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Regulatory Activity of Polyunsaturated Fatty Acids in T-Cell Signaling

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Abstract

n-3 polyunsaturated fatty acids (PUFA) are considered to be authentic immunosuppressors and appear to exert beneficial effects with respect to certain immune-mediated diseases. In addition to promoting T-helper 1 (Th1) cell to T-helper 2 (Th2) cell effector T-cell differentiation, n-3 PUFA may also exert anti-inflammatory actions by inducing apoptosis in Th1 cells. With respect to mechanisms of action, effects range from the modulation of membrane receptors to gene transcription via perturbation of a number of second messenger cascades. In this review, the putative targets of anti-inflammatory n-3 PUFA, activated during early and late events of T-cell activation will be discussed. Studies have demonstrated that these fatty acids alter plasma membrane micro-organization (lipid rafts) at the immunological synapse, the site where T-cells and antigen presenting cells (APC) form a physical contact for antigen initiated T-cell signaling. In addition, the production of diacylglycerol and the activation of different isoforms of protein kinase C (PKC), mitogen activated protein kinase (MAPK), calcium signaling, and nuclear translocation/activation of transcriptional factors, can be modulated by n-3 PUFA. Advantages and limitations of diverse methodologies to study the membrane lipid raft hypothesis, as well as apparent contradictions regarding the effect of n-3 PUFA on lipid rafts will be critically presented.

1. Introduction

Polyunsaturated fatty acids (PUFA) are important for the structure and function of many plasma membrane proteins, including receptors, enzymes, and active transport molecules. Since mammals cannot synthesize long chain n-3 or n-6 PUFA de novo, these fatty acids should be consumed in the diet. These two groups of PUFA are defined depending on the position of the double bond nearest the methyl end of the fatty acid. The n-6 PUFA are synthesized from

linoleic acid (18:2n-6), involving an enzymatic pathway which includes desaturases and elongases and are abundantly present in meat and vegetable oils [1]. The most biologically active n-6 PUFA is arachidonic acid (AA, 20:4n-6), which is implicated in many cellular functions and inflammation. AA has been reported to give rise to prostaglandins and thromboxanes via cyclooxygenase pathways and to leukotrienes, hydroperoxyeicosatetraenoic acids (HPETE) and hydroxyeicosatetraenoic acids (HETE) via lipoxygenase pathways [2-4].

Marine algae are rich in enzymes involved in n-3 PUFA synthesis and, therefore, can elongate alpha-linolenic acid (ALA, 18:3n-3) into eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6, n-3) which are consumed by humans through the marine fish food chain [4]. EPA and DHA are major fatty acids involved in cellular functions. EPA can also serve as a substrate for both cyclooxygenase and 5- and 15-lipoxygenases, giving rise to eicosanoids with a slightly different structure from those formed from arachidonic acid [3, 5]. Interestingly, DHA has been recently shown to give rise to anti-inflammatory docosanoids coined as resolvins, protectins and maresins [6]. The detailed accounts on synthesis and bioconversion of these fatty acids are described in recent review articles [6-7].

2. n-3 PUFA as immunosuppressors

Over the last 30 years, we have witnessed an enormous increase in the consumption of n-6 PUFA due to the increased intake of vegetable oils from corn, sunflower seeds, cottonseed, and soybeans. These oils are a rich source of n-6 PUFA. In contrast, the intake of n-3 PUFA has declined because of the decrease in fish consumption and the industrialization of meat and eggs which are rich in n-6 and devoid of n-3 PUFA. At present, the dietary ratio of n-6 to n-3 fatty acids ranges from 20-30:1 instead of the historical range of 1-2:1 in western countries [8]. A number of experimental and clinical studies have shown that a high n-6/n-3 PUFA ratio may contribute to the prevalence of many diseases, e.g., cardiovascular pathology, chronic inflammatory/autoimmune disorders and cancers. As far as n-3 PUFA are concerned, epidemiological studies suggest a decreased incidence of inflammatory diseases in Greenland Inuits and Japanese [9]. Dietary supplementation with fish oils has been reported to exert anti-inflammatory effects in patients suffering from rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD) [10-13]. RA is a chronic inflammatory autoimmune disease manifested by swollen and painful joints, bone erosion and functional impairment. The joint lesions are characterised by infiltration of T-cells, macrophages and B-cells into the synovium. Hence, n-3 PUFA seem to exert their beneficial effects both via their anti-inflammatory and anti-autoimmune properties [14]. In a dual-centre, double-blind placebo-controlled randomized study of 9 months' duration, ninety-seven patients with RA were randomized to take 10 g of cod liver oil containing 2.2 g of n-3 PUFA. It was concluded that these fatty acids can be used as non-steroidal anti-inflammatory drug (NSAID)-sparing agents in this pathology [15]. It appears that a minimum daily dose of 3 g of EPA / DHA is necessary to derive the expected benefits. These doses of n-3 PUFA are associated with significant reductions in the release of leukotriene B(4) from stimulated neutrophils and IL-1 from monocytes [16]. Sometimes, the ameliorative effects of n-3 PUFA are marginal with respect to the treatment of RA. For instance, a study conducted on forty-five subjects, in a double-blind, placebo-controlled cross-over manner, has shown that an n-3 PUFA-containing diet did not remarkably improve the severity of RA, although there was evidence of cardioprotective effects in these subjects [17].

n-3 PUFA exert anti-inflammatory properties, in part, via their actions on macrophages. For example, feeding of n-3 PUFA-containing oils has been shown to decrease the production of IL-1, IL-6 and TNF- α in rats [18]. In humans, Endres et al. [19] and Meydani et al. [20] have observed that fish oil supplementation curtailed the ability of peripheral blood monocytes to produce TNF- α , IL-1 α and IL-1 β . In addition, in mouse T-cells, an EPA and DHA rich diet has been shown to decrease both the secretion of IL-2 and expression of IL-2 mRNA [21]. n-3

PUFA modulate nuclear translocation of NF- κ B, inhibit IL-1 and TNF- α production and give rise to resolvins and protectins, which are endogenous anti-inflammatory molecules [14].

Preliminary data suggest that n-3 PUFA also exert beneficial effects with respect to psoriasis [22], atopic dermatitis [23], and multiple sclerosis (MS) [24]. For example, a study on a positive correlation between the risk of MS and the sales of meat and dairy products has been reported [25]. On the other hand, an inverse correlation was observed between MS risk and the sales of vegetables and fish products. In a Norwegian study, it was shown that the consumption of fish is associated with a decreased risk for developing MS [26]. In fact, a recent survey conducted on MS patients has revealed that 37% of 1,573 patients had used n-3 PUFA at some point in their lives [27]. However, confirmatory, definitive studies are needed in order to make recommendations for clinical practice. In the following sections, we have principally focused on the modulation of T-cell signalling by n-3 PUFA at the cellular and molecular level. Review articles on immunosuppression can be consulted for additional details [28-30].

3. T-cell differentiation (Th1/Th2 dichotomy) and n-3 PUFA

T-helper 1 (Th1) cells produce pro-inflammatory cytokines and n-3 PUFA may orient this phenotype to the Th2 phenotype, considered to be anti-inflammatory. Th1 cells secrete IL-2, IFN- γ and TNF- β while Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13 [31]. The pathogenic role of Th1 and the protective role of Th2 cells have been described in RA, MS, and insulin-dependent diabetes mellitus [32]. Besides, Th1/Th2 balance seems to be an important indicator of the disease state [32].

In mice, an n-3 PUFA-enriched diet was reported to induce the downregulation of IL-2 driven CD4 and CD8 activation, and upregulation of Th2 T-cell subpopulation [33]. In another study, dietary fish oil significantly increased the percentage of Th2 polarized cells and suppressed the Th1 cell number. Kleemann et al. [34], who fed BB rats a fish oil rich diet, observed a shift in Th1/Th2 cytokines ratio towards Th2 due to an increase in IL-10 mRNA expression in the Peyer's patches. Wallace et al. [35] have observed that feeding fish oil to mice induced a shift in the IFN- γ /IL-4 ratio by a factor of four as compared to animals fed low fat diets. Mizota et al. [36] have recently conducted a very elegant study in both humans and mice that were fed n-3 PUFA enriched diets. These investigators observed that the ratio of IFN- γ /IL-4 was significantly increased after n-3 PUFA supplementation. The effects of dietary n-3 PUFA on innate and specific immune response in models of contact and atopic dermatitis have also been examined [37]. In this study, mice were fed for 3 weeks either n-6 or n-3 PUFA-fortified diets. n-3 PUFA reduced oedema thickness, leukocyte infiltration, and enhanced antioxidant defences and IL-10 production in the inflamed ears of mice from both models. Zhang et al. [38] have suggested that anti-inflammatory effects of n-3 PUFA, explained by a shift in Th1/Th2 balance, might be due to the direct suppression of Th1 development, and not by an enhanced polarization of T-cells toward a Th2 phenotype, at least ex vivo. It is also possible that n-3 PUFA may eliminate Th1 cells by activation-induced cell death (AICD) as observed in murine splenocytes in which these fatty acids, after their incorporation into lipid rafts, induced AICD in Th1 cells [39-40].

There is evidence that the effects of n-3 PUFA depend on the pathological conditions, soliciting Th1 or Th2 phenotypes. EPA and/or DHA may shift the Th1 phenotype to Th2 in autoimmune or inflammatory situations, as opposed to in septic conditions. For instance, Tsou et al. [41] have observed no immunosuppressive effects of n-3 PUFA in sepsis. This study examined the effect of fish oil (FO)-enriched diets before and/or n-3 PUFA-containing total parenteral nutrition (TPN) after sepsis on the distribution of T-cell subpopulations and splenocyte cytokine mRNA expressions in rats with polymicrobial sepsis. These investigators concluded that FO administration before and/or after sepsis maintained blood T-cell subpopulations and

modulated both Th1 and Th2 cytokine mRNA expressions in rats. Similarly, Maes et al. [42] have concluded that n-3 PUFA induce a Th1-like response which may not be compatible with what is expected from antidepressive substances. In fact, preoperative oral intake supplementation with a formula containing n-3 PUFA maintained the Th1/Th2 ratio after surgical intervention [43]. Parenteral n-3 PUFA may also modulate inflammatory and immune responses in rats undergoing total gastrectomy. Linn et al. [44] have reported that FO administration promotes not only Th1 cytokine production but also enhances peritoneal macrophage phagocytic activity, and reduces leukocyte adhesion molecule expression in rats with total gastrectomy. Using CFSE-labelled DO11.10 CD4⁺ cells adoptively transferred into fish oil fed mice immunized with Th1 polarizing agents, Zhang et al demonstrated that n-3 PUFA reduced the number of cell division in vivo [45]. These data indicate that the attenuated inflammatory response which accompanies fish oil feeding may be explained, in part, by suppression of Th1 clonal expansion. It is important to point out that physiologically relevant levels of n-3 PUFA were used in this study, corresponding to ~1.4 and 1.0 energy % as EPA and DHA, respectively.

