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n-3 Fatty acids, inflammation and immunity: new mechanisms to explain old actions

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Numerous effects of *n*-3 fatty acids EPA and DHA on functional responses of cells involved in inflammation and immunity have been described. Fatty acid-induced modifications in membrane order and in the availability of substrates for eicosanoid synthesis are long-standing mechanisms that are considered important in explaining the effects observed. More recently, effects on signal transduction pathways and on gene expression profiles have been identified. Over the last 10 years or so, significant advances in understanding the mechanisms of action of *n*-3 fatty acids have been made. These include the identification of new actions of lipid mediators that were already described and of novel interactions among those mediators and the description of an entirely new family of lipid mediators, resolvins and protectins that have anti-inflammatory actions and are critical to the resolution of inflammation. It is also recognised that EPA and DHA can inhibit activation of the prototypical inflammatory transcription factor NF-κB. Recent studies suggest three alternative mechanisms by which *n*-3 fatty acids might have this effect. Within T-cells, as well as other cells of relevance to immune and inflammatory responses, EPA and DHA act to disrupt very early events involving formation of the structures termed lipid rafts which bring together various proteins to form an effective signalling platform. In summary, recent research has identified a number of new mechanisms of action that help to explain previously identified effects of *n*-3 fatty acids on inflammation and immunity.

Cytokine: Eicosanoid: Fish oil: Resolvin: Lipid raft

Overview of inflammation and immunity in health and disease

The immune system is the means by which the sources of non-threatening antigens are identified and tolerated and by which threatening antigens are identified and their sources eliminated. The immune system is principally thought of in the context of protection against pathogenic bacteria, viruses, fungi and parasites, but it also plays roles in identification and elimination of tumour cells and in the response to physical insults such as injury, surgery, burns and irradiation. The immune system is highly complex, involving many different specialised cell types dispersed throughout the body and moving between body compartments as part of routine immune surveillance or in response to specific stimuli. The cells of the immune system interact with one another and with other cell types (e.g. epithelial cells, endothelial cells, platelets, hepatocytes and adipocytes) in order to elicit and regulate local and systemic responses to infection, injury or insult. Many chemical mediators are produced during the course of an immune response; some of these are directly damaging to infectious organisms, others play a regulatory role promoting the activity of particular cell types, while others serve to terminate the response when the source of the initial immune stimulation has been eliminated.

The immune response can be classified into two general arms termed the innate (or natural) and the acquired (or specific). The innate immune response can be activated via recognition of certain general structural features of pathogens; these features may be shared by numerous pathogens. For example, lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria and also known as endotoxin, is recognised by Toll-like receptor (TLR)-4 on the surface of innate immune cells. In contrast, the acquired immune response is specific for a single antigen which must be presented by an antigen-presenting cell to an antigen-specific T-cell. Thus, the innate response is induced quickly and is not improved by prior exposure to the triggering pathogen, while the acquired response is induced slowly but is enhanced by prior exposure to the antigen. The two arms of the immune system communicate during an immune response because innate immune cells can present antigen, thus inducing the acquired response, while the acquired immune response produces chemicals that activate innate immune cells or make their processes more efficient. Inflammation is part of a normal innate immune response. Obviously, immune responses, including the inflammatory component, are protective and hence are beneficial to health. However, active immune responses triggered by normally benign structures or by host antigens can cause tissue damage and disease. These diseases will often involve infiltration and activation of immune cells (both innate and acquired) within particular tissue compartments initiating and perpetuating tissue damage which can become pathological. This can be caused by in-appropriate activation of the immune response, perhaps because of wrong recognition of an immune trigger (e.g. a host antigen), or by an inability to shut-off an inappropriate immune response because of loss of a terminating or resolving factor. Examples of diseases where an inappropriate immune response is central to the pathology are rheumatoid arthritis (autoimmune destruction of the joints), inflammatory bowel diseases (loss of tolerance to commensal gut bacteria resulting in an active and dama-ging immune response within the gastrointestinal mucosa) and asthma (adverse immune response to a normally benign environmental antigen causing airways damage). Classic inflammatory cells and chemical mediators pro-duced by those cells are central to the pathology of these diseases and hence they are often referred to as 'inflam-matory diseases'[1–3]. Nevertheless it should not be over-looked that cells of the acquired immune response also play an important, often key regulatory, role in these diseases.

