single administration of a FO concentrate (750 mg/kg body weight) by oesophageal catheter to mice before, or immediately after, the inoculation with allogenic cells. Hinds & Sanders (1993) showed a suppressed H*v*.G response in mice fed on a 160 g FO/kg diet compared with those fed on a standard chow diet. Recently, Sanderson *et al.* (1995*b*) reported significantly diminished G*v*.H and H*v*.G

Table 6. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on measurements of immune function* in vivo

Species	Details of diet used	Stimulus	Effect	Reference
Antibody production:				
Rat	250 g FO/kg, body wt increase from 100 to 250 g	Ovalbumin	↑ IgG and IgE	Prickett *et al.* (1982)
Rat	250 g FO/kg, 5–6 weeks	Type II collagen	↓ IgG	Prickett *et al.* (1984)
Rat	500 mg EPA + 333 mg DHA/kg per d, 7 weeks	BSA	No effect	Yoshino & Ellis (1987)
Rabbit	76 g FO/kg, 20 weeks	BSA	No effect	Kelley *et al.* (1988)
Man	Encapsulated FO (6 g/d), 6 weeks	Tetanus toxin	↓ IgM and IgG	Virella *et al.* (1989)
Man	Encapsulated FO (8 g/d), 6 weeks	Tetanus toxin	No effect	Virella *et al.* (1991)
Chicken	70 g FO/kg, 3 weeks	Sheep erythrocytes	↑ Antibodies	Fritsche *et al.* (1991)
Mouse	200 g FO/kg, 8, 9 weeks	Rat erythrocytes	↓ Antibodies	Atkinson & Maisey (1995)
Delayed-type hypersensitivity:				
Rat	500 mg EPA + 333 mg DHA/kg per d, 7 weeks	BSA	↓ Response	Yoshino & Ellis (1987)
Rabbit	76 g FO/kg, 20 weeks	DNCB	No effect	Kelley *et al.* (1988)
Mouse	FO, daily subcutaneous injections of small volumes for 6 d	TNBS	↓ Response	Kelley *et al.* (1989)
Man	LO-rich diet, 8 weeks	Seven recall antigens	↓ Response	Kelley *et al.* (1991)
Mouse	Tail vein injection of Tri-EPA, Tri-DHA*	Sheep erythrocytes	↓ Response	Taki *et al.* (1992)
Man	Low-fat, low-cholesterol diet + *n*-3 PUFA (1·23 g/d), 24 weeks	Seven recall antigens	↓ Response	Meydani *et al.* (1993)
Mouse	10 g EPA or DHA ethyl ester/kg, 10 d	PPD	↓ Response	Fowler *et al.* (1993)
Host *v.* graft and graft *v.* host responses†:				
Mouse	750 mg FO/kg orally 6 h before initiation	Allogenic cells	↓ H*v*.G, G*v*.H responses	Mertin *et al.* (1985)
Mouse	160 g FO/kg, 4 weeks	Allogenic cells	↓ H*v*.G response	Hinds & Sanders (1993)
Rat	200 g FO/kg, 4 weeks	Allogenic cells	↓ H*v*.G, G*v*.H responses	Sanderson *et al.* (1995*b*)
Rat	200 g LO/kg, 4 weeks	Allogenic cells	↓ G*v*.H response	Jeffery *et al.* (1996)
Organ transplantation:				
Rat	FO (1 ml/d orally), 3 weeks	Renal transplant	↑ Graft survival	Elzinga *et al.* (1987)
Mouse	FO (1 ml/d orally), 1 week	Cardiac transplant	↑ Graft survival	Kelley *et al.* (1989)
Rat	125 g FO, from 2–4 weeks before transplant until rejection	Cardiac transplant	↑ Graft survival	Otto *et al.* (1990)
Rat	300 g FO/kg, from 3 weeks before transplant + 12 weeks post-transplant	Renal transplant	↑ Graft survival	Kort *et al.* (1991)

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Table 6. *Effects of dietary* n-*3 polyunsaturated fatty acids (PUFA) on measurements of immune function* in vivo

Species	Details of diet used	Stimulus	Effect	Reference
Organ transplantation: (cont)				
Rat	Continuous intravenous infusion of 200 g FO/l emulsion (9 g fat/kg per d)	Cardiac transplant	↑ Graft survival	Grimm *et al.* (1993)
Man	FO (6 g/d), 12 months from 3 d post-transplant	Renal transplant	↑ Graft survival	Homan van der Heide *et al.* (1993)

FO, marine fish oil; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LO, linseed oil, DNCB, dinitrochlorobenzene; BSA, bovine serum albumin; PPD, purified protein derivative of *Mycobacterium tuberculosis;* TNBS, trinitrobenzenesulphonic acid; Ig, immunoglobulin; H, host; G, graft; ↓, ↑, depression and promotion respectively.

* Triacylglycerol with EPA or DHA at all three *sn*-positions.

† Assessed using a popliteal lymph node weight assay.

responses (by 34 and 20% respectively) in rats fed on 200 g FO/kg diet compared with those fed on a low-fat diet or diets containing 200 g coconut, olive, safflower or evening primrose oils/kg. Such observations accord with the demonstrations of significantly diminished *ex vivo* T (and B; Yaqoob *et al.* 1995*a*) lymphocyte proliferation, NK cell activity and CTL activity following FO feeding (for references, see Table 2). Feeding rats on a 200 g FO/kg diet resulted in less IL-2R$^+$ cells and CD16$^+$/CD3$^-$ cells in the PLN following the G*v*.H response, indicating an inhibition of lymphocyte activation and a decrease in the proportion of NK cells respectively (Sanderson *et al.* 1995*b*).