The differentiation of T-cells into Th1 and Th2 subsets is tightly regulated through the activities of specific signaling pathways and transcription factors [46]. The T-box transcription factor, T-bet, represents a key regulator of Th1 cell development through its ability to transactivate IFN- γ gene while, concomitantly, repressing IL-4 gene expression [47]. GATA-3 represents a key transcription factor for the development of Th2 cells. The conditional knockout of GATA-3 in mice reduces the expression of Th2 cytokines in vitro and in vivo [48]. We have observed that the absence of PPAR α resulted in the upregulation of T-bet mRNA and downregulation of GATA-3 transcripts. Since n-3 PUFA are putative PPAR α ligands [49], it is possible that these agents may modulate T-cell differentiation via PPAR α . By conducting a study on PPAR α knockout mice, we have observed that DHA downregulates T-bet and upregulates GATA-3 both at the protein and transcription levels, independently of PPAR α activation, through suppression of MAPK activation [50].

Though the exact mechanism of action of n-3 PUFA with respect to T-cell differentiation is not well-understood, the ability of these fatty acids to up-regulate the Th2 phenotype may modulate activated T-cells to respond to environmental factors that drive disease development/progression.

4. T-cells contain n-3 PUFA-specific PLA₂

It has been shown that DHA is incorporated into membrane phospholipids (PL), which are segregated to form their own domains. These “DHA-domains” appear to drive proteins from their resident lipid raft-rich environment to DHA-rich non-raft phases, and vice versa [51-52]. In Jurkat T-cells, n-3 PUFA are incorporated into phosphatidylcholine (PC) < phosphatidylethanolamine (PE) < phosphatidylinositol (PI) / phosphatidylserine (PS) [53]. This is noteworthy because these PL classes are targets of phospholipase A₂ (PLA₂, EC 3.1.1.4) and, therefore, can modulate PKC, MAPK and calcium channel-dependent signalling cascades.

There are essentially two large subgroups within the PLA₂ family: the small and secretory PLA₂ (sPLA₂) among which are pancreatic type IB and type V, and the intracellular Ca²⁺-dependent type IV PLA₂ and Ca²⁺-independent type VI PLA₂ [54]. For a long time, the role of different isoforms of PLA₂ in T-cell activation and proliferation remained ambiguous [55-56]. Ca²⁺-dependent type IV PLA₂ was previously shown to be involved in T-cell activation [56]. More recently, the presence of type VI PLA₂ in peripheral human blood Band T-cells has been implicated [57]. This PLA₂ isoform plays a key role in the control of cell growth [57]. Tessier et al. [58] have shown that human Jurkat T-cells possess two secretory and two cytosolic PLA₂ isoforms, which collectively modulate T-cell proliferation. With

respect to n-3 PUFA, type IV cPLA₂ is capable of the releasing DHA whereas type VI liberates EPA from T-cells [53]. These results suggest that upon T-cell activation, n-3 PUFA may be released from the plasma membrane and, therefore, may modulate cell signalling. Interestingly, several PLA₂ isoforms have been shown to be involved in the capacitative calcium influx in human T-cells [59]. The suggestion of the existence of an n-3 PUFA-specific PLA₂ in T-cells is provocative, and suggests that the timely release of EPA and/or DHA may be tightly regulated.

5. T-cell activation and n-3 PUFA

T-cell activation is tightly regulated by the signals transmitted via the T-cell receptor (TCR)/CD3 complex [60]. In addition to re-organization of signaling proteins (like the negative inhibitor CD45), there is also significant concentration of the CD3/TCR complex in the middle of immunological synapses or supra molecular activation clusters (SMAC). The cytoplasmic domains of TCR-associated proteins contain a motif, immunoreceptor tyrosine-based activation motif (ITAM) or antigen recognition activation motif (ARAM), that is sufficient and necessary to initiate the early and late signaling events in T-cells [60-61]. There are three ITAM motifs in the ζ chain of CD3 and one ITAM each in the cytoplasmic domain of CD3 γ , δ and ϵ .

One of the earliest events in T-cell activation is the induction of protein tyrosine kinase (PTK) activity. TCR subunits possess no intrinsic PTK activity. It has been shown that ITAMs mediate the interaction between TCR complexes and PTK Src-like (lck, fyn, yes) and ZAP70 proteins [60-62]. The latter binds to the phosphorylated ITAM whereas lck protein non-covalently associates with CD4 and CD8. Thus, src family PTK (lck or fyn) most likely phosphorylate the ITAM upon TCR stimulation. Phosphorylated ITAM then recruits ZAP70, which activates the kinase activity of ZAP70. Recruitment of ZAP70 subsequently activates phospholipase C- γ 1 (PLC γ 1) by phosphorylating select tyrosine residues. CD45 is a membrane phosphatase that removes Y394 and Y505 phosphate from lck, keeping it in a primed state. Csk is a kinase that phosphorylates Y505 and maintains lck in an inactive state [63].

The antagonist of ITAM is immunoreceptor tyrosine-based inhibition motif (ITIM), which negatively regulates cell signalling. Examples of ITIM proteins are cytotoxic T-lymphocyte antigen (CTLA)-4 in T-cells and Ly-49/KIR in NK cells. Most ITIM receptors bind to SHP-1 while CTLA-4 was reported to bind to SHP-2. Both SHP-1 and SHP-2 are SH2 containing phosphatases. PTK activation leads to formation of a complex with many adapters like LAT and SLP-76, and PLC γ 1 docks onto LAT [64].

5.1. Modulation of PKC activation by n-3 PUFA

In T-cells, PLC γ 1 hydrolyses phosphatidylinositol bisphosphate (PIP₂) and gives rise to diacylglycerols (DAGs) which are the activators of PKC. During the last decade, a number of isoforms of PKC have been discovered and, on the basis of their requirement for calcium and phosphatidylserine (PS), they have been classified into three large families including conventional PKC (cPKC), novel or new PKC (nPKC) or atypical PKC (aPKC). The cPKCs are composed of PKC α , PKC β _I, PKC β _{II}, PKC γ , and nPKCs, PKC δ , PKC ϵ , PKC ζ , PKC η , PKC μ , followed by aPKCs, PKC θ and PKC ξ [65]. Interestingly, nPKC can be activated in the absence of DAG whereas cPKC require this lipid for their activation [65].

5.1.1. Putative effects of n-3 PUFA—Since EPA and DHA can be released from the sn-2 position of membrane phospholipids by the action of n-3 PUFA specific-PLA₂, these fatty acids may directly modulate PKC activation [66]. Though early studies produced contradictory results on the modulation of PKC activation by n-3 PUFA, it is generally accepted that these fatty acids, during long incubation periods [67], curtail the PKC enzymatic activity (Figure 1).

Interestingly, in the absence of PL, EPA and DHA enhanced the catalytic activity of PKC and supported the binding of radioactive phorbol esters to membrane binding sites. In contrast, in the presence of PL, n-3 PUFA reduced both the catalytic activity of PKC and phorbol ester binding. Hence, it was concluded that n-3 PUFA compete for PS binding sites present on PKC [68].

One of the most important PKC genes in T-cells is PKC- θ , which is recruited rapidly to SMAC during T cell activation. Deletion of this gene leads to defective T cell activation. Dietary supplementation of n-3 PUFA resulted in their incorporation into PL and, consequently, inhibited the recruitment of PKC- θ to lipid rafts and subsequent signalling in T-cells [69].

In human Jurkat T-cells, DHA and EPA have been found to inhibit PKC activation [70-71], particularly the membrane recruitment of PKC- α and PKC- ϵ [53]. These two PKC isoforms (PKC- α and PKC- ϵ) were coupled to MAPK activation, upstream of ERK1/2, and the action of n-3 PUFA led to a decrease in nuclear translocation of nuclear factor κ B (NF- κ B), and subsequently to inhibition of IL-2 gene expression and cell proliferation [53].

5.1.2. DAGs-containing n-3 PUFA—It is expected that during a prolonged phase of in vivo supplementation with n-3 PUFA, activation of PLC γ 1 or PLD will give rise to DAG which will contain EPA and/or DHA. We have demonstrated that dietary n-3 PUFA suppress the formation of DAG in mouse splenic T-cell [72]. It has also been implicated that DAG-containing n-3 PUFA may act differently on PKC activation. One question is which phospholipase will give rise to DAG? In human T-cells, DHA has been shown to activate PLD [73]. This observation is noteworthy, as PLD generates DAG via its action on PC, which is highly enriched with exogenous n-3 PUFA. In Jurkat T-cells, DHA, following its incorporation into PC, significantly increased (by 35%) basal DAG levels [74].

To better understand the action of DAGs containing n-3 PUFA, we synthesized SAG, 1-stearoyl-2-arachidonyl-sn-glycerol; SDG, 1-stearoyl-2-docosahexaenoyl-sn-glycerol; SEG, 1-stearoyl-2-eicosapentaenoyl-sn-glycerol and assessed their efficiency with respect to the activation of cPKC (α, β, γ) and nPKC (ϵ, δ) PKC [75]. We observed that the three DAG species were not acting similarly with respect to activation of different isoforms of PKC. SAG, SDG and SEG curtailed the phorbol ester-induced activation of β I, γ , and δ , but not α and ϵ isoforms of PKC. Since SDG and SEG in the absence or presence of SAG differentially modulated PKC isoforms, it appeared that there might be two types of binding sites for DAG species which, in the presence of PS and calcium, might signal differently. These data suggest that the actions of DAG species on PKC activation are complex and may exert both inhibitory and stimulatory effects [75,76]. In dogs fed a fish oil diet, DAG containing n-3 PUFA were associated with a reduction in the expression of PKC- δ and PKC- ϵ isoforms [76]. Whether n-3 PUFA-containing DAG species are capable of effectively recruiting DAG-binding C1 domain-containing proteins and regulate polarization remains to be determined [77].

5.2. Calcium signalling and n-3 PUFA

In T-cells, sustained Ca^{2+} entry is necessary for complete and long-lasting activation of calcineurin/nuclear factor of activated T cells (NFAT) pathways. After TCR engagement, initially, calcium is released from intracellular pools, mainly the endoplasmic reticulum (ER), and extruded into the extracellular medium [78-79]. In turn, cells refill their intracellular emptied pool by opening calcium channels [79], termed store-operated (SOC) or Ca^{2+} -release activated- Ca^{2+} (CRAC) channels. A recent breakthrough in our understanding of CRAC channel function is the identification of STIM and ORAI, two essential regulators of CRAC channel function. A growing number of studies have emphasized that the Ca^{2+} /calcineurin/NFAT pathway is crucial for both development and function of all T-cell lineage cells, such

as conventional T cells (α,β + TCR), Foxp3+ regulatory T cells, and invariant natural killer T cells [80].