n-3 Fatty acids, inflammation and immunity

Research on the influence of fatty acids on immunity started in the 1970s, with the earliest studies evaluating and comparing the effects of common SFA and the n-6 fatty acid linoleic acid[4–7]. The effects observed were considered to involve modifications of the physical state of the plasma membrane of immune cells: membrane order ('fluidity') could clearly be involved in a mem-brane-mediated process such as phagocytosis[8–11] but has also been thought to be important in T-lymphocyte responses to activation[12,13]. The discovery that eicosa-noids, including PGE_2, play roles in inflammation and in the regulation of immune cell function[14,15] initiated research into the effects of the common eicosanoid pre-cursor arachidonic acid and also raised the possibility that the effects of some fatty acids on immune cell responses could be due to modification of eicosanoid production[15,16]. Thus, the two long-standing mechanisms to describe the effects of fatty acids on inflammation and immunity involve alterations of membrane order and modulation of eicosanoid production, both driven by modification of the fatty acid composition of the phospholipids within membranes of inflammatory and immune cells.

The first studies of the effects of n-3 fatty acids on inflammation and immunity were published in the 1980s. In vitro studies revealed that both EPA and DHA could influence the functional responses of immune cells to stimulation (see Calder[5] for references) and early studies of fish oil in experimental models of autoimmunity[17] and clinical trials of fish oil in patients with rheumatoid arthritis[18,19] demonstrated significant anti-inflammatory activity of the combination of EPA and DHA. The effects of EPA and DHA on inflammation and immunity were considered to be consistent with the existing mechanisms of fatty acid action. First, the highly unsaturated nature of EPA and DHA means that they have the potential to have marked effects on membrane order in immune cells[13]. Secondly, incorporation of EPA and DHA into cells involved in inflammation and immunity is partly at the expense of arachidonic acid[20–37] hence decreasing the amount of substrate available to produce inflammatory and immunoregulatory eicosanoids[26–29,38–42]. Over the last 25 years, the effects of n-3 fatty acids on aspects of inflammation and immunity have been extensively exam-ined. They have been demonstrated to affect the functions of a range of cell types involved in innate and acquired immunity, to modify the expression of key cell surface proteins and to modulate the production of reactive oxygen species, eicosanoids and cytokines (Table 1). The effects of n-3 fatty acids on inflammation and immunity have been reviewed many times[5,7,43–54], and the reader is referred to these reviews for a detailed coverage of the topic. The current article will provide an update on the mechanisms of action of n-3 fatty acids with respect to inflammation and immunity, putting these in the context of the earlier understandings of n-3 fatty acid actions.

EPA and DHA are rapidly incorporated into phospholipids of immune cells in human subjects

It is well known that increased oral supply of EPA and DHA results in an increase in the amount of those fatty acids in immune cells in laboratory animals[20–24,39–41] and human subjects[26–33,35–37,55]. This increase occurs in a dose–response manner[32,37,55] and time-course studies reported that near maximum incorporation of both EPA and DHA occurred within a few weeks in human subjects[31,37,55]. The incorporation of these highly unsatu-rated long chain n-3 fatty acids is mainly at the expense of

Table 1. Summary of the effects of *n*-3 fatty acids (EPA + DHA) on immune and inflammatory cells

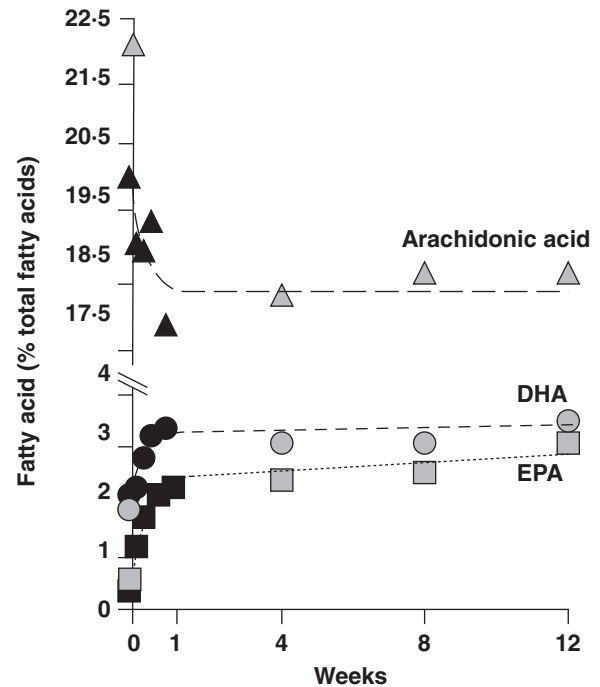| Cell type	Effects seen
Endothelial cells	Decreased adhesion molecule expression
	Decreased adhesion of leucocytes
	Decreased production of inflammatory cytokines
Neutrophils	Decreased chemotaxis
	Decreased adhesion to endothelial cells
	Decreased respiratory burst
	Decreased production of eicosanoids from arachidonic acid
Monocytes and macrophages	Decreased chemotaxis
	Decreased adhesion molecule expression
	Decreased adhesion to endothelial cells
	Decreased respiratory burst
	Increased phagocytosis
	Decreased production of eicosanoids from arachidonic acid
	Decreased production of inflammatory cytokines
Antigen presenting cells	Decreased MHC I and II expression
	Decreased antigen presentation
T-cells	Decreased adhesion molecule expression
	Decreased adhesion to endothelial cells
	Decreased production of T-helper 1 type cytokines
	Decreased proliferation