Animal models of organ transplantation. Graft rejection in transplantation surgery is caused by an immune reaction to the foreign material introduced to 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 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 (for reviews, see Feogh *et al.* 1986; Feogh, 1988). Therefore, since *n*-3 PUFA affect the levels and types of eicosanoids formed (see p. 759) they would be expected to influence graft survival. In addition, *n*-3 PUFA exert immunomodulatory effects which might be independent of eicosanoids and so these effects might play a role in enhancing graft survival. Animal studies of DTH, G*v*.H and H*v*.G responses (see p. 751) indicate that dietary *n*-3 PUFA can suppress the response to foreign antigens. The immunosuppressive effect of dietary FO has also been evaluated, usually favourably, in studies of heart and kidney transplantation in laboratory animals (for references, see Table 6). In a typical result, it was reported that intravenous fat emulsions rich in *n*-3 PUFA prolonged the survival of rat cardiac transplants by up to 60% (Grimm *et al.* 1993).

Do n-*3 polyunsaturated fatty acids diminish host defence?*

The immunosuppressive effects of *n*-3 PUFA described previously mean that there could be some detrimental effects resulting from their consumption; this is evident from animal

experiments. Albina *et al.* (1993) found that the wound-healing response was diminished in rats fed for 21 d pre-wounding and 30 d post-wounding on a 170 g FO, 30 g maize oil/kg diet compared with those fed on 200 g maize oil/kg. In a study investigating resistance to bacteria (oral administration of *Salmonella typhimurium*), Chang *et al.* (1992*b*) reported that mice fed on 200 g FO/kg showed lower survival over 15 d (48%) than those fed on maize oil (62·5%), coconut oil (87·5%) or a low-fat diet (88%); spleens from the FO-fed animals had a greater number of bacteria per spleen than those from animals fed on the other diets. Similarly, a study of experimental tuberculosis in guinea-pigs reported an increased number of bacteria in the spleen of FO-fed animals and it was concluded that this represented persistence of the experimental infection (Mayatepek *et al.* 1994).

CLINICAL ASPECTS OF IMMUNOMODULATION BY *N*-3 POLYUNSATURATED FATTY ACIDS

Trials of n-*3 polyunsaturated fatty acids supplementation in inflammatory and autoimmune diseases*

The low incidence of cardiovascular disorders amongst populations consuming large quantities of oily fish has been well documented (Dyerberg *et al.* 1975; Kromann & Green, 1980; Kromhout *et al.* 1985), but the intense interest in FO and heart disease has overshadowed the unusual pattern of the incidence of some other diseases in native Greenland Eskimos. Kromann & Green (1980) 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 (MS), in a population of Greenland Eskimos compared with sex- and age-matched groups living in Denmark. Thus, the *n*-3 PUFA-containing FO in the Eskimo diet could have a protective role towards these types of diseases. Most of these diseases are characterized 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 MS) 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 FO in animal models of inflammatory and autoimmune diseases (see p. 751) indicates that there may be some benefit from supplementation of the diet of suitable patients with *n*-3 PUFA. There have been a number of clinical trials assessing the benefits of dietary supplementation with FO in several inflammatory and autoimmune diseases (for references, see Table 7).

Rheumatoid arthritis. A number of trials of supplementation with *n*-3 PUFA in rheumatoid arthritis patients have been performed (for references, see Table 7). These trials all show significant biochemical changes resulting from the supplementation and most show modest clinical benefits (for details, see Table 7). These studies have been reviewed elsewhere (Kremer, 1993).

Psoriasis. A number of trials of supplementation with *n*-3 PUFA in psoriasis have been performed (for references, see Table 7). All these trials show significant changes in biochemical variables measured and most show some clinical improvement. These studies have been reviewed in detail elsewhere (Soyland & Drevon, 1993; Grimminger & Mayser, 1995).

Multiple sclerosis. A link between fat consumption and MS was first proposed by Swank (1950), who suggested that the higher prevalence of MS in 'more northern countries' was

Table 7. *Trials involving* n*-3 polyunsaturated fatty acid (PUFA) supplementation in inflammatory and autoimmune diseases*