In Jurkat T-cells, n-3 PUFA induced an early increase in $[Ca^{2+}]_i$ [81]. A correlation was observed between both the carbon chain length and the number of double bonds with respect to the ability to mobilize cytosolic free $[Ca^{2+}]_i$. Interestingly, n-3 PUFA mobilized the release of Ca^{2+} from the inositol 1,4,5-trisphosphate-sensitive Ca^{2+} pool. We have also observed that DHA enhances calcium release from endoplasmic reticulum (ER), and the prolonged calcium response was contributed by the opening of CRAC channels in Jurkat T-cells [82-83]. The DHA-induced increase in $[Ca^{2+}]_i$ is implicated in the development of acidosis in Jurkat T-cells (Figure 1) [84]. Hence, DHA-induced acidification is brought about by its deprotonation and increase in $[Ca^{2+}]_i$ which activate the Ca^{2+}/H^+ ATPase, thereby driving Ca^{2+} out and H^+ into the cell [84]. It is tempting to speculate that perturbations in calcium homeostasis induced by n-3 PUFA, may also play a role in health and disease. For example, T-cells from spontaneously hypertensive rats (SHR) exhibit abnormal calcium signalling when calcium homeostasis is assessed by employing agents like ionomycin and thapsigargin. When SHR were fed an n-3 PUFA diet, we observed that these fatty acids exerted antihypertensive effects by modulating, in part, T-cell calcium signalling [85]. Along these lines, T-cell calcium signalling is altered in gestational diabetes and macrosomia. Hence, diets enriched with n-3 PUFA may partially restore T-cell calcium signalling in these two pathologies [86].

In general, in many studies, T-cells incubated with n-3 PUFA have been immediately assessed in a real-time mode, ranging from seconds to minutes. These observations on the increases in calcium signalling by n-3 PUFA demonstrate immediate actions; however, some investigators have incubated T-cells for 2 or 3 days or isolated cells from animals fed fish oils or n-3 PUFA enriched diets, and subsequently conducted experiments on calcium signalling, triggered by CD3/TCR. In these experiments, a decrease in CD3-induced calcium signalling was observed in these T-cells [87-88]. We have recently demonstrated that n-3 PUFA modulate T-cell activation by limiting mitochondrial translocation to the IS and reducing Ca^{2+} entry (unpublished results).

5.2.1. DAGs-containing n-3 PUFA—Transient receptor potential (TRP) channel proteins constitute a large and diverse family of proteins that are expressed in many tissues and cell types [89]. Activation by a PLC-dependent pathway characterizes members of the canonical TRP (i.e., TRPC) family, designated TRPC1 through TRPC7. The TRPC3/6/7 channels were shown to be directly activated by DAGs [90]. Gamberucci et al. [91] demonstrated that both Jurkat and human peripheral blood T-cells possess the TRPC6 protein, detected by Western blotting in a purified plasma membrane fraction. We have also detected mRNA encoding TRPC3/6 channels in Jurkat T-cells. In Jurkat T-cells, the expression of TRPC3 and TRPC6 mRNA is maximal during the G1 phase of the cell cycle (unpublished results). In order to elucidate the role of TRPC6 channels, we ectopically expressed TRPC6 in HEK cells and assessed the role of different molecular species of DAG with respect to calcium signalling [92]. We further synthesized SAG and SDG and assessed their role in the modulation of TRPC6 channels. We observed that SDG and 1,2-dioctanoyl-sn-glycerol (DOG), a DAG analogue, evoked a mutated increase in $[Ca^{2+}]_i$ via TRPC6 channels, compared to SAG. However, activation of TRPC6 channels by all DAG molecular species (SAG, DOG and SDG) required Src kinases [92]. Moreover, the integrity of lipid rafts was required for the optimal action of SAG, DOG and SDG. Further studies are needed to document the interaction of different molecular species of DAG with the opening of TRPC3 and TRPC6 channels in human T-cells.

5.3. MAPK cascade and n-3 PUFA

In T-cells, the small GTPase Ras has been shown to be activated in response to stimulation via TCR [93]. GTPase Ras plays a central role in coupling signalling to a number of different transduction pathways including the three major mitogen-activated protein kinase (MAPK) cascades. The activity of these MAPK, e.g., extracellular signal-related kinase (ERK), the Jun N-terminal kinase (JNK) and p38, is regulated through distinct, hierarchically organized modules of kinases [94]. Effectors for Ras include the serine/threonine kinase Raf-1, which via MEK activates ERK, and the GTPase Rac, which via different MKKs activates JNK and p38.

It has been observed in a number of different cell lines, that n-6 PUFA activate MAP kinase phosphorylation [95-97]. In vascular smooth muscle cells, AA enhanced MAP kinase activation via the production of 15-HETE [96]. In monocyte cell lines, AA up-regulated the formation of prostaglandins via a MAP kinase pathway [97]. In renal proximal epithelial cells, the same n-6 fatty acid induced tyrosine phosphorylation and MAP kinase activation via association with the adapter proteins Shc/Grb2/Sos [98]. In contrast, n-3 PUFA were found to diminish ERK1/2 phosphorylation in Jurkat T- cells [70,99] and 3T3 fibroblasts [71]. In these studies, n-3 PUFA exerted their action via PKC, which was directly coupled to MAPK phosphorylation [70-71,99]. Furthermore, in human peripheral T-cells, the phosphorylation of ERK1/2 induced by IL-2 was decreased (by 83 and 27%) by DHA and EPA treatment, respectively [100]. Several other reports have also confirmed the inhibitory effects of n-3 PUFA on ERK1/2 in endothelial cells [101], renal cells [102], dermal fibroblasts [103], macrophages [104] and rat smooth muscle cells [105]. In foxp3-positive Treg cells, DHA was observed to diminish, in a dose-dependent manner, the capacity of these cells to inhibit T effector cell proliferation [106]. In these studies, DHA exerted its action, in part, by inhibiting ERK1/2 and Akt phosphorylation [106]. Thus, n-3 PUFA inhibit MAPK activation and, therefore, modulate T-cell function.

5.3.1. DAGs-containing with n-3 PUFA—In T-cells, Ras can be activated by two Ras exchange factors, SOS and RasGRP, which are recruited through the adapters Grb2 and LAT, and via DAG, respectively. MAP kinase phosphorylation patterns induced by active Ras can vary and contribute to distinct cellular responses [107]. DAG also recruits and activates PKC-?, thereby turning on the ERK1/2 pathway. RasGRP relies on its DAG-binding domain to selectively activate ERK1/2. The activity of PKC-? depends on RasGRP sufficiency to effectively trigger downstream signals [108]. DAG-PKC-RasGRP-driven Ras-ERK1/2 activation in T-cells represents a unique signalling module [109]. RasGRP belongs to GEFs and possesses a pair of atypical EF-hands (a calcium-binding motif), and the C1 domain, which represents a signature motif that is involved in the recognition of DAG [108,110]. We have shown that RasGRP exhibits, in a cell free system, the ability to differentially bind SAG, SEG, and SDG [74]. SDG (K_i , $8.37 \pm 1.02 \mu\text{M}$) appears to be less potent than SAG (K_i , $4.49 \pm 0.01 \mu\text{M}$) and SEG (K_i , $4.97 \pm 1.04 \mu\text{M}$) as far as the activation of RasGRP is concerned. Interestingly, incubation of T-cells with different fatty acids resulted in the production of n-3 PUFA-enriched DAG upon activation. Since RasGRP possesses a DAG-binding domain and DAG/n-3 PUFA inhibited the action of RasGRP with respect to activation of MAP kinase, we have hypothesized that T-cell activation can be modulated by altering the molecular species composition of DAG.

6. Lipid rafts: modulation by n-3 PUFA

Most cellular functions are highly dependent on the membrane lipid environment [111]. Over the past decade, cholesterol and sphingolipid enriched “rafts” in the “sea” of plasma membrane have been identified as lipid rafts [112]. They were first defined as detergent-resistant membrane microdomains (DRM) or liquid ordered (l_o) subdomains, owing to their insolubility

in cold nonionic detergents or gel-like properties compared to liquid disordered (l_d) “bulk” membrane, respectively. Despite ongoing debate regarding the existence and experimental isolation of these domains [113-115], a growing body of data indicates that these microdomains function in a variety of cellular events including intracellular signaling cascades and protein trafficking [112,116-118].

6.1. Experimental approaches for measuring rafts

A variety of experimental approaches to dissect the structure and/or function of lipid rafts have been developed. We describe these approaches below.

6.1.1. Biochemical isolation—Lipid rafts were first identified by insolubility in non-ionic detergents. After density gradient centrifugation, separate fractions, in which cholesterol and sphingolipids are highly enriched, were observed. Interestingly, signalling proteins pre-dominantly relocalize into these fractions following stimuli. However, artificial effects of detergents on the formation of cholesterol rich sub-domains are also reported [119], resulting in the need for direct visualization of lipid rafts in living cell membranes.

6.1.2. Lipid raft marker labelling—Ganglioside GM1, to which cholera toxin subunit B specifically binds, is widely used as a lipid raft marker. Burack et al. demonstrated the accumulation of GM1 into cSMAC following IS formation [120]. However, Hullin-Matsuda and Kobayashi demonstrated that sphingomyelin-rich domains were spatially and functionally distinct from the GM1 ganglioside-rich domains in Jurkat T-cells [121]. Given that both sphingomyelin and GM1, as well as cholesterol, are major “building blocks” of lipid rafts, this study imply that lipid rafts are heterogeneous. Therefore, the direct visualization of lipid rafts independent of a GM-1 specific marker is required.

6.1.3. FRET—Fluorescence resonance energy transfer (FRET) technology is based on the principle that a higher energy donor fluorophore is capable of transferring energy directly to a lower energy acceptor molecule. As a result, by imaging photo-activated donors and energy-transferred acceptors, specific co-clustering of donor and acceptor probes are evaluated. With respect to FRET application to investigate lipid raft formation in living cells, Chichili et al. utilized fluorophore fused to raft-associated proteins, i.e. Lck and Src, to visualize the spatial colocalization and energy transfer in lipid rafts [122]. Due to the limitation of energy transfer in a certain distance between donor and acceptor, the size of lipid rafts can be calculated; 70 nm in dimension or smaller in CHO cells [123]. Theoretically, FRET approaches can be utilized for any fluorophore-fused proteins to examine their colocalization with known raft marker proteins. However, these efforts are complicated by lipid raft heterogeneity issues.

6.1.4. Polarity sensitive probes—Owing to the liquid-ordered properties of lipid rafts, polarity sensitive probes are also utilized to visualize the degree of liquid-orderedness in the cell membrane [124]. For example, 6-propionyl-2-dimethylaminonaphthalene (prodan) [125] and its derivatives, 6-acyl-2-dimethylaminonaphthalene (laurdan) [126] and 6-dodecanoyl-2-[N-methyl-N-(carboxymethyl)amino]naphthalene (C-laurdan) [127] have been used. Gaus et al. demonstrated, using laurdan, the existence and functions of lipid rafts in macrophages [128]. With respect to the activation-induced condensation of the cell membrane in T-lymphocytes, they further demonstrated an increased liquid-orderedness of the plasma membrane at the immunological synapse, a site of T-lymphocyte activation [129]. Importantly, cholesterol depletion by methyl- β -cyclodextrin diminished the increase of liquid-orderedness, reaffirming that lipid rafts accumulate at the immunological synapse.