Fig. 1. Time course of incorporation of EPA and DHA and of disappearance of arachidonic acid in human mononuclear cells in healthy volunteers consuming fish oil. Healthy human volunteers consumed fish oil providing 2·1 g EPA and 1·1 g DHA per d for 1 week[56] or for 12 weeks[31]. Blood was sampled at several time points in each study and mononuclear cells prepared. Fatty acid composition of the cells was determined by GC. Mean values are shown. ■ and □, EPA; ● and ○, DHA; ▲ and △, arachidonic acid; Black symbols represent data from Faber *et al*.[56]; Grey symbols represent data from Yaqoob *et al*.[31].

n-6 fatty acids, especially arachidonic acid[20–37]. A recent study evaluated the incorporation of EPA and DHA into human mononuclear cells (a mixture of 85 % lymphocytes and 15 % monocytes isolated from peripheral blood) over one week[56]. By coincidence this study provided the same daily amounts of EPA and DHA as used in an earlier study by Yaqoob *et al*.[31], allowing a direct comparison of the findings of these two studies in healthy human volunteers. Faber *et al*.[56] report significant incorporation of EPA and DHA after just 1 d of supplementation and combining the findings of these two studies reveals that the near maximum incorporation of both *n*-3 fatty acids into human mononuclear cells occurs at about 7 d of supplementation (Fig. 1). This rapid incorporation of EPA and DHA suggests that subsequent functional effects on cell responsiveness and function may occur more quickly than previously considered.

Opposing actions of eicosanoids produced from arachidonic acid and EPA regulate leucocyte-endothelial interaction

Eicosanoids are biologically active lipid mediators produced from PUFA, most commonly the *n*-6 fatty acid arachidonic acid. Eicosanoids play wide ranging roles in inflammation and regulation of immune function[57,58]. To produce these eicosanoids, arachidonic acid is released from membrane phospholipids through the action of phospholipase A_2 enzymes, and then acts as a substrate for cyclooxygenase (COX), lipoxygenase or cytochrome P450 enzymes (Fig. 2). COX enzymes lead to PG and thromboxanes, lipoxygenase enzymes lead to leucotrienes (LT) and cytochrome P450 enzymes lead to hydroxy-eicosatetraenoic and epoxyeicosatrienoic acids[57–59]. The decrease in arachidonic acid content of cell membrane phospholipids that occurs with incorporation of EPA and DHA (Fig. 1) reduces the availability of the usual eicosanoid substrate. Thus, increased incorporation of *n*-3 fatty acids into cell membranes is associated with decreased production of the major 2-series PG and 4-series LT[26–29,30–42]. This represents a key anti-inflammatory effect of *n*-3 fatty acids, and has been long recognised.