Supplementation	Biochemical observations	Clinical observations	Reference
Rheumatoid arthritis:			
EPA + DHA (1·8 + 0·9 g/d respectively), 12 weeks	↑ Serum *n*-3 PUFA	No improvement	Kremer *et al.* (1985)
EPA + DHA (2·7 + 1·8 g/d respectively), 14 weeks	↑ *n*-3 PUFA in serum; ↓ LTB_4 production by neutrophils	Fewer tender joints	Kremer *et al.* (1987)
FO (20 g/d), 6 weeks	↑ *n*-3 PUFA in neutrophils; ↓ LTB_4 production by neutrophils	Reduced severity of some symptoms	Sperling *et al.* (1987)
n-3 PUFA (3·2 g/d), 12 weeks	↓ LTB_4 production by neutrophils	Some improvement	Cleland *et al.* (1988)
n-3 PUFA (2·7 or 5·4 g/d), 24 weeks	↓ IL-1 and LTB_4 production by macrophages and neutrophils	Improvement in joint inflammation	Kremer *et al.* (1990)
n-3 PUFA (3·4 g/d), 12 weeks	↓ LTB_4 production	Modest decrease in morning stiffness and joint swelling	van der Tempel *et al.* (1990)
n-3 PUFA (3·6 g/d), 12 weeks	↓ IL-1β in plasma	Improvement	Esperson *et al.* (1992)
Psoriasis:			
n-3 PUFA (15 g/d), 12 weeks	↑ *n*-3 PUFA in serum	Moderate improvement	Allen *et al.* (1985)
n-3 PUFA (19–23 g/d), 8 weeks	↑ *n*-3 PUFA in serum, neutrophils and epidermis	Mild to moderate improvement in lesions	Ziboh *et al.* (1986)
n-3 PUFA (10–15 g/d), 6 weeks	↓ LTB_4 production	Some improvement in scaling	Maurice *et al.* (1987)
EPA (18 g/d), 8 weeks	None investigated	Improvement in itching	Bittiner *et al.* (1988)
n-3 PUFA (3 g/d), 6 weeks	↑ *n*-3 PUFA in serum phospholipids	No improvement	Bjorneboe *et al.* (1988)
EPA (3·6 g/d), 12–24 weeks	↓ PGE_2 and LTB_4 in inflammatory exudate	Improvement in scaling	Terano *et al.* (1989)
n-3 PUFA (5 g/d), 16 weeks	↑ *n*-3 PUFA in serum phospholipids	No improvement	Soyland *et al.* (1993*a*)
n-3 PUFA ethyl esters (6 g/d), 16 weeks	No effect on IL-2, IL-6, TNF production by lymphocytes or on lymphocyte proliferation	None investigated	Soyland *et al.* (1994)
Atopic dermatitis:			
n-3 PUFA (3 g/d), 12 weeks	None investigated	Improvement	Bjorneboe *et al.* (1987)
Systemic lupus erythematosus:			
FO (6 or 18 g/d), 10 weeks	↑ *n*-3 PUFA in platelets, ↓ LTB_4 production by neutrophils	No improvement	Clark *et al.* (1989)
EPA + DHA (162 + 144 mg/d respectively), several months to years	None investigated	Significant improvement	Das (1994)

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Table 7. *Trials involving* n-*3 polyunsaturated fatty acid (PUFA) supplementation in inflammatory and autoimmune diseases*

Supplementation	Biochemical observations	Clinical observations	Reference
Multiple sclerosis:			
EPA + DHA (1·7 + 1·1 g/d respectively), 2 years	↑ *n*-3 PUFA in serum and adipose tissue	Slight benefit in relapse rate and disease progression	Bates *et al.* (1989)
FO (6 g/d), 6 months	↓ PGE_2, LTB_4, IL-1β, IL-2, IFN-γ, TNF-α production by monocytes	None investigated	Gallai *et al.* (1993)

EPA, eicosopentaenoic acid; DHA, docosahexaenoic acid; FO, marine fish oil; LTB_4, leukotriene B_4; IL, interleukin; PGE_2, prostaglandin E_2; TNF, tumour necrosis factor; IFN-γ, interferon-γ.

due to the higher saturated fat intake of these populations. This suggestion is supported by a number of observations. For example, the incidence of MS is lower in fishing communities of Norway than in inland agricultural areas, which indicates a lower incidence amongst groups with a lower overall fat intake and a higher intake of PUFA (for references, see Mertin & Meade, 1977). The aim of dietary trials in MS patients has been to correct the apparent lipid abnormalities by increasing intake of PUFA and decreasing the intake of saturated fats. A controlled trial using *n*-3 PUFA in MS patients showed some beneficial effects, particularly with regard to the frequency of relapses (Bates *et al.* 1989). Gallai *et al.* (1993) showed that supplementation of the diet of MS patients with 6 g FO/d resulted in diminished ability of blood monocytes to produce a range of cytokines *ex vivo* (see Table 7).

Bronchial asthma, atopic dermatitis, systemic lupus erythematosus and ulcerative colitis. Other inflammatory and autoimmune diseases which have been subject to trials of *n*-3 PUFA supplementation include bronchial asthma (Parm *et al.* 1988; Picado *et al.* 1988), atopic dermatitis (Bjorneboe *et al.* 1987; Soyland & Drevon, 1993), systemic lupus erythematosus (Clark *et al.* 1989; Westberg & Tarkowski, 1990) and ulcerative colitis (Hawthorne *et al.* 1992). Das (1994) reported that long-term (many months to several years) consumption of *n*-3 PUFA led to very marked reduction in the symptoms of systemic lupus erythematosus.

Use of dietary n-*3 polyunsaturated fatty acids to prevent graft rejection*

The animal studies described previously indicate that FO could be used to prolong the survival of organ transplants. This was recently confirmed in the study of Homan van der Heide *et al.* (1993). These authors reported that renal transplant patients who received FO (6 g/d for the first post-operative year) in combination with cyclosporin A had better kidney function and fewer rejections over 1 year compared with patients who received coconut oil and cyclosporin A. The beneficial effects of *n*-3 PUFA supplementation in renal transplantation in human subjects have been recently confirmed (Bennett *et al.* 1995; Maachi *et al.* 1995).