6.1.5. Lipid raft-associated protein labelling—Stauffer and Meyer demonstrated that green fluorescent protein (GFP)-tagged SH2 domains from Syk or PLC γ 1 colocalized with

GM1 in antigen-activated rat basophilic leukaemia cells [130]. Harder et al. demonstrated that glycosyl-phosphatidylinositol (GPI)-anchored proteins placental alkaline phosphatase (PLAP) and GM1 colocalized in BHK cells and Jurkat T-cells following cross-linking of each marker using antibodies and/or cholera toxin. Interestingly, cross-linking of GPI-anchored PLAP further accumulated src-like protein tyrosine kinase fyn, a putative lipid raft marker [131]. These data indicate that raft-specific proteins can be used as a marker of location and size of lipid rafts.

6.1.6. Membrane rip-offs—Hancock et al. developed a gold-particle electron microscopy based technique to determine the spatial localization of reporter proteins in the inner leaflet of the plasma membrane [132-133]. Briefly, these investigators generated plasma membrane sheets on electron microscopy grids with the inner leaflet facing up (rip-off). Following fixation and labelling with gold-conjugated antibodies, the localization of proteins are imaged to determine the coordinates of gold particles within a standard sized area. By statistical analysis using Ripley's univariate K-function analysis, the clusters of gold patterning were determined. Alternatively, transfected GFP-fused truncated H-Ras (tH, cholesterol-dependent) or K-Ras (tK, cholesterol-independent) as lipid rafts or bulk membrane markers, respectively, were utilized in the place of gold particles [134]. This approach has an advantage in that the location of membrane subdomain marker proteins in the inner leaflet of the cell membrane is directly visualized. However, a limitation is that low confluent cells can generate peeled-off membranes instead of separated ripped-off inner leaflets [132].

6.2. Modulation of immune synapse signalling by n-3 PUFA

Lipid rafts are enriched in the immunological synapse, the site of T-cell and antigen presenting cell (APC) contact for antigen recognition. This range of contact modes and distinct molecular arrangements serve as a platform for the migration of T-cell receptors, co-activating receptors and cell signaling mediators [135]. Of interest, the addition of fatty acids in culture or diet modulated the formation of the immunological synapses and targeting of signaling proteins into lipid rafts. Geyeregger et al. [136] demonstrated that purified PUFA, i.e. linoleic acid (LA, 18:2 n-6), alpha-linolenic acid (ALA, 18:3 n-3), arachidonic acid (AA, 20:4 n-6), EPA and DHA suppressed CD3-induced migration of Src family kinases, i.e. Lck and Fyn, into DRM compared to the saturated stearic acid (18:0) or monounsaturated oleic acid (18:1 n-9) treated control human Jurkat CD4⁺ T-cells. In contrast, the intracellular localization of ganglioside GM1 was not affected by PUFA treatments. Kim et al. also observed that, following IS formation by anti-CD3 (clone 145-2C11) expressing hybridoma cells, GM1 localization in CD4⁺ T-cells from endogenous n-3 PUFA producing *fat-1* transgenic mice was not altered, as compared to wild type control T-cells [137]. Since GM-1 is localized to the outer (exofacial) leaflet of the plasma membrane, these data imply that PUFA treatment exclusively altered the fatty acid composition in the inner leaflet (cytofacial) of the plasma membrane.

Fan et al. from our laboratory [69,138] demonstrated that a diet containing 4% FO suppresses the colocalization of PKC9 into GM-1 specific lipid rafts in mitogen-stimulated murine CD4⁺ T-cells. Kim et al. further demonstrated that n-3 PUFA down-modulated the migration and activation status of PKCθ and PLCγ-1, as assessed by immunostaining of pan- or phospho-proteins in fixed cells, respectively [137]. These data suggest that following the formation of an immunological synapse between CD4⁺ T-cells and antigen presenting cells, dietary n-3 PUFA alters the temporal location of signalling proteins at the immunological synapse by affecting the formation of lipid rafts.

6.3. Apparent contradictions of PUFA effects on lipid rafts

Data are lacking regarding how n-3 PUFA modulate lipid rafts per se due to the limitations of experimental approaches as discussed above and the dynamic formation/dissociation

properties of lipid rafts. To determine the effect of n-3 PUFA on the formation of lipid rafts, Kim et al. demonstrated that n-3 PUFA increased the formation of lipid rafts in endogenous n-3 PUFA enriched CD4⁺ T-cells [137]. Shaikh et al. also reported that lipid raft size was increased by DHA treatment in EL-4 B-cell cultures [52]. Collectively, these data are consistent with the findings of Wassall et al. [139] who demonstrated that the unique hairpin structure of DHA is incompatible with cholesterol and subsequently forms DHA-rich microdomains, which may explain the above observations. We have analyzed the effects of dietary lipids on cholesterol-dependent (GFP-tH) and cholesterol-independent (GFP-tK) nanoclusters within the inner plasma membrane leaflet of HCT-116 colonic epithelial cells. These results were compared with equivalent data obtained using cultured HeLa cells [140]. In both cases, PUFA significantly increased clustering within cholesterol-dependent (GFP-tH) nanoclusters [Figure 2]. The assay relies on reasonably good transfection efficiency of the nanocluster marker ($\geq 40\%$ of cells expressing the label) to allow a sufficient recovery of immuno-gold labeled plasma membranes for data analysis [141]. In contrast, in cell line experiments using human Jurkat CD4⁺ T-cells, EPA (50 μ M) incubation impaired lipid raft formation following co-culture with anti-CD3 coated beads as assessed by Laurdan labelling [142]. Along these lines, we recently analyzed the lipid raft formation in Laurdan labelled Jurkat cells at the IS following co-culture with superantigen Staphylococcal Enterotoxin E-pulsed human Raji B-cells. General polarization (GP) values at the IS were significantly suppressed in DHA (50 μ M) treated cells, as compared to AA treated control cells (Figure 3). These data suggest that malignant transformed Jurkat cell lines may be inherently different from primary T-cells with respect to specific plasma membrane properties, and therefore may not be a suitable model to probe fatty acid effects on lipid raft-dependent signalling. Undoubtedly, the complexity of experiments using different cell types, activation methodologies, and fatty acids doses, contribute to the apparent disparate results.

7. T-cell co-activation and n-3 PUFA

Increasing evidence in both murine models and humans suggest that the interaction of CD28/CTLA4 with their ligands B7.1 and B7.2, present on B-cells and antigen presenting cells, is the critical costimulatory pathway involved in the induction of maximal T-cell activation and the prevention of anergy. In addition, CD28/CTLA4 signalling also assures the termination of T-cell:APC conjugation and T-cell cycle.

PLC γ 1 and PLD along with c-jun terminal kinase (JNK) and p38/MAP kinase are activated via CD28 co-stimulation [144]. JNK is now considered to be a point of convergence for early (TCR signalling) and late (CD28 signalling) events. Co-stimulatory signals also phosphorylate several PTK, mediating early cascading events initiated via the TCR. CD28 signalling also regulates the activity of phosphatidylinositol (PI)-3 kinase, which synergizes with TCR-driven signals to activate JNK in human T-cells [143]. JNK and p38/MAPK are regulated by MEK, analogous to ERK1/ERK2 signalling. Similar to GTPase-p21ras, which is proximal to ERK1/ERK2 in TCR-triggered signalling, the p21ras-related GTPases, Rho and Rac, may be critical for the activation of JNK [144]. It has been demonstrated that n-3 PUFA are capable of inhibiting the phosphorylation of JNK in Jurkat T-cells [145]. In addition, we have shown that EPA may suppress CD4⁺ T-cell activation/proliferation by enhancing the down-regulatory receptor CTLA-4, while not altering the levels of CD28 [143]. However, the possibility that IL-10 may partly mediate the inhibitory action of n-3 PUFA, during co-stimulation of T-cells, has not been ruled out [146].

8. T-cell apoptosis and n-3 PUFA

Apoptosis, (programmed cell death), triggered either by extracellular signals or by chronic antigenic stimulation, is considered a normal component of T-cell development. Activation-

induced cell death (AICD) is a form of apoptosis resulting from chronic antigen stimulation and is responsible for the peripheral deletion of previously activated lymphocytes. This process plays an essential role in immunological tolerance as this is implicated in negative selection of T-cells in the thymus [147].

With respect to n-3 PUFA, feeding lupus-prone (NZB/NZW)F₁ (B/W) female mice a fish oil diet not only extended their life span and exerted anti-inflammatory actions but also induced apoptosis and prevented the accumulation of self-reactive immune cells in lymphoid organs [148]. n-3 PUFA-induced apoptosis was higher than that initiated by anti-CD3 and anti-Fas antibodies in mouse spleen T-cells. It was reported that these fatty acids exert their action, in part, by increasing the generation of lipid peroxides and elevating Fas-L expression along with decreasing Bcl-2 expression [148,149]. In Jurkat T-cells, DHA, but not n-6 PUFA, induced apoptosis, which was inhibited by tautomycin and cypermethrin, suggesting that DHA acts via a PP1/PP2B protein phosphatase-dependent manner [150]. In leukemic Ramos cells, EPA promoted apoptosis via the intrinsic pathway by increasing activity of caspase-3 and -9 [151].

In mice fed an n-3 PUFA diet, in vitro stimulation of T-cells revealed an enhancement of AICD only in cells expressing a Th1-like cytokine profile without influencing Th2 cells [39]. This observation is very interesting as it indicates that n-3 PUFA suppress Th1 phenotype, which may ultimately contribute to an elevation of Th2 cells. This could be beneficial with respect to autoimmune diseases. Consistent with this outcome, n-3 PUFA enhanced the polarization and deletion of proinflammatory Th1 cells, in part, by modulating plasma membrane microdomain fatty acid composition [40].

9. Conclusions and perspectives

Defining the molecular and cellular mechanisms which regulate T-cell homeostasis is the focus of intense research. While evidence for the existence of lipid rafts in the plasma membrane has provoked debate, new imaging approaches have started to define cell surface nanoscale organization. Recently, a number of investigators have documented the unique membrane altering properties of long chain n-3 PUFA. Data from these and other studies demonstrate that EPA and DHA are unique fatty acids, capable of altering basic properties of cell membranes, thereby modulating phospholipases and PTKs which are an integral part of lipid microdomains and immunological synapses. This, in turn, appears to modulate downstream cell signalling cascades (Figure 1).