EPA is also a substrate for the COX, lipoxygenase and cytochrome P450 enzymes, but the mediators produced have a different structure from those made from arachidonic acid (e.g. PGE_3 rather than PGE_2 and LTB_5 rather than LTB_4). Increased generation of 5-series LT has been demonstrated using macrophages from fish oil-fed mice[40] and neutrophils from human subjects taking fish oil supplements for several weeks[26,28,29]. The functional significance of this is that the eicosanoids produced from EPA are often much less biologically active than those produced from arachidonic acid[60–62]. One reason for this reduced biological potency is that eicosanoid receptors typically have a much lower affinity for the EPA-derived mediator than for the arachidonic acid-derived one[63]. Thus, EPA results in decreased production of potent
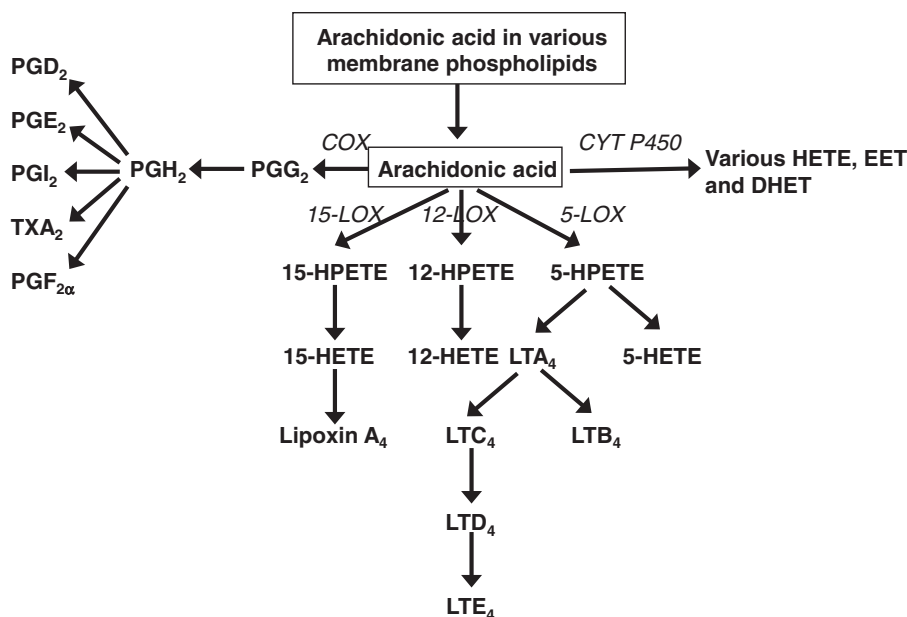
Fig. 2. Summary of eicosanoid synthesis from arachidonic acid. COX, cyclooxygenase; CYT P450, cytochrome P450 enzymes; DHET, dihydroxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; LOX, lipoxygenase; LT, leucotriene; TX, thromboxane. Note that not all enzymes are named and that not all metabolites are shown.

eicosanoids from arachidonic acid and increased production of weak eicosanoids. Recently, the effect of arachidonic acid-derived PGD_2 and EPA-derived PGD_3 on neutrophil adhesive interactions with endothelial cells was investigated in an *in vitro* setting[64]. Both EPA and PGD_3 were able to inhibit neutrophil transmigration through the endothelial cell monolayers, an effect which could be prevented by either arachidonic acid or PGD_2. An antagonist to the PGD receptor DP1 also inhibited transmigration, while a DP1 agonist overcame the inhibitory effect of EPA. It was concluded that PGD_2 acts to up-regulate neutrophil transmigration, analogous to neutrophilic infiltration into inflammatory sites, acting through DP1 while PGD_3 acts to prevent this effect of PGD_2 again acting at DP1. The observation that PGD_3 can effectively compete with PGD_2 is supported by the findings of Wada *et al.*[63] that DP1 has a greater affinity for PGD_3 than for PGD_2.

New families of anti-inflammatory and inflammation resolving mediators are produced from EPA and DHA

Historically, research has focused on the initiation of inflammatory responses and strategies to promote or suppress. In the last 10 years or so a greater appreciation of the importance of 'turning off' inflammation has developed. This process is termed resolution and it seems likely that failure to resolve inflammation is an important factor in determining the course of inflammatory responses and their transition to disease states. During the recent past, lipid mediators produced from EPA and DHA have been discovered that seem to play a central role in resolution of inflammation. Hence, these mediators have been termed resolvins. Those produced from EPA are called E-series

while those produced from DHA are termed D-series. Related compounds called protectins (also referred to as neuroprotectins when generated within neural tissue) are produced from DHA. The synthesis of resolvins and protectins involves the COX and lipoxygenase pathways operating across two cell types, with different epimers being produced in the presence and absence of aspirin[65–68]. Resolvin synthesis is increased by feeding fish-oil rich diets to laboratory rodents[69] and was shown to occur in fat-1 mice in which colitis had been induced[70]. A recent study revealed significant concentrations of resolvins E1 and D1 in the plasma of healthy human volunteers after supplementation with fish oil for 3 weeks[71].