Other clinical aspects of n-3 *polyunsaturated fatty acids involving cells of the immune system*

n-3 PUFA have been shown to have beneficial effects on the development of atherosclerosis (for a review, see Leaf & Weber, 1988) and to protect against some forms of cancer (for a review, see Nettleton, 1995). Monocytes, macrophages and lymphocytes are all involved in the development of the atherosclerotic plaque; activated macrophages and T-cells and the products of their activation (cytokines, eicosanoids, reactive oxygen species) are found at the site of endothelial damage and in the atherosclerotic plaque (for reviews, see Ross, 1993; Wick *et al.* 1995). This is a situation analogous to the site of tissue damage in an inflammatory disease. Thus, one aspect of the beneficial effects of dietary *n*-3 PUFA in atherosclerosis could relate to their anti-inflammatory and immunosuppressive actions. Such immunosuppressive actions, particularly the significantly lowered cytolytic activities of NK cells, CTL and macrophages reported following FO feeding (for references, see Tables 2 and 3) might be expected to make an individual more susceptible to tumour cell growth. However, at least as far as some types of tumour cell are concerned, this does not appear to be the case and a lowered incidence of some types of cancer is associated with increased consumption of *n*-3 PUFA in the diet (for reviews, see Henderson, 1991; Nettleton, 1995). In addition, dietary FO reduces growth of certain types of tumours in animal models (for reviews, see Reddy, 1991; Nettleton, 1995).

MECHANISMS BY WHICH *N*-3 POLYUNSATURATED FATTY ACIDS MIGHT EXERT IMMUNOMODULATORY ACTIONS

Fatty acids as eicosanoid precursors

The precursors and pathways of eicosanoid synthesis. Eicosanoids are a family of oxygenated derivatives of dihomo-γ-linolenic and arachidonic acids and EPA. Eicosanoids include PG and thromboxanes (TX), which together are termed prostanoids, and LT, lipoxins (LX), hydroperoxyeicosatetraenoic acids (HPETE) and hydroxyeicosatetraenoic acids (HETE). In most conditions the principal precursor for these compounds is arachidonic acid and the eicosanoids produced from arachidonic acid appear to have more potent biological functions than those released from dihomo-γ-linolenic acid or EPA. The precursor PUFA is released from membrane phosphatidylcholine (PC) by the action of phospholipase A_2 (*EC* 3.1.1.4) or from membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) by the actions of phospholipase C (*EC* 3.1.4.3) and a diacylglycerol (DAG) lipase (*EC* 3.1.1.34).

The pathways of eicosanoid synthesis begin with cyclooxygenase, which yields the PG and TX, or with the 5-, 12- or 15-lipoxygenases, which yield the LT, HPETE, HETE and LX (Fig. 2). A third pathway which operates through the microsomal cytochrome P-450 results in formation of epoxides which are converted to HETE. The amounts and types of eicosanoids synthesized are determined by the availability of arachidonic acid, by the activities of phospholipase A_2 and phospholipase C and by the activities of cyclooxygenase and the lipoxygenases.

The major biologically-active products of the cyclooxygenase pathway are PGA_2, PGE_2, PGI_2 (prostacyclin), $PGF_{2\alpha}$ and TXA_2, although these are produced in a cell-specific manner. These compounds usually have a short half-life and act locally to the cell from which they are produced. Their production is initiated by particular stimuli (e.g.

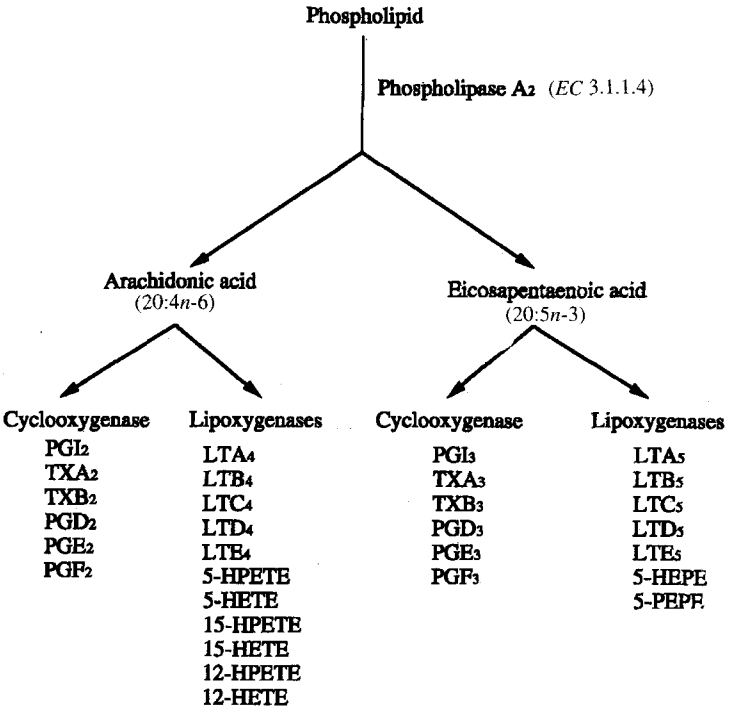

Fig. 2. Synthesis of eicosanoids from arachidonic and eicosapentaenoic acids. PG, prostaglandin; TX, thromboxane; LT, leukotriene; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid.

cytokines, growth factors, endotoxin, zymosan, oxygen free radicals, antigen–antibody complexes, bradykinin, collagen, thrombin) and, once produced, they themselves are able to modify the response to the stimulus. Different prostanoids have different, sometimes opposite, effects; for example, TXA_2 increases platelet aggregation whereas PGI_2 inhibits platelet aggregation. PG are associated with the inflammatory response and cyclooxygenase inhibitors include aspirin and related non-steroidal anti-inflammatory drugs. It appears that each of the lipoxygenase enzymes has a particular cellular distribution: 5-lipoxygenase is found in mast cells, monocytes, macrophages and granulocytes, 12-lipoxygenase is found in platelets and some epithelial cells and 15-lipoxygenase is found in young myeloid cells and some epithelial cells.