PKCs are associated both the early (seconds to minutes) and late (hours to days) phases of cell proliferation [152]. In the early phase, PKC is activated via the PLC γ 1-mediated PIP₂ hydrolysis whereas in the late phase, the prolonged activation of PKC is contributed by PC hydrolysis, catalysed by PLD via the production of phosphatidic acid, PA [153]. PLD, in the late phase, may be activated by PKC which is dependent on PLC γ 1 activation during the early phase [154]. Since n-3 PUFA in T-cells are preferentially incorporated into PC, these fatty acids will give rise, by the action of PLD, to DAG-n-3 PUFA, which are capable of modulating PKC activation in the late phase of cell activation. We postulate that n-3 PUFA will modulate early events of T-cell activation when the organism is maintained on a diet containing these agents and stimulated via the TCR. Moreover, n-3 PUFA will also interfere with the late phase of cell activation, involving the action of PLD and, therefore, will interfere with the progression of inflammatory and autoimmune diseases. In both early and late events, PC might also be the target of PLA₂ which contains n-3 PUFA (Figure 1) and, thereby, induce the release of these fatty acids which will again modulate PKC activation and, consequently, disease progression. n-3 PUFA, while impacting the early or late events of T-cell signalling, also inhibit nuclear translocation of transcriptional factors. For example, CD3/CD28-induced activity of NF-AT was markedly reduced by n-3 PUFA treatment [145,155]. Furthermore, IL-2 promoter activity,

IL-2 and IL-13 mRNA levels, IL-2 secretion, and IL-2R alpha-chain expression were significantly diminished by PUFA treatment [145].

Although the mechanisms of n-3 PUFA action are still not fully defined in molecular terms, it is becoming increasingly clear that these long chain fatty acids alter T-cell membrane lipid microdomain properties, alter the spectrum of intracellular signaling, and modulate nuclear receptor activation, which collectively may explain their pleiotropic properties. Unfortunately, at present there are limited data to support the notion that n-3 PUFA ameliorate clinical symptoms in patients affected by diseases characterized by active inflammation. It is highly likely that genetic differences contribute to the variable response to n-3 PUFA supplementation, making it difficult to determine how best to use EPA/DHA in the prevention and/or treatment of chronic inflammatory and autoimmune diseases.

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References

1. Chapkin, RS. Reappraisal of the essential fatty acids. In: Chow, CK., editor. Fatty acids in foods and their health implications. 3rd. CRC Press; New York: 2008. p. 675-691.
2. Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med* 1990;323:645-655. [PubMed: 2166915]
3. Lands WE. Biosynthesis of prostaglandins. *Annu Rev Nutr* 1991;11:41-60. [PubMed: 1892707]
4. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006;83:1505S-1519S. [PubMed: 16841861]
5. Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc* 2002;61:345-358. [PubMed: 12296294]
6. Serhan CN. Systems approach to inflammation resolution: identification of novel anti-inflammatory and pro-resolving mediators. *J Thromb Haemost* 2009;7:44-48. [PubMed: 19630766]
7. Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie* 2009;91:791-795. [PubMed: 19455748]
8. Spector AA. Essentiality of fatty acids. *Lipids* 1999;34(Suppl):S1-3. [PubMed: 10419080]
9. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991;54:438-463. [PubMed: 1908631]
10. Kremer JM. Clinical studies of omega-3 fatty acid supplementation in patients who have rheumatoid arthritis. *Rheum Dis Clin North Am* 1991;17:391-402. [PubMed: 1862247]
11. Nielsen GL, Faarvang KL, Thomsen BS, Teglbjaerg KL, Jensen LT, Hansen TM, et al. The effects of dietary supplementation with n-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: a randomized, double blind trial. *Eur J Clin Invest* 1992;22:687-691. [PubMed: 1459173]
12. Skoldstam L, Borjesson O, Kjallman A, Seiving B, Akesson B. Effect of six months of fish oil supplementation in stable rheumatoid arthritis. A double-blind, controlled study. *Scand J Rheumatol* 1992;21:178-185. [PubMed: 1529284]
13. Geusens P, Wouters C, Nijs J, Jiang Y, Dequeker J. Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. A 12-month, double-blind, controlled study. *Arthritis Rheum* 1994;37:824-829. [PubMed: 8003055]
14. Calder PC. Session 3: Joint Nutrition Society and Irish Nutrition and Dietetic Institute Symposium on 'Nutrition and autoimmune disease' PUFA, inflammatory processes and rheumatoid arthritis. *Proc Nutr Soc* 2008;67:409-418. [PubMed: 18847518]
15. Galarraga B, Ho M, Youssef HM, Hill A, McMahon H, Hall C, et al. Cod liver oil (n-3 fatty acids) as a non-steroidal anti-inflammatory drug sparing agent in rheumatoid arthritis. *Rheumatology (Oxford)* 2008;47:665-669. [PubMed: 18362100]

16. Kremer JM. n-3 fatty acid supplements in rheumatoid arthritis. *Am J Clin Nutr* 2000;71:349S–351S. [PubMed: 10617995]
17. Dawczynski C, Schubert R, Hein G, Muller A, Eidner T, Vogelsang H, et al. Long-term moderate intervention with n-3 long-chain PUFA-supplemented dairy products: effects on pathophysiological biomarkers in patients with rheumatoid arthritis. *Br J Nutr* 2009;101:1517–1526. [PubMed: 19245735]
18. Yaqoob P, Calder P. Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cell Immunol* 1995;163:120–128. [PubMed: 7758122]
19. Endres S, Meydani SN, Ghorbani R, Schindler R, Dinarello CA. Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J Leukoc Biol* 1993;54:599–603. [PubMed: 8245713]
20. Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrill-Labrode A, et al. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J Nutr* 1991;121:547–555. [PubMed: 2007907]
21. Jolly CA, McMurray DN, Chapkin RS. Effect of dietary n-3 fatty acids on interleukin-2 and interleukin-2 receptor alpha expression in activated murine lymphocytes. *Prostaglandins Leukot Essent Fatty Acids* 1998;58:289–293. [PubMed: 9654402]
22. Bittiner SB, Tucker WF, Cartwright I, Bleehe SS. A double-blind, randomised, placebo-controlled trial of fish oil in psoriasis. *Lancet* 1988;1:378–380. [PubMed: 2893189]
23. Bjorneboe A, Soyland E, Bjorneboe GE, Rajka G, Drevon CA. Effect of dietary supplementation with eicosapentaenoic acid in the treatment of atopic dermatitis. *Br J Dermatol* 1987;117:463–469. [PubMed: 2823859]
24. Bates D, Cartlidge NE, French JM, Jackson MJ, Nightingale S, Shaw DA, et al. A double-blind controlled trial of long chain n-3 polyunsaturated fatty acids in the treatment of multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1989;52:18–22. [PubMed: 2540285]
25. Lauer K. The risk of multiple sclerosis in the U.S.A. in relation to sociogeographic features: a factor-analytic study. *J Clin Epidemiol* 1994;47:43–48. [PubMed: 8283194]
26. Kampman MT, Wilsgaard T, Mellgren SI. Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle. *J Neurol* 2007;254:471–477. [PubMed: 17377831]
27. Schwarz S, Knorr C, Geiger H, Flachenecker P. Complementary and alternative medicine for multiple sclerosis. *Mult Scler* 2008;14:1113–1119. [PubMed: 18632773]
28. Cleland LG, James MJ, Proudman SM. The role of fish oils in the treatment of rheumatoid arthritis. *Drugs* 2003;63:845–853. [PubMed: 12678571]
29. Mehta LR, Dworkin RH, Schwid SR. Polyunsaturated fatty acids and their potential therapeutic role in multiple sclerosis. *Nat Clin Pract Neurol* 2009;5:82–92. [PubMed: 19194388]
30. Fetterman JW Jr, Zdanowicz MM. Therapeutic potential of n-3 polyunsaturated fatty acids in disease. *Am J Health Syst Pharm* 2009;66:1169–1179. [PubMed: 19535655]
31. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996;17:138–146. [PubMed: 8820272]
32. Liblau RS, Singer SM, McDevitt HO. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 1995;16:34–38. [PubMed: 7880387]
33. Arrington JL, Chapkin RS, Switzer KC, Morris JS, McMurray DN. Dietary n-3 polyunsaturated fatty acids modulate purified murine T-cell subset activation. *Clin Exp Immunol* 2001;125:499–507. [PubMed: 11531960]
34. Kleemann R, Scott FW, Worz-Pagenstert U, Nimal Ratnayake WM, Kolb H. Impact of dietary fat on Th1/Th2 cytokine gene expression in the pancreas and gut of diabetes-prone BB rats. *J Autoimmun* 1998;11:97–103. [PubMed: 9480727]
35. Wallace FA, Miles EA, Evans C, Stock TE, Yaqoob P, Calder PC. Dietary fatty acids influence the production of Th1- but not Th2-type cytokines. *J Leukoc Biol* 2001;69:449–457. [PubMed: 11261793]
36. Mizota T, Fujita-Kambara C, Matsuya N, Hamasaki S, Fukudome T, Goto H, et al. Effect of dietary fatty acid composition on Th1/Th2 polarization in lymphocytes. *JPEN J Parenter Enteral Nutr* 2009;33:390–396. [PubMed: 19221048]