The biological effects of resolvins and protectins have been examined extensively in cell culture and in animal models of inflammation, where they have been shown to be anti-inflammatory and inflammation resolving. For example, resolvins D1 and E1 and protectin D1 all inhibited transendothelial migration of neutrophils, preventing the infiltration of neutrophils into sites of inflammation; resolvin D1 inhibited IL-1β production; and protectin D1 inhibited TNF and IL-1β production[65–68]. Resolvins reduce inflammation and exert protection in experimental animals in models of inflammatory disease including arthritis[72], colitis[73] and asthma[74,75]. The potent effects of resolvins and protectins may explain many of the anti-inflammatory effects of *n*-3 fatty acids.

n-3 Fatty acids inhibit activation of the pro-inflammatory transcription factor NF-κB

Cell culture studies with EPA and DHA show inhibition of LPS-induced production of COX-2, inducible nitric oxide

synthase, TNF, IL-1, IL-6, IL-8 and IL-12 in endothelial cells[76,77] and monocytes[78,79]. Animal feeding studies with fish oil, a source of EPA and DHA, support the observations made *in vitro* with respect to the effects of *n*-3 fatty acids on inflammatory cytokine production. For example, dietary fish oil decreased the production of TNF, IL-1β and IL-6 by LPS-stimulated mouse macrophages[39,80,81]. Some studies in healthy human subjects have demonstrated that oral fish oil supplements can decrease production of TNF, IL-1β, IL-6 and various growth factors by LPS-stimulated monocytes or mononuclear cells[26,27,30,82–84], although not all studies confirm this effect.

NF-κB is a key transcription factor involved in up-regulation of inflammatory cytokine, adhesion molecule and COX-2 genes[85,86]. Inactive NF-κB is a trimer localised within the cytosol; it is activated via a signalling cascade triggered by extracellular inflammatory stimuli which involves phosphorylation of an inhibitory subunit of NF-κB (IκB) which then dissociates allowing translocation of the remaining NF-κB dimer to the nucleus[87]. LPS induces inflammation by activating NF-κB, as do some inflammatory cytokines and UV irradiation. EPA or fish oil has been shown to decrease LPS-induced activation of NF-κB in human monocytes[78,88,89] and this was associated with decreased IκB phosphorylation[88,89].

Until fairly recently it has not been clear how *n*-3 fatty acids could influence NF-κB activation. However, recent studies suggest several possible mechanisms that might be involved. PPARγ is a transcription factor that acts in an anti-inflammatory manner[90]. It is able to directly regulate inflammatory gene expression, but it also interferes physically with the activation of NF-κB[91]. DHA induced PPARγ in dendritic cells and this was associated with inhibition of NF-κB activation and reduced production of the pro-inflammatory cytokines TNF and IL-6 following LPS stimulation[92]. In addition, DHA induced a number of known PPARγ target genes in dendritic cells, suggesting this as an important anti-inflammatory mechanism of action of DHA and perhaps also of EPA[93].

The *n*-3 fatty acid mediated inhibition of NF-κB activation is associated with decreased IκB phosphorylation[88,94]. In contrast with the effects of EPA and DHA on IκB phosphorylation and subsequent activation of NF-κB, SFA, especially lauric acid, enhanced IκB phosphorylation and NF-κB activation in macrophages[94] and dendritic cells[95] and so promoted inflammatory gene expression. Lee *et al.*[94] found that EPA and DHA were able to prevent the NF-κB mediated pro-inflammatory effect of lauric acid in macrophages. They also showed that the activation of NF-κB and induction of COX-2 expression by lauric acid did not occur in macrophages expressing a dominant-negative mutant of the cell surface LPS receptor, TLR-4, suggesting that lauric acid somehow interacts with TLR-4. Myeloid differentiation primary response gene 88 is a cell membrane-associated adapter protein used by TLR-4 to activate NF-κB. DHA inhibited COX-2 expression in macrophages bearing constitutively active TLR-4 but not in those bearing constitutively active myeloid differentiation primary response gene 88 suggesting that the effects of DHA are at the level of TLR-4[94]. More

recently, Wong *et al.*[96] demonstrated that exposure of macrophages to lauric acid-induced association of TLR-4, myeloid differentiation primary response gene 88 and other signalling proteins into membrane rafts in much the same way as LPS acts. Furthermore, they showed that DHA inhibited the ability of both LPS and lauric acid to promote recruitment of these signalling proteins into rafts. Thus, the differential effects of fatty acids on inflammatory signalling initiated through TLR-4 and impacting on NF-κB appear to relate to their ability to promote or disrupt raft formation within the membrane of inflammatory cells.