The *n*-3 PUFA, EPA and DHA, competitively inhibit the oxygenation of arachidonic acid by cyclooxygenase. In addition, EPA (but not DHA) is able to act as a substrate for both cyclooxygenase and 5-lipoxygenase (Fig. 2). Ingestion of FO which contain *n*-3 PUFA will result in a decrease in membrane arachidonic acid levels and a concomitant decrease in the capacity to synthesize eicosanoids from arachidonic acid (see p. 759); EPA gives rise to the 3-series PG and TX and the 5-series LT (Fig. 2). The eicosanoids produced from EPA do not always have the same biological properties as the analogues produced from arachidonic acid. For example, TXA_3 is less active than TXA_2 in aggregating platelets and constricting blood vessels and LTB_5 is less active than LTB_4 with regard to chemotactic and aggregatory properties in human neutrophils. In contrast, PGI_3 is as active as PGI_2 in inhibiting platelet aggregation and promoting vasodilation.

The synthesis of eicosanoids by cells of the immune system. Macrophages synthesize a range of cyclooxygenase and lipoxygenase products (for reviews, see Goldyne & Stobo, 1981; Hwang, 1989); the exact profile of eicosanoids formed depends on the species and anatomical site of origin of the macrophages and the nature of the stimulus used. Studies concerning eicosanoid synthesis by lymphocytes have proved controversial (see Goldyne, 1988). Many studies indicate that lymphocytes do not synthesize cyclooxygenase or lipoxygenase products (for references, see Goldyne, 1988; Calder, 1996*b*). Nevertheless, lymphocytes contain similar amounts of arachidonic acid in their membrane phospholipids to macrophages and mitogen-stimulated lymphocytes release arachidonic acid extracellularly. There is evidence that macrophages utilize this released arachidonic acid for eicosanoid synthesis. Indeed, in the presence of lymphocytes, macrophages produce increased amounts of PGE_2 *in vitro*. Thus, it seems that among immunocompetent cells, macrophages are the principal, and perhaps the only, source of eicosanoids, but that an interaction between lymphocytes and macrophages exists.

Effects of eicosanoids on cells of the immune system. Not only are immune cells a source of eicosanoids, but they are subject to their regulatory effects; the most-well-documented effects are those of PGE_2. *In vivo*, PG are involved in modulating the intensity and duration of inflammatory and immune responses; PGE_2 has a number of pro-inflammatory effects including fever, erythema, increased vascular permeability, vasodilation and enhancement of pain and oedema caused by other agents such as bradykinin and histamine. In chronic inflammatory conditions increased activity of suppressor T-cells and increased rates of PGE_2 production are observed, and elevated PGE_2 production has been observed in patients suffering from infections, whose T-cells show depressed functional responses. It is generally accepted that PGE_2 is a regulator of immune cell functions; the age and type of target cell and the concentration of PGE_2 determine the nature of the response.

PG appear to play a role in regulating the differentiation of both T and B lymphocytes; for example, PGE_2 induces immature thymocytes to differentiate into mature T-cells. In addition, the functions of T-cells, B-cells, NK cells and macrophages are modulated by eicosanoids; these effects are reviewed elsewhere (Goodwin & Cueppens, 1983; Goldyne, 1988; Hwang, 1989). T-lymphocytes have receptors for PGE_1 and PGE_2 and these compounds suppress T-lymphocyte proliferation, T-cell-mediated cytotoxicity, IL-2 production and NK cell activity *in vitro* (for reviews, see Goodwin & Cueppens, 1983; Goldyne, 1988; Hwang, 1989). B-cells have receptors for the E-series PG and PGE_2 can influence antibody production. PG inhibit production of IL-1 and TNF by macrophages (Kunkel *et al.* 1982). Some macrophage enzyme activities are also modulated by PG, as is expression of MHC II receptors. There are conflicting reports about the effects of LT on lymphocyte proliferation (for reviews, see Goodwin & Cueppens, 1983; Goldyne, 1988; Hwang, 1989; Calder, 1996*b*), but NK cell activity is enhanced by LTB_4 (Rola-Pleszczynski *et al.* 1983). LTB_4 and LTC_4 enhance IL-1 production by macrophages (Kunkel *et al.* 1982) and LTB_4 enhances IFN-γ production by lymphocytes (Rola-Pleszczynski *et al.* 1983). Since they influence eicosanoid production (see p. 759), it is clear that *n*-3 PUFA can modulate immune cell functions by eicosanoid-mediated effects.