37. Sierra S, Lara-Villoslada F, Comalada M, Olivares M, Xaus J. Dietary fish oil n-3 fatty acids increase regulatory cytokine production and exert anti-inflammatory effects in two murine models of inflammation. *Lipids* 2006;41:1115–1125. [PubMed: 17269557]
38. Zhang P, Smith R, Chapkin RS, McMurray DN. Dietary (n-3) polyunsaturated fatty acids modulate murine Th1/Th2 balance toward the Th2 pole by suppression of Th1 development. *J Nutr* 2005;135:1745–1751. [PubMed: 15987859]
39. Switzer KC, McMurray DN, Morris JS, Chapkin RS. (n-3) Polyunsaturated fatty acids promote activation-induced cell death in murine T lymphocytes. *J Nutr* 2003;133:496–503. [PubMed: 12566490]
40. Switzer KC, Fan YY, Wang N, McMurray DN, Chapkin RS. Dietary n-3 polyunsaturated fatty acids promote activation-induced cell death in Th1-polarized murine CD4⁺ T-cells. *J Lipid Res* 2004;45:1482–1492. [PubMed: 15145980]
41. Tsou SS, Chiu WC, Yeh CL, Hou YC, Yeh SL. Effects of omega-3 fatty acids on inflammatory mediators and splenocyte cytokine mRNA expressions in rats with polymicrobial sepsis. *Nutrition* 2008;24:484–491. [PubMed: 18329852]
42. Maes M, Mihaylova I, Kubera M, Bosmans E. Why fish oils may not always be adequate treatments for depression or other inflammatory illnesses: docosahexaenoic acid, an omega-3 polyunsaturated fatty acid, induces a Th-1-like immune response. *Neuro Endocrinol Lett* 2007;28:875–880. [PubMed: 18063921]
43. Matsuda A, Furukawa K, Takasaki H, Suzuki H, Kan H, Tsuruta H, et al. Preoperative oral immune-enhancing nutritional supplementation corrects TH1/TH2 imbalance in patients undergoing elective surgery for colorectal cancer. *Dis Colon Rectum* 2006;49:507–516. [PubMed: 16421661]
44. Lin MT, Yeh SL, Yeh CL, Lai YN, Chang KJ, Lee PH, et al. Parenteral n-3 fatty acids modulate inflammatory and immune response in rats undergoing total gastrectomy. *Shock* 2006;25:56–60. [PubMed: 16369187]
45. Zhang P, Kim W, Zhou L, Wang N, Ly L, McMurray M, Chapkin RS. Dietary fish oil inhibits antigen-specific Th1 cell development by suppression of clonal expansion. *Journal of Nutrition* 2006;136:2391–2398.
46. Ho IC, Glimcher LH. Transcription: tantalizing times for T cells. *Cell* 2002;109(Suppl):S109–120. [PubMed: 11983157]
47. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000;100:655–669. [PubMed: 10761931]
48. Yamashita M, Ukai-Tadenuma M, Miyamoto T, Sugaya K, Hosokawa H, Hasegawa A, et al. Essential role of GATA3 for the maintenance of type 2 helper T (Th2) cytokine production and chromatin remodeling at the Th2 cytokine gene loci. *J Biol Chem* 2004;279:26983–26990. [PubMed: 15087456]
49. Issemann I, Prince RA, Tugwood JD, Green S. The peroxisome proliferator-activated receptor:retinoid × receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs. *J Mol Endocrinol* 1993;11:37–47. [PubMed: 8240670]
50. Attakpa E, Hichami A, Simonin AM, Sanson EG, Dramane KL, Khan NA. Docosahexaenoic acid modulates the expression of T-bet and GATA-3 transcription factors, independently of PPARalpha, through suppression of MAP kinase activation. *Biochimie* 2009;91:1359–1365. [PubMed: 19799960]
51. Shaikh SR, Dumaual AC, Castillo A, LoCascio D, Siddiqui RA, Stillwell W, et al. Oleic and docosahexaenoic acid differentially phase separate from lipid raft molecules: a comparative NMR, DSC, AFM, and detergent extraction study. *Biophys J* 2004;87:1752–1766. [PubMed: 15345554]
52. Shaikh SR, Rockett BD, Salameh M, Carraway K. Docosahexaenoic acid modifies the clustering and size of lipid rafts and the lateral organization and surface expression of MHC class I of EL4 cells. *J Nutr* 2009;139:1632–1639. [PubMed: 19640970]
53. Denys A, Hichami A, Khan NA. n-3 PUFAs modulate T-cell activation via protein kinase C-alpha and -epsilon and the NF-kappaB signaling pathway. *J Lipid Res* 2005;46:752–758. [PubMed: 15627650]
54. Balboa MA, Balsinde J, Jones SS, Dennis EA. Identity between the Ca²⁺-independent phospholipase A(2) enzymes from P388D(1) macrophages and Chinese hamster ovary cells. *J Biol Chem* 1997;272:8576–8580. [PubMed: 9079688]

55. Le Gouvello S, Colard O, Theodorou I, Bismuth G, Tarantino N, Debre P. CD2 triggering stimulates a phospholipase A₂ activity beside the phospholipase C pathway in human T lymphocytes. *J Immunol* 1990;144:2359–2364. [PubMed: 1968928]
56. Boilard E, Surette ME. Anti-CD3 and concanavalin A-induced human T cell proliferation is associated with an increased rate of arachidonate-phospholipid remodeling. Lack of involvement of group IV and group VI phospholipase A₂ in remodeling and increased susceptibility of proliferating T cells to CoA-independent transacyclase inhibitor-induced apoptosis. *J Biol Chem* 2001;276:17568–17575. [PubMed: 11278296]
57. Roshak AK, Capper EA, Stevenson C, Eichman C, Marshall LA. Human calcium-independent phospholipase A₂ mediates lymphocyte proliferation. *J Biol Chem* 2000;275:35692–35698. [PubMed: 10964913]
58. Tessier C, Hichami A, Khan NA. Implication of three isoforms of PLA(2) in human T-cell proliferation. *FEBS Lett* 2002;520:111–116. [PubMed: 12044881]
59. Hichami A, Joshi B, Simonin AM, Khan NA. Role of three isoforms of phospholipase A₂ in capacitative calcium influx in human T-cells. *Eur J Biochem* 2002;269:5557–5563. [PubMed: 12423354]
60. Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. *Annu Rev Immunol* 2009;27:591–619. [PubMed: 19132916]
61. Acuto O, Di Bartolo V, Michel F. Tailoring T-cell receptor signals by proximal negative feedback mechanisms. *Nat Rev Immunol* 2008;8:699–712. [PubMed: 18728635]
62. Horejsi V, Zhang W, Schraven B. Transmembrane adaptor proteins: organizers of immunoreceptor signalling. *Nat Rev Immunol* 2004;4:603–616. [PubMed: 15286727]
63. Sieh M, Batzer A, Schlessinger J, Weiss A. GRB2 and phospholipase C-gamma 1 associate with a 36- to 38-kilodalton phosphotyrosine protein after T-cell receptor stimulation. *Mol Cell Biol* 1994;14:4435–4442. [PubMed: 7516467]
64. Rudd CE, Taylor A, Shneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. *Immunol Rev* 2009;229:12–26. [PubMed: 19426212]
65. Nishizuka Y. Discovery and prospect of protein kinase C research: epilogue. *J Biochem* 2003;133:155–158. [PubMed: 12761176]
66. Yoshida K, Asaoka Y, Nishizuka Y. Platelet activation by simultaneous actions of diacylglycerol and unsaturated fatty acids. *Proc Natl Acad Sci U S A* 1992;89:6443–6446. [PubMed: 1631141]
67. Shinomura T, Asaoka Y, Oka M, Yoshida K, Nishizuka Y. Synergistic action of diacylglycerol and unsaturated fatty acid for protein kinase C activation: its possible implications. *Proc Natl Acad Sci U S A* 1991;88:5149–5153. [PubMed: 1905018]
68. Speizer LA, Watson MJ, Brunton LL. Differential effects of omega-3 fish oils on protein kinase activities in vitro. *Am J Physiol* 1991;261:E109–114. [PubMed: 1858866]
69. Fan YY, Ly LH, Barhoumi R, McMurray DN, Chapkin RS. Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production. *J Immunol* 2004;173:6151–6160. [PubMed: 15528352]
70. Denys A, Hichami A, Khan NA. Eicosapentaenoic acid and docosahexaenoic acid modulate MAP kinase (ERK1/ERK2) signaling in human T cells. *J Lipid Res* 2001;42:2015–2020. [PubMed: 11734574]
71. Denys A, Hichami A, Maume B, Khan NA. Docosahexaenoic acid modulates phorbol ester-induced activation of extracellular signal-regulated kinases 1 and 2 in NIH/3T3 cells. *Lipids* 2001;36:813–818. [PubMed: 11592732]
72. Jolly CA, Jiang YH, Chapkin RS, McMurray DN. Dietary (n-3) polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion, and the formation of diacylglycerol and ceramide. *J Nutr* 1997;127:37–43. [PubMed: 9040541]
73. Bechoua S, Dubois M, Nemoz G, Lagarde M, Prigent AF. Docosahexaenoic acid lowers phosphatidate level in human activated lymphocytes despite phospholipase D activation. *J Lipid Res* 1998;39:873–883. [PubMed: 9555950]
74. Madani S, Hichami A, Cherkaoui-Malki M, Khan NA. Diacylglycerols containing Omega 3 and Omega 6 fatty acids bind to RasGRP and modulate MAP kinase activation. *J Biol Chem* 2004;279:1176–1183. [PubMed: 14583629]

75. Madani S, Hichami A, Legrand A, Belleville J, Khan NA. Implication of acyl chain of diacylglycerols in activation of different isoforms of protein kinase C. *FASEB J* 2001;15:2595–2601. [PubMed: 11726535]
76. Jude S, Martel E, Vincent F, Besson P, Couet C, Ogilvie GK, et al. Dietary long-chain n-3 fatty acids modify blood and cardiac phospholipids and reduce protein kinase-C-delta and protein kinase-C-epsilon translocation. *Br J Nutr* 2007;98:1143–1151. [PubMed: 17663802]
77. Quann EJ, Merino E, Furuta T, Huse M. Localized diacylglycerol drives the polarization of the microtubule-organizing center in T cells. *Nat Immunol* 2009;10:627–635. [PubMed: 19430478]
78. Oh-hora M, Rao A. Calcium signaling in lymphocytes. *Curr Opin Immunol* 2008;20:250–258. [PubMed: 18515054]
79. Putney JW Jr. Type 3 inositol 1,4,5-trisphosphate receptor and capacitative calcium entry. *Cell Calcium* 1997;21:257–261. [PubMed: 9105735]
80. Oh-hora M. Calcium signaling in the development and function of T-lineage cells. *Immunol Rev* 2009;231:210–224. [PubMed: 19754899]
81. Chow SC, Jondal M. Polyunsaturated free fatty acids stimulate an increase in cytosolic Ca^{2+} by mobilizing the inositol 1,4,5-trisphosphate-sensitive Ca^{2+} pool in T cells through a mechanism independent of phosphoinositide turnover. *J Biol Chem* 1990;265:902–907. [PubMed: 2153118]
82. Bonin A, Khan NA. Regulation of calcium signalling by docosahexaenoic acid in human T-cells. Implication of CRAC channels. *J Lipid Res* 2000;41:277–284. [PubMed: 10681412]
83. Denys A, Aires V, Hichami A, Khan NA. Thapsigargin-stimulated MAP kinase phosphorylation via CRAC channels and PLD activation: inhibitory action of docosahexaenoic acid. *FEBS Lett* 2004;564:177–182. [PubMed: 15094063]
84. Aires V, Hichami A, Moutairou K, Khan NA. Docosahexaenoic acid and other fatty acids induce a decrease in pHi in Jurkat T-cells. *Br J Pharmacol* 2003;140:1217–1226. [PubMed: 14645139]
85. Triboulot C, Hichami A, Denys A, Khan NA. Dietary (n-3) polyunsaturated fatty acids exert antihypertensive effects by modulating calcium signaling in T cells of rats. *J Nutr* 2001;131:2364–2369. [PubMed: 11533280]
86. Guermouche B, Yessoufou A, Soulimane N, Merzouk H, Moutairou K, Hichami A, et al. n-3 fatty acids modulate T-cell calcium signaling in obese macrosomic rats. *Obes Res* 2004;12:1744–1753. [PubMed: 15601968]
87. Zeyda M, Staffler G, Horejsi V, Waldhausl W, Stulnig TM. LAT displacement from lipid rafts as a molecular mechanism for the inhibition of T cell signaling by polyunsaturated fatty acids. *J Biol Chem* 2002;277:28418–28423. [PubMed: 12029091]
88. Stulnig TM, Berger M, Sigmund T, Raederstorff D, Stockinger H, Waldhausl W. Polyunsaturated fatty acids inhibit T cell signal transduction by modification of detergent-insoluble membrane domains. *J Cell Biol* 1998;143:637–644. [PubMed: 9813086]
89. Montell C. Drosophila TRP channels. *Pflugers Arch* 2005;451:19–28. [PubMed: 15952038]
90. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* 1999;397:259–263. [PubMed: 9930701]
91. Gamberucci A, Giurisato E, Pizzo P, Tassi M, Giunti R, McIntosh DP, et al. Diacylglycerol activates the influx of extracellular cations in T-lymphocytes independently of intracellular calcium-store depletion and possibly involving endogenous TRP6 gene products. *Biochem J* 2002;364:245–254. [PubMed: 11988098]
92. Aires V, Hichami A, Boulay G, Khan NA. Activation of TRPC6 calcium channels by diacylglycerol (DAG)-containing arachidonic acid: a comparative study with DAG-containing docosahexaenoic acid. *Biochimie* 2007;89:926–937. [PubMed: 17532549]
93. Cantrell DA. GTPases and T cell activation. *Immunol Rev* 2003;192:122–130. [PubMed: 12670400]
94. Robbins DJ, Zhen E, Cheng M, Xu S, Ebert D, Cobb MH. MAP kinases ERK1 and ERK2: pleiotropic enzymes in a ubiquitous signaling network. *Adv Cancer Res* 1994;63:93–116. [PubMed: 8036991]
95. Hii CS, Ferrante A, Edwards YS, Huang ZH, Hartfield PJ, Rathjen DA, et al. Activation of mitogen-activated protein kinase by arachidonic acid in rat liver epithelial WB cells by a protein kinase C-dependent mechanism. *J Biol Chem* 1995;270:4201–4204. [PubMed: 7876176]