Activation of PPARγ and disruption of lipid rafts that initiate inflammatory signalling represent two mechanisms by which *n*-3 fatty acids could inhibit activation of NF-κB. Recently, a third mechanism has been identified[97]. This involves a cell surface G-protein coupled receptor called GPR120 which is highly expressed on inflammatory macrophages. The GPR120 agonist GW9508 inhibited responsiveness of macrophages to LPS[97]. The effect of GW9508 involved reduced phosphorylation of IκB kinase and IκB, IκB maintenance in the cytosol and reduced TNF and IL-6 production. These observations suggest that GPR120 signalling is anti-inflammatory. Both, EPA and DHA, but not arachidonic, palmitic or myristic acids, promoted GPR120-mediated gene activation, although they were less potent than GW9508. The effects of DHA were further explored[97]. Its inhibitory effects on LPS-induced IκB kinase phosphorylation, IκB phosphorylation and degradation, and TNF, IL-6 and monocyte chemotactic protein-1 production did not occur in GPR120 knockdown cells. These observations suggest that the inhibitory effect of DHA (and probably also those of EPA) on responsiveness to LPS occur via GPR120, which induces signalling that interferes with the pathway that activates NF-κB.

Thus, recent studies suggest three alternative mechanisms by which EPA and DHA might act to suppress inflammatory signalling via NF-κB: activation of PPARγ which physically interacts with NF-κB preventing its nuclear translocation, interfering with early membrane events involved in activation of NF-κB via TLR-4 and action via GPR120 which initiates an anti-inflammatory signalling cascade that inhibits signalling leading to NF-κB activation (Fig. 3). The extent to which these three mechanisms are interlinked is not clear at this stage.

n-3 Fatty acids affect the formation of signalling platforms (rafts) in the plasma membrane of T-cells and in other immune cells

In cell cultures both EPA and DHA inhibit T-cell proliferation[13,16,98–100] and the production of the key T-helper 1 type cytokine IL-2[13,99,100]. Animal feeding studies with fairly high amounts of fish oil, or of individual *n*-3 fatty acids, have also reported reduced T-cell proliferative responses[101–104] and alterations in T-helper 1 cytokine gene expression[102] and production[103]. Studies in human subjects are less consistent, although some studies have shown that increased intake of EPA + DHA decreases human T-cell proliferation[33,38] and IL-2 production[38]. These functional effects of *n*-3 fatty acids on
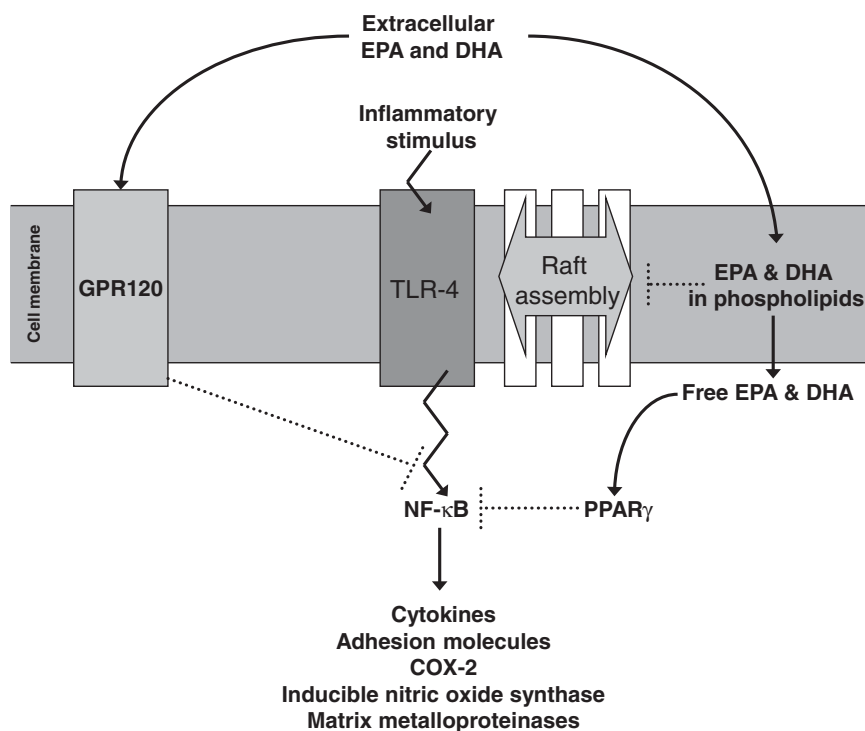
Fig. 3. Summary of different mechanisms by which *n*-3 fatty acids inhibit activation of the pro-inflammatory transcription factor NF-κB. COX, cyclooxygenase; GPR, G-protein coupled receptor; PPAR, peroxisome proliferator activated receptor. Dotted lines indicate inhibition.