Modulation of eicosanoid synthesis by n-*3 polyunsaturated fatty acids.* Culture of macrophages or lymphocytes with *n*-3 PUFA results in replacement of arachidonic acid in phospholipids by the *n*-3 PUFA provided (Magrum & Johnston, 1983, 1985; Lokesh *et al.* 1988; Calder *et al.* 1990*c*, 1994*b*). As a result of this modification, less arachidonic acid-

derived eicosanoids are produced by these cells. Dietary lipid modulation also results in significant modification of the fatty acid composition of macrophages isolated from the peritoneal cavity of mice (Lokesh *et al.* 1986; Chapkin *et al.* 1992; Hardardottir & Kinsella, 1992), rats (Magrum & Johnston, 1983; Brouard & Pascaud, 1990; Watanabe *et al.* 1991; Sherrington *et al.* 1995*a*) or hamsters (Surette *et al.* 1995), of macrophages isolated from the lungs of pigs (Fritsche *et al.* 1993), of lymphocytes isolated from rodent lymphoid tissues (Brouard & Pascaud, 1990; Robinson *et al.* 1993*a*; Yaqoob, 1993; Yaqoob *et al.* 1995*b*) and of monocytes isolated from human peripheral blood (Lee *et al.* 1985; Endres *et al.* 1989). It is widely reported that feeding laboratory animals *n*-3 PUFA-containing oils such as LO or FO results in decreased production of arachidonic acid-derived eicosanoids (Magrum & Johnston, 1983; Lokesh *et al.* 1986; Brouard & Pascaud, 1990; Hardardottir & Kinsella, 1991, 1992; Black & Kinsella, 1993; Fritsche *et al.* 1993; Yaqoob & Calder, 1995*b*; see Fig. 3). FO supplementation of the human diet results in similar changes (Lee *et al.* 1985; Endres *et al.* 1989). The suppression in the amount of arachidonic acid-derived eicosanoids is mirrored by an elevation in the level of EPA-derived eicosanoids (Chapkin *et al.* 1990). As indicated earlier, these latter compounds are often less biologically potent than the analogues synthesized from arachidonic acid. Thus, significant effects on processes such as platelet aggregation, vasoconstriction, neutrophil function, inflammation and immunity result. A thorough review of the effects of dietary lipids on eicosanoid production, particularly by macrophages, may be found elsewhere (Kinsella *et al.* 1990).

n-*3 Polyunsaturated fatty acids and membrane fluidity*

Fatty acids have important roles in membrane structure and there are several ways by which they can potentially influence the functions of membrane proteins. The fluid mosaic model of membrane structure describes biological membranes as dynamic and responsive structures. It is now also recognized that domains exist in membranes, where lipid–protein and lipid–lipid interactions may be highly specific. The composition of phospholipids in cell membranes is usually characteristic for the cell type, but may change with stage of the cell cycle, with age, in response to stimuli or to changes in the environment or the diet (Stubbs & Smith, 1984); these changes may have functional consequences. A number of membrane-bound enzymes have been shown to be particularly sensitive to their fatty acid environments; these include adenylate cyclase (*EC* 4.6.1.1), 5′-nucleotidase (*EC* 3.1.3.5) and the Na^+/K^+ ATPase (*EC* 3.6.1.37; for reviews, see Brenner, 1984; Stubbs & Smith, 1984; Murphy, 1990). The same is true for a number of receptors, including adrenergic and insulin receptors. The mechanisms by which membrane lipids modulate enzyme activity or receptor function are not fully understood, but suggestions include changes in membrane fluidity (Brenner, 1984; Stubbs & Smith, 1984) and fatty acid-dependent effects on the conformation of the protein complex (Murphy, 1990).

The stimulation of lymphocytes is accompanied by *de novo* synthesis and turnover of membrane phospholipids (Resch *et al.* 1971, 1972; Resch & Ferber, 1972; Ferber *et al.* 1975). Within minutes of stimulation, remodelling of the membrane phospholipids begins, with saturated fatty acids in phospholipids being substituted by PUFA (Ferber *et al.* 1975). As a result, changes in the fatty acid composition of lymphocyte phospholipids are detectable within minutes of stimulation and are quite marked by 4 h post-stimulation (Ferber *et al.* 1975). Culture of mitogen-stimulated lymphocytes for several days results in a very significant increase in the content of unsaturated fatty acids in the membrane phos-

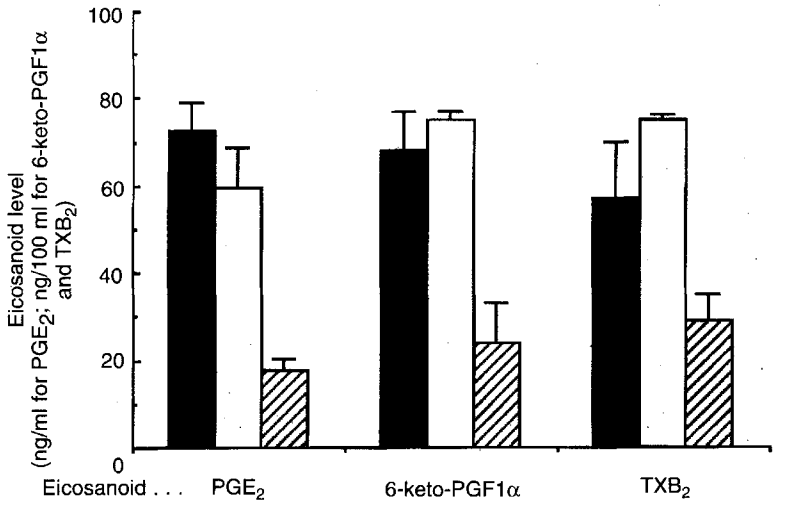

Fig. 3. Effect of dietary fish oil on the production of arachidonic acid-derived eicosanoids by murine peritoneal macrophages. Mice were fed for 8 weeks on a low-fat (25 g/kg; ■) diet or on diets containing 200 g either safflower oil (▨) or fish oil (▨)/kg. Thioglycollate-elicited peritoneal macrophages were prepared and cultured with bacterial lipopolysaccharide for 8 h. The medium was collected and assayed using ELISA. PGE_2, prostaglandin E_2; 6-keto-PGF1α, 6-keto-prostaglandin F1α; TXB_2, thromboxane B_2. Values are means with their standard errors represented by vertical bars. Data are taken from Yaqoob & Calder (1995*b*).