96. Rao GN, Baas AS, Glasgow WC, Eling TE, Runge MS, Alexander RW. Activation of mitogen-activated protein kinases by arachidonic acid and its metabolites in vascular smooth muscle cells. *J Biol Chem* 1994;269:32586–32591. [PubMed: 7798262]
97. Dulin NO, Sorokin A, Douglas JG. Arachidonate-induced tyrosine phosphorylation of epidermal growth factor receptor and Shc-Grb2-Sos association. *Hypertension* 1998;32:1089–1093. [PubMed: 9856979]
98. Barry OP, Kazanietz MG, Pratico D, FitzGerald GA. Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. *J Biol Chem* 1999;274:7545–7556. [PubMed: 10066822]
99. Denys A, Hichami A, Khan NA. Eicosapentaenoic acid and docosahexaenoic acid modulate MAP kinase enzyme activity in human T-cells. *Mol Cell Biochem* 2002;232:143–148. [PubMed: 12030372]
100. Gorjao R, Hirabara SM, de Lima TM, Cury-Boaventura MF, Curi R. Regulation of interleukin-2 signaling by fatty acids in human lymphocytes. *J Lipid Res* 2007;48:2009–2019. [PubMed: 17592174]
101. Massaro M, Habib A, Lubrano L, Del Turco S, Lazzerini G, Bourcier T, et al. The omega-3 fatty acid docosahexaenoate attenuates endothelial cyclooxygenase-2 induction through both NADP(H) oxidase and PKC epsilon inhibition. *Proc Natl Acad Sci U S A* 2006;103:15184–15189. [PubMed: 17018645]
102. Hagiwara S, Makita Y, Gu L, Tanimoto M, Zhang M, Nakamura S, et al. Eicosapentaenoic acid ameliorates diabetic nephropathy of type 2 diabetic KKAY/Ta mice: involvement of MCP-1 suppression and decreased ERK1/2 and p38 phosphorylation. *Nephrol Dial Transplant* 2006;21:605–615. [PubMed: 16282336]
103. Kim HH, Shin CM, Park CH, Kim KH, Cho KH, Eun HC, et al. Eicosapentaenoic acid inhibits UV-induced MMP-1 expression in human dermal fibroblasts. *J Lipid Res* 2005;46:1712–1720. [PubMed: 15930517]
104. Lo CJ, Chiu KC, Fu M, Chu A, Helton S. Fish oil modulates macrophage P44/P42 mitogen-activated protein kinase activity induced by lipopolysaccharide. *JPEN J Parenter Enteral Nutr* 2000;24:159–163. [PubMed: 10850941]
105. Bousserouel S, Brouillet A, Bereziat G, Raymondjean M, Andreani M. Different effects of n-6 and n-3 polyunsaturated fatty acids on the activation of rat smooth muscle cells by interleukin-1 beta. *J Lipid Res* 2003;44:601–611. [PubMed: 12562859]
106. Yessoufou A, Ple A, Moutairou K, Hichami A, Khan NA. Docosahexaenoic acid reduces suppressive and migratory functions of CD4+CD25+ regulatory T-cells. *J Lipid Res* 2009;50:2377–2388. [PubMed: 19561360]
107. Zhang XF, Settleman J, Kyriakis JM, Takeuchi-Suzuki E, Elledge SJ, Marshall MS, et al. Normal and oncogenic p21ras proteins bind to the amino-terminal regulatory domain of c-Raf-1. *Nature* 1993;364:308–313. [PubMed: 8332187]
108. Ebinu JO, Bottorff DA, Chan EY, Stang SL, Dunn RJ, Stone JC. RasGRP, a Ras guanyl nucleotide-releasing protein with calcium- and diacylglycerol-binding motifs. *Science* 1998;280:1082–1086. [PubMed: 9582122]
109. Carrasco S, Merida I. Diacylglycerol-dependent binding recruits PKCtheta and RasGRP1 C1 domains to specific subcellular localizations in living T lymphocytes. *Mol Biol Cell* 2004;15:2932–2942. [PubMed: 15064353]
110. Lorenzo PS, Beheshti M, Pettit GR, Stone JC, Blumberg PM. The guanine nucleotide exchange factor RasGRP is a high-affinity target for diacylglycerol and phorbol esters. *Mol Pharmacol* 2000;57:840–846. [PubMed: 10779365]
111. Phillips R, Ursell T, Wiggins P, Sens P. Emerging roles for lipids in shaping membrane-protein function. *Nature* 2009;459:379–385. [PubMed: 19458714]
112. Pike LJ. Rafts defined: a report on the Keystone symposium on lipid rafts and cell function. *J Lipid Res* 2006;47:1597–1598. [PubMed: 16645198]
113. Shaw AS. Lipid rafts: now you see them, now you don't. *Nat Immunol* 2006;7:1139–1142. [PubMed: 17053798]

114. Heerklotz H. Triton promotes domain formation in lipid raft mixtures. *Biophys J* 2002;83:2693–2701. [PubMed: 12414701]
115. Kenworthy AK. Have we become overly reliant on lipid rafts? Talking Point on the involvement of lipid rafts in T-cell activation. *EMBO Rep* 2008;9:531–535. [PubMed: 18516088]
116. Jury EC, Flores-Borja F, Kabouridis PS. Lipid rafts in T cell signalling and disease. *Sem Cell & Devel Biol* 2007;18:608–615.
117. Hanzal-Bayer MF, Hancock JF. Lipid rafts and membrane traffic. *FEBS Letters* 2007;581:2098–2104. [PubMed: 17382322]
118. He HT, Marguet D. T-cell antigen receptor triggering and lipid rafts: a matter of space and time scales. Talking Point on the involvement of lipid rafts in T-cell activation. *EMBO Rep* 2008;9:525–530. [PubMed: 18516087]
119. Munro S. Lipid rafts: elusive or illusive? *Cell* 2003;115:377–388. [PubMed: 14622593]
120. Burack WR, Lee KH, Holdorf AD, Dustin ML, Shaw AS. Cutting edge: quantitative imaging of raft accumulation in the immunological synapse. *J Immunol* 2002;169:2837–2841. [PubMed: 12218094]
121. Hullin-Matsuda F, Kobayashi T. Monitoring the distribution and dynamics of signaling microdomains in living cells with lipid-specific probes. *Cell Mol Life Sci* 2007;64:2492–2504. [PubMed: 17876518]
122. Chichili GR, Rodgers W. Clustering of membrane raft proteins by the actin cytoskeleton. *J Biol Chem* 2007;282:36682–36691. [PubMed: 17947241]
123. Jacobson K, Dietrich C. Looking at lipid rafts? *Trends Cell Biol* 1999;9:87–91. [PubMed: 10201072]
124. Owen DM, Neil MA, French PM, Magee AI. Optical techniques for imaging membrane lipid microdomains in living cells. *Semin Cell Dev Biol* 2007;18:591–598. [PubMed: 17728161]
125. Weber G, Farris FJ. Synthesis and spectral properties of a hydrophobic fluorescent probe: 6-propionyl-2-(dimethylamino)naphthalene. *Biochemistry* 1979;18:3075–3078. [PubMed: 465454]
126. Gaus K, Zech T, Harder T. Visualizing membrane microdomains by Laurdan 2-photon microscopy. *Mol Membr Biol* 2006;23:41–48. [PubMed: 16611579]
127. Kim HM, Choo HJ, Jung SY, Ko YG, Park WH, Jeon SJ, et al. A two-photon fluorescent probe for lipid raft imaging: C-laurdan. *Chembiochem* 2007;8:553–559. [PubMed: 17300111]
128. Gaus K, Gratton E, Kable EP, Jones AS, Gelissen I, Kritharides L, et al. Visualizing lipid structure and raft domains in living cells with two-photon microscopy. *Proc Natl Acad Sci U S A* 2003;100:15554–15559. [PubMed: 14673117]
129. Gaus K, Chklovskaya E, Fazekas de St Groth B, Jessup W, Harder T. Condensation of the plasma membrane at the site of T lymphocyte activation. *J Cell Biol* 2005;171:121–131. [PubMed: 16203859]
130. Stauffer TP, Meyer T. Compartmentalized IgE receptor-mediated signal transduction in living cells. *J Cell Biol* 1997;139:1447–1454. [PubMed: 9396750]
131. Harder T, Scheiffele P, Verkade P, Simons K. Lipid domain structure of the plasma membrane revealed by patching of membrane components. *J Cell Biol* 1998;141:929–942. [PubMed: 9585412]
132. Hancock JF, Prior IA. Electron microscopic imaging of Ras signaling domains. *Methods* 2005;37:165–172. [PubMed: 16288888]
133. Prior IA, Parton RG, Hancock JF. Observing cell surface signaling domains using electron microscopy. *Sci STKE* 2003;2003:PL9. [PubMed: 12684529]
134. Prior IA, Harding IA, Yan J, Sluimer J, Parton RG, Hancock JF. GTP-dependent segregation of H-ras from lipid rafts is required for biological activity. *Nature Cell Biol* 2001;3:368–375. [PubMed: 11283610]
135. Friedl P, den Boer AT, Gunzer M. Tuning immune responses: diversity and adaptation of the immunological synapse. *Nat Rev Immunol* 2005;5:532–545. [PubMed: 15999094]
136. Geyerregger R, Zeyda M, Zlabinger GJ, Waldhausl W, Stulnig TM. Polyunsaturated fatty acids interfere with formation of the immunological synapse. *J Leukoc Biol* 2005;77:680–688. [PubMed: 15703198]
137. Kim W, Fan YY, Barhoumi R, Smith R, McMurray DN, Chapkin RS. n-3 polyunsaturated fatty acids suppress the localization and activation of signaling proteins at the immunological synapse