T-cells have been linked with changes in membrane order[13], altered patterns of eicosanoid production[16] and modification of early signal transduction events within the plasma membrane, including reduced generation of dia-cylglycerol[103,104] and inhibition of the activation of specific isoforms of protein kinase C[105,106] and of mito-gen-activated protein kinases[107,108]. Until fairly recently, the earliest event reported to be affected by *n*-3 fatty acids following T-cell activation was the phosphorylation of the signalling enzyme phospholipase C-γ1 which was decreased by fish oil feeding in rats[109]. This latter effect was confirmed in a T-cell line exposed to EPA[110] and was extended to demonstrate further upstream events of EPA on signalling proteins in T-cells[110–112]. The earliest event affected by EPA was reported to be the inhibition of the anchoring of the protein called linker of activated T-cells into the plasma membrane[112]. These *in vitro* studies identified that the effects of EPA on early signalling events in T-cells seem to involve the disruption of the formation of signalling platforms in the plasma membrane termed lipid rafts[110–115].

Lipid rafts are regions of membranes with a distinct, characteristic structural composition[116,117]. They are par-ticularly rich in sphingolipids and cholesterol, and the side chains of the phospholipids are usually highly enriched in SFA compared with the surrounding non-raft regions of the membrane. As a result of the presence of cholesterol and SFA, lipid rafts are more ordered ('less fluid') than the surrounding (non-raft) portions of the membrane. Cyto-plasmic proteins that are covalently modified by SFA (palmitoyl or myristoyl moieties) and cell surface proteins

that are attached via a glycosyl phosphatidylinositol anchor are highly concentrated within lipid raft regions. Many proteins involved in signal transduction, such as Src family kinases, G proteins, growth factor receptors, mitogen-activated protein kinases and protein kinase C are pre-dominantly found in lipid rafts, which appear to act as signalling platforms by bringing together (i.e. co-localising) various signalling components, facilitating their interac-tion. The importance of rafts has been well demonstrated with respect to T-lymphocyte responses to activation[118–121] and research now suggests that raft disruption under-lies the mechanism of action of *n*-3 fatty acids on T-cells[53,114,115,121] and other immune cells[54,122,123]. As indicated earlier, cell culture studies have demon-strated that exposure to EPA modifies raft formation in T-cells in a way that impairs the intracellular signall-ing mechanisms in these cells[110–115]. Feeding studies with *n*-3 fatty acids in mice confirm modifications of raft structure and function, linked to altered T-cell responses[124,125].

In addition to *n*-3 fatty acids affecting lipid rafts in T-cells in ways that are linked to functional changes in the cells[110–115,124,125], effects on lipid rafts are also relevant to the influence of *n*-3 fatty acids on other cells of the immune system including inflammatory macrophages, dendritic cells and B-cells. In an earlier section it was described how Wong *et al.*[96] had demonstrated that opposing effects of lauric acid and DHA and of LPS and DHA on responses of macrophages involved differential effects on lipid raft formation, ultimately linked to the NF-κB activation cascade. A series of studies has evaluated

Table 2. Summary of the mechanisms of action of *n*-3 fatty acids (EPA + DHA) on immune and inflammatory cells