pholipids (Anel *et al.* 1990; Calder *et al.* 1994*b*). This change in fatty acid composition is accompanied by an increase in membrane fluidity (Anel *et al.* 1990; Calder *et al.* 1994*b*), although this has been a controversial subject for many years (for references, see Calder *et al.* 1994*b*). In support of the proposal that membrane fluidity changes are an inherent component of lymphocyte activation and subsequent proliferation (Anel *et al.* 1990; Calder *et al.* 1994*b*), Curtain *et al.* (1978) found that a non-mitogenic lectin (wheatgerm agglutinin) does not affect lymphocyte membrane fluidity, in contrast to the effect of the mitogenic lectins Con A and PHA. That plasma membrane fluidity does influence lymphocyte activity was shown by the studies of Maccecchini & Burger (1977) and Huber *et al.* (1991). In contrast to the situation with lymphocytes, stimulation of macrophages with LPS appears to cause a decrease in plasma membrane fluidity (Grimble & Tappia, 1995).

As described earlier, culture of lymphocytes, macrophages or monocytes in the presence of *n*-3 PUFA results in significant alterations in the fatty acid composition of the membrane phospholipids. Such changes result in increased lymphocyte plasma membrane fluidity (Calder *et al.* 1994*b*). In contrast, even though they elicit significant changes in the fatty acid composition of membrane phospholipids (for references, see p. 760), diets rich in *n*-3 PUFA do not appear to significantly alter the fluidity of the plasma membranes of lymphocytes (Yaqoob, 1993; Yaqoob *et al.* 1995*b*), macrophages (Grimble & Tappia, 1995; Sherrington *et al.* 1995*a*) or monocytes (Lee *et al.* 1985). There are at least three possible reasons for this. First, substituting *n*-3 PUFA for *n*-6 PUFA in membrane phospholipids may have only a minimal effect on fluidity. Second, the effect of increasing the PUFA content of membrane phospholipids might be counteracted by altering the cholesterol content of the membrane and/or by altering the proportions of different types of phospholipids present. Third, the techniques used yield values for the fluidity of the plasma membrane as a whole and it is possible that undetectable changes occur in particular regions of the membrane.

n-3 *Polyunsaturated fatty acids and signal transduction*

Apart from influencing the pattern of eicosanoids produced (see p. 759), it is possible that *n*-3 PUFA may influence signalling within cells of the immune system in other ways. Many lipids are involved directly in intracellular signalling pathways; for example, hydrolysis of membrane phospholipids such as PIP_2 and PC by phospholipases generates second messengers such as DAG. Other phospholipids have roles in activating or stabilizing enzymes involved in intracellular signalling; for example, phosphatidylserine (PS) is required for PKC activation. Since PIP_2, PC, PS 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. Indeed, PKC is more active in the presence of dioleoylglycerol or diarachidonoylglycerol than in the presence of DAG containing two saturated fatty acids or one saturated and one unsaturated fatty acid (Kishimoto *et al.* 1980). Bell & Sargent (1987) showed that rat spleen PKC was less active in the presence of an *n*-3 PUFA-rich PS compared with a PUFA-poor PS irrespective of the fatty acid composition of the DAG. Recently, Fowler *et al.* (1993) reported that feeding mice for 10 d on a diet containing 10 g purified ethyl ester of either EPA or DHA/kg resulted in enrichment of spleen lymphocyte DAG species with the fatty acid fed; the total mass of DAG was elevated in the DHA-fed mice.

In addition to the effects of fatty acids on intracellular signalling mechanisms due to changes in the fatty acid composition of the phospholipids which are involved, it has been proposed that unsaturated fatty acids themselves may have a direct effect (for references, see Sumida *et al.* 1993). This direct modulatory effect of fatty acids has been most extensively documented in relation to PKC activity which was shown to be enhanced by DHA (Shinomura *et al.* 1991). In contrast, although Speizer *et al.* (1991) reported that EPA and DHA increased brain PKC activity in the absence of PS and DAG, they found that in the presence of both PS and DAG each of these fatty acids caused up to 60% inhibition of PKC activity. Another study has shown that EPA and DHA inhibit rat lymphocyte PKC activity in the presence of Ca, PS and DAG (May *et al.* 1993); protein kinase A (PKA) activity was unaffected by EPA and DHA (May *et al.* 1993). Recently, rat peritoneal macrophage PKC activity was shown to be inhibited by EPA and DHA; again PKA activity was unaffected (Tappia *et al.* 1995). In accordance with both the direct effects of *n*-3 PUFA *in vitro* (May *et al.* 1993; Tappia *et al.* 1995) and the effects of enriching PS with *n*-3 PUFA (Bell & Sargent, 1987), Van Meter *et al.* (1994) found that feeding mice FO resulted in diminished spleen lymphocyte PKC activity.