- in murine CD4⁺ T cells by affecting lipid raft formation. *J Immunol* 2008;181:6236–6243. [PubMed: 18941214]
138. Fan YY, McMurray DN, Ly LH, Chapkin RS. Dietary (n-3) polyunsaturated fatty acids remodel mouse T-cell lipid rafts. *J Nutr* 2003;133:1913–1920. [PubMed: 12771339]
 139. Wassall SR, Stillwell W. Docosahexaenoic acid domains: the ultimate non-raft membrane domain. *Chem Phys Lipids* 2008;153:57–63. [PubMed: 18343224]
 140. Chapkin RS, Wang N, Fan YY, Lupton JR, Prior IA. Docosahexaenoic acid alters the size and distribution of cell surface microdomains. *Biochim Biophys Acta (BBA) - Biomembranes* 2008;1778:466–471.
 141. Omerovic J, Prior IA. Compartmentalized signalling: Ras proteins and signalling nanoclusters. *FEBS J* 2009;276:1817–1825. [PubMed: 19243428]
 142. Zech T, Ejsing CS, Gaus K, de Wet B, Shevchenko A, Simons K, et al. Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signalling. *EMBO J* 2009;28:466–476. [PubMed: 19177148]
 143. Ly LH, Smith R, Switzer KC, Chapkin RS, McMurray DN. Dietary eicosapentaenoic acid modulates CTLA-4 expression in murine CD4⁺ T-cells. *Prostaglandins Leukot Essent Fatty Acids* 2006;74:29–37. [PubMed: 16221546]
 144. Cooper JA. MAP kinase pathways. Straight and narrow or tortuous and intersecting? *Curr Biol* 1994;4:1118–1121. [PubMed: 7704576]
 145. Zeyda M, Szekeres AB, Saemann MD, Geyeregger R, Stockinger H, Zlabinger GJ, et al. Suppression of T cell signaling by polyunsaturated fatty acids: selectivity in inhibition of mitogen-activated protein kinase and nuclear factor activation. *J Immunol* 2003;170:6033–6039. [PubMed: 12794131]
 146. Ly LH, Smith R 3rd, Chapkin RS, McMurray DN. Dietary n-3 polyunsaturated fatty acids suppress splenic CD4(+) T cell function in interleukin (IL)-10(-/-) mice. *Clin Exp Immunol* 2005;139:202–209. [PubMed: 15654818]
 147. Green DR, Droin N, Pinkoski M. Activation-induced cell death in T cells. *Immunol Rev* 2003;193:70–81. [PubMed: 12752672]
 148. Fernandes G, Chandrasekar B, Luan X, Troyer DA. Modulation of antioxidant enzymes and programmed cell death by n-3 fatty acids. *Lipids* 1996;31(Suppl):S91–96. [PubMed: 8729101]
 149. Avula CP, Zaman AK, Lawrence R, Fernandes G. Induction of apoptosis and apoptotic mediators in Balb/C splenic lymphocytes by dietary n-3 and n-6 fatty acids. *Lipids* 1999;34:921–927. [PubMed: 10574656]
 150. Siddiqui RA, Jensi LJ, Neff K, Harvey K, Kovacs RJ, Stillwell W. Docosahexaenoic acid induces apoptosis in Jurkat cells by a protein phosphatase-mediated process. *Biochim Biophys Acta* 2001;1499:265–275. [PubMed: 11341974]
 151. Heimli H, Giske C, Naderi S, Drevon CA, Hollung K. Eicosapentaenoic acid promotes apoptosis in Ramos cells via activation of caspase-3 and -9. *Lipids* 2002;37:797–802. [PubMed: 12371751]
 152. Imboden J, Weyand C, Goronzy J. Antigen recognition by a human T cell clone leads to increases in inositol trisphosphate. *J Immunol* 1987;138:1322–1324. [PubMed: 3492548]
 153. Billah MM, Anthes JC. The regulation and cellular functions of phosphatidylcholine hydrolysis. *Biochem J* 1990;269:281–291. [PubMed: 2201284]
 154. Khan NA, Hichami A. Ionotrophic 5-hydroxytryptamine type 3 receptor activates the protein kinase C-dependent phospholipase D pathway in human T-cells. *Biochem J* 1999;344(Pt 1):199–204. [PubMed: 10548551]
 155. Kim W, Fan Y, Smith R, Patil B, Jayaprakash GK, McMurray DN, Chapkin RS. Dietary curcumin and limonin suppress T-cell proliferation and interleukin-2 production in mice. *J Nutr* 2009;139:1042–1048. [PubMed: 19321585]

Abbreviations

AA	arachidonic (n-6) acid
AICD	activation-induced cell death

ALA	alpha-linolenic acid
APC	antigen-presenting cells
ARAM	antigen recognition activation motif
CD	cluster of differentiation
CRAC	Ca ²⁺ -release activated- Ca ²⁺ channels
CTLA	cytotoxic T-lymphocyte antigen
DAG	diacylglycerol
DHA	docosahexaenoic (n-3) acid
DOG	1,2-dioctanoyl-sn-glycerol
DRM	detergent-resistant membrane microdomains
EPA	eicosapentaenoic (n-3) acid
ER	endoplasmic reticulum
FO	fish oil
FRET	fluorescence resonance energy transfer
GFP	green fluorescent protein
GPI	glycosyl-phosphatidylinositol
HETE	hydroxyeicosatetraenoic acids
HPETE	hydroperoxyeicosatetraenoic acids
IBD	inflammatory bowel diseases
IL	interleukin
IS	immunological synapse
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibition motif
MAPK	mitogen-activated protein kinase
MS	multiple sclerosis
NFAT	nuclear factor of activated T-cells
NF-κB	nuclear factor-kappa B
NK	natural killer
NSAID	non-steroidal anti-inflammatory drugs
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PIP ₂	phosphatidylinositol 4,5-bisphosphate
PKC	protein kinase C
PL	phospholipids
PLA	phospholipase A

PLC	phospholipase C
PLD	phospholipase D
PLAP	placental alkaline phosphatase
PS	phosphatidylserine
PTK	protein tyrosine kinase
PUFA	polyunsaturated fatty acids
RA	rheumatoid arthritis
SAG	1-stearoyl-2-arachidonyl-sn-glycerol
SDG	1-stearoyl-2-docosaheptaenoyl-sn-glycerol
SEG	1-stearoyl-2-eicosapentaenoyl-sn-glycerol
SHR	spontaneously hypertensive rats
SMAC	supra molecular activation clusters
SOC	store-operated Ca^{2+} channels
TCR	T-cell receptor
Th	T helper cells
TNF	tumor necrosis factor
TPN	total parenteral nutrition
TRP	transient receptor potential
ZAP	zeta-chain-associated protein kinase

Figure 1. Modulatory effects of n-3 PUFA on T-cell signalling

n-3 PUFA suppress T-cell activation by modulating lipid rafts at the immunological synapse. This is linked to the displacement of PTKs and diminishment of their phosphorylation/activation status. Alternatively, “free” n-3 PUFA are released from n-3 PUFA-enriched phospholipid domains by the action of n-3 PUFA-specific PLA₂ and, consequently, modulate PKC and pHi. n-3 PUFA mobilize Ca²⁺ from the intracellular pool (i.e., endoplasmic reticulum), which perturbs [Ca²⁺]_i, followed by the opening of CRAC/SOC channels. n-3 PUFA also modulate the translocation of PKC isoforms, and alter the phosphorylation of MAPK, thereby inhibiting translocation of transcription factors (e.g., NF-kB, NF-AT, etc.) to the nucleus. n-3 PUFA, by the action of PLD, give rise to DAG-containing n-3 PUFA (DAG-DHA/EPA) which, in turn, 1) evoke increases in [Ca²⁺]_i via TRPC channels, 2) modulate RasGRP, and/or 3) modulate PKC isoforms. Collectively, n-3 PUFA alter gene transcription, leading to immunosuppression. Dashed lines indicate the action of n-3 PUFA.

Figure 2. Dietary PUFA alter inner-leaflet raft (tH) and non-raft (tK) markers in HCT-116 cells
 Ripley's K-function analysis of immuno-gold co-ordinates enables the extent of clustering within a 2D point pattern to be determined. Positive deflection from $L(r)-r = 0$ indicates a tendency for clustering. All cultures were treated with OA (18:1n-9), LA (18:2n-6), AA (20:4n-6), EPA (20:5n-3) or DHA (22:6n-3) (50 μ M) for 96 h, control-untreated. (A) GFP-truncated H-ras (located exclusively to inner leaflet rafts); displays an increase in clustering with LA and AA pre-treatment. Refer to panel (D) for statistical differences between treatments. (B) GFP-truncated H-ras, comparative effects of EPA vs DHA treatment. (C) GFP-truncated K-ras (non-raft marker) exhibits a small decrease in cluster size (EPA) or total clustering. K-functions (8-16 lawns) are means ($n > 500$ particles) from 2 separate experiments. Difference between (D) inner-leaflet raft (tH) or (E) inner-leaflet non-raft (tK) treatments: Pairwise comparison plots. The red solid line is the mean difference between two $L(r)$ functions; the dashed green lines mark the 95% confidence interval. There exists a statistically significant difference between the two $L(r)$ functions at the 0.05 level ($p < 0.05$) when part of the solid black zero line is outside the two dashed green curves, e.g., panel (D), LA vs control and panel (E), OA vs EPA. X-axis indicates the radius r (nm) in which the $L(r)$ function is calculated. Y-axis indicates the difference between the two $L(r)$ functions.

Figure 3. Assessment of lipid raft formation at the immunological synapse using a T-cell culture system

Human Jurkat T-cell and superantigen Staphylococcal Enterotoxin E (SEE)-pulsed Raji B-cell co-cultures were assessed by laurdan labeling and generalized polarization (GP)-values. Jurkat T-cells were pre-treated with 50 μ M DHA (n-3 PUFA) or AA (n-6, control) for 72 h. Culture medium was changed every 24 h with fresh fatty acid-BSA complex to avoid lipid peroxidation and consequent oxidative stress. (A) Representative two-photon microscopy RGB image of laurdan labelled Jurkat T-cells and red cell tracker labelled Raji B-cells. Three different regions of interest, i.e., (a) the immunological synapse, (b) contact whole T-cell, or (c) non-contact whole T-cell were drawn to calculate (B) GP-values for lipid raft formation assessment over time (10-30 min co-culture period). Detailed protocols for laurdan labelling, two-photon microscopic imaging, and calculation of GP-values at the regions of interest have been previously described (140). *Significant difference between AA vs DHA at the immunological synapse ($P < 0.05$).