Effect	Mechanism(s) likely to be involved
Decreased production of eicosanoids from arachidonic acid	Decreased arachidonic acid in membrane phospholipids Inhibition of arachidonic acid metabolism Decreased expression of cyclooxygenase-2 gene (via decreased activation of NF-κB)
Increased production of (weakly inflammatory) eicosanoids from EPA	Increased EPA in membrane phospholipids
Increased production of inflammation resolving resolvins and protectins	Increased EPA and DHA in membrane phospholipids Inhibition of arachidonic acid metabolism
Decreased production of inflammatory cytokines	Decreased expression of inflammatory cytokine genes (via decreased activation of NF-κB)
	— Induction and activation of PPARγ
	— Decreased generation of intracellular signals due to disruption of membrane lipid rafts
	— Activation of GPR120 signalling
Decreased leucocyte chemotaxis	Decreased production of some chemoattractants Decreased expression of chemoattractant receptors
Decreased T-cell reactivity	Decreased generation of intracellular signals due to disruption of membrane lipid rafts
Decreased antigen presentation	Decreased MHC expression due to disruption of membrane lipid rafts

the *in vitro* effects of EPA and DHA and the effect of fish-oil feeding to mice on raft clustering in B-cells and the functional significance of the effects seen[126–131]. An *in vitro* study using a B-cell line found that DHA, but not EPA, decreased raft clustering and that this was associated with increased movement of MHC I into rafts[127], although these findings contrast with earlier observations that DHA decreased MHC I expression on the surface of B-cells due to an impairment of trafficking of MHC I from the endoplasmic reticulum to the Golgi and suppressed B-cell conjugation with T-cells[126]. Feeding mice diets containing fish oil resulted in increased CD69 expression on the surface of stimulated splenic B-cells and increased the *ex vivo* production of inflammatory cytokines[128–130], effects associated with impaired raft clustering and modification of raft size[129,130]. Most recently Gurzell *et al.*[131] reported that feeding colitis-prone mice an *n*-3 fatty acid rich diet increased the EPA and DHA content of splenic B-cells, diminished the clustering of rafts in B-cells and resulted in larger membrane raft regions. These membrane effects were associated with enhanced B-cell activation *ex vivo* and increased inflammatory cytokine production. Thus, there are generally similar effects observed with a B-cell line *in vitro*[127] and with B-cells after feeding *n*-3 fatty acids to mice[128–131]. The observations suggest that *n*-3 fatty acids enhance B-cell function and that this may be due to modulation of structure–function relationships within the plasma membrane. If this is correct then the effects of *n*-3 fatty acids on T-cells and B-cells might be different, although lipid raft disruption may be involved in both cases.

Summary and conclusions

Numerous effects of EPA and DHA on functional responses of cells involved in inflammation and immunity have been established over the last 40 years. These include

inhibition of leucocyte chemotaxis, adhesion molecule expression and leucocyte-endothelial adhesive interactions, production of eicosanoids such as PG and LT from the *n*-6 fatty acid arachidonic acid, production of inflammatory cytokines like TNF and IL-6 and T-cell reactivity and enhanced phagocytosis (Table 1). These effects have been interpreted in the context of reducing inflammation that would lead to benefit in inflammatory conditions, as discussed elsewhere[46]. Fatty acid-induced modifications in membrane order and in the availability of substrates for eicosanoid synthesis are long-standing mechanisms that are considered important in explaining the anti-inflammatory and immunomodulatory actions of EPA and DHA. More recently, effects on signal transduction pathways and on gene expression profiles were identified to play a role. Over the last 10 years or so, significant advances in understanding the mechanisms of action of *n*-3 fatty acids have been made (Table 2). These include the identification of new actions of lipid mediators that were already described and of novel interactions among those mediators and the description of an entirely new family of lipid mediators, resolvins and protectins that have anti-inflammatory actions and, perhaps more importantly, are critical to the resolution of inflammation. These mediators may explain many of the existing actions of EPA and DHA. It is also recognised that EPA and DHA can inhibit activation of the prototypical inflammatory transcription factor NF-κB within classic inflammatory cells such as macrophages. Recent studies suggest three alternative mechanisms by which *n*-3 fatty acids might have this effect: activation of PPARγ which physically interacts with NF-κB preventing its nuclear translocation; interfering with early membrane events involved in activation of NF-κB via TLR-4; action via GPR120 which initiates an anti-inflammatory signalling cascade that inhibits signalling leading to NF-κB activation. Within T-cells, as well as other cells of relevance to immune and inflammatory responses, EPA

and DHA act to disrupt very early events involving formation of the structures termed lipid rafts which bring together various proteins to form an effective signalling platform. The discovery of actions of EPA and DHA on lipid rafts and on the earliest signalling events in membranes and of a membrane receptor for *n*-3 fatty acids (GPR120) will re-focus attention on the membrane as the key site of action of these important bioactive fatty acids.

Acknowledgements

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