A change in the concentration of intracellular free Ca 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, Richieri & Kleinfeld (1989) reported that oleic acid, but not stearic acid, inhibited the target cell- or Con A-stimulated rise in intracellular free Ca concentration in a CTL cell line. Several unsaturated fatty acids including ALA, EPA and DHA inhibit the anti-CD3-induced increase in intracellular free Ca concentration in the JURKAT T-cell line (Chow *et al.* 1990; Breittmayer *et al.* 1993); the fatty acids appeared to act by blocking Ca entry into the cells and it was concluded that they act directly on receptor-operated Ca channels (Chow *et al.* 1990).

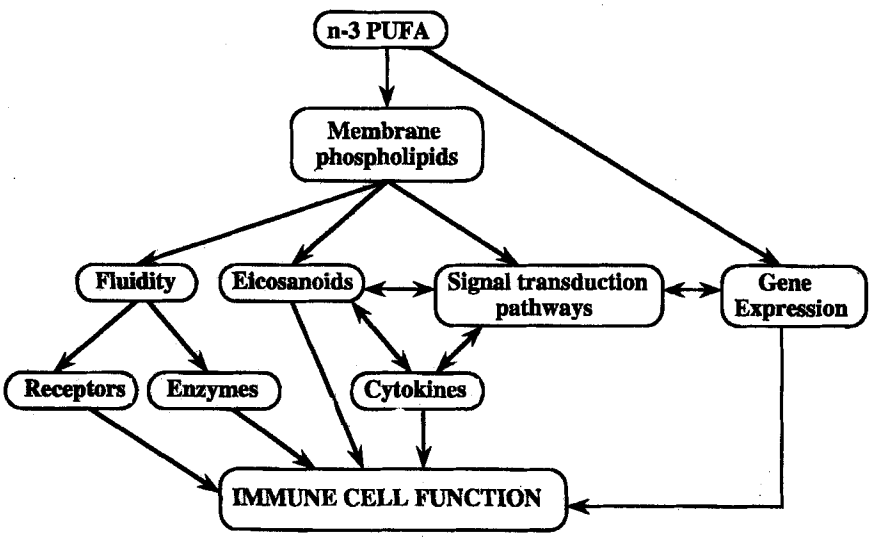

Fig. 4. Mechanisms by which *n*-3 polyunsaturated fatty acid (PUFA) could exert immunomodulatory actions.

n-*3 Polyunsaturated fatty acids and gene expression*

It is now well documented that fatty acids affect the expression of genes involved in hepatic fatty acid and lipoprotein metabolism (for a review, see Jump *et al.* 1995) and the genes involved in adipocyte differentiation and development (for review, see Ailhaud *et al.* 1995). Dietary *n*-3 PUFA have particularly potent effects on the expression of genes for proteins involved in hepatic peroxisomal proliferation, fatty acid oxidation and lipoprotein assembly (Berthou *et al.* 1995). They may act in a fashion analogous to that of steroid hormones, possessing intracellular receptors which directly influence transcription (for reviews, see Nunez, 1995; Sumida, 1995). Alternatively, unsaturated fatty acids may act by increasing or decreasing the levels of other mediators (e.g. eicosanoids, cytokines) which affect gene expression. Studies of the effects of fatty acids on the expression of genes important in immune cell functioning are few. However, a recent study showed that inclusion of FO in the diet of autoimmune-disease-prone mice results in elevated levels of mRNA for IL-2, IL-4 and transforming growth factor-β and reduced levels of mRNA for c-*myc* and c-*ras* in the spleen (Fernandes *et al.* 1994). The same workers showed that dietary FO completely abolished mRNA production for IL-1β, IL-6 and TNF-α in the kidneys of these animals (Chandrasekar & Fernandes, 1994). Robinson *et al.* (1995) reported that feeding mice on a FO-rich diet significantly diminished *ex vivo* IL-1β mRNA production by LPS- or PMA-stimulated spleen lymphocytes; the lower IL-1β mRNA level was not due to accelerated degradation but to impaired synthesis. FO feeding to mice lowered basal TNF-α mRNA and LPS-stimulated TNF-α and IL-1β mRNA levels (Renier *et al.* 1993). These studies suggest that *n*-3 PUFA might affect immune cell functioning by control at the transcriptional level.

CONCLUDING REMARKS

The amount and type of eicosanoids made can be affected by the type of fat consumed in the diet. It is now apparent that both eicosanoids and *n*-3 PUFA are potent modulators of lymphocyte and macrophage functions *in vitro*. Inclusion in the diet of high levels of certain lipids containing *n*-3 PUFA markedly affects the functions of cells of the immune system subsequently tested *in vitro*. Cellular components of both natural and acquired immunity are affected. *In vivo* tests are perhaps the most appropriate approach for determining the effect of different dietary *n*-3 PUFA on immune function. Several studies indicate that diets rich in *n*-3 PUFA are anti-inflammatory and immunosuppressive *in vivo*, although there have been relatively few studies in man. Although some of the effects of *n*-3 PUFA may be brought about by modulation of the amount and types of eicosanoids made, it is clear that these fatty acids can also elicit their effects by eicosanoid-independent mechanisms (Fig. 4). Such *n*-3 PUFA-induced effects may be of use as a therapy for acute and chronic inflammation, for disorders which involve an inappropriately-activated immune response and for the enhancement of graft survival.

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