Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation[1–3]

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ABSTRACT

Dietary supplementation with polyunsaturated fatty acids (PUFAs), especially those of the n−3 class, has immunosuppressive effects on both innate and adaptive immunity through various mechanisms. In this review, we focus on the PUFA modulation of membrane architecture and its consequent effects on both T cell responses and antigen presentation. We first use data from in vitro and in vivo experiments to make the case that the immunosuppressive effects of PUFAs begin with membrane incorporation and modulation of lipid-protein lateral organization. This in turn inhibits downstream signaling mediated by T cell receptors and suppresses T cell activation and proliferation. Next, we review evidence for PUFA-mediated alteration of major histocompatibility complex class I and II surface expression and antigen presentation. We propose that PUFAs influence the expression of major histocompatibility complex by altering its conformation, orientation, lateral organization, and trafficking, with consequences for recognition by effector T cells. Finally, we present data from model membrane studies to explain the physical principles that make PUFA acyl chains unique in modifying membrane lateral organization and protein function. An important concept to emerge from these studies is that PUFA acyl chains and cholesterol molecules are sterically incompatible. By applying this concept to the T cell activation and signaling model, mechanisms emerge by which PUFAs can modulate membrane lipid-protein lateral organization. Our data-based models show that membrane modification of both effectors and targets is an important, often overlooked, mechanism of immunomodulation by PUFAs. *Am J Clin Nutr* 2006;84:1277–89.

KEY WORDS Polyunsaturated fatty acids, antigen-presenting cells, T cells, immunosupression, cell membranes

INTRODUCTION

Dietary intakes of fats containing conjugated, long-chain polyunsaturated, oxidized, and *trans* acyl chains influence immune function (1, 2). Polyunsaturated fatty acids (PUFAs), in particular, are reported to have potential beneficial effects in a variety of autoimmune and inflammatory disorders. However, the molecular mechanisms that underlie these effects have remained elusive. In this review, we focus on how PUFAs can alter T cell activation and signaling by altering the lateral organization of the membrane bilayer. We also show how PUFAs can alter antigen presentation through the major histocompatibility complex (MHC) class I and II pathways through specific changes in the plasma membrane and endomembranes. Finally, from model membrane experiments, we present the physical principles that make PUFA acyl chains unique in their effects on membrane organization and function.

PUFA nomenclature and dietary sources

PUFAs are classified as n−3 or n−6 on the basis of the location of the last double bond relative to the terminal methyl (ω) end of the molecule (**Figure 1**). Some common PUFAs and their abbreviations are listed in **Table 1**. Intake of PUFAs results in uptake into essentially every cell of the body, where they bind fatty acid–binding proteins and undergo metabolic conversions (3). There are 2 PUFAs: linoleic acid (LA, 18:2n−6) and α-linolenic (ALA, 18:3n−3), which cannot be synthesized de novo by human cells and, hence, are designated as essential fatty acids. These essential fatty acids undergo a series of saturation, desaturation, and elongations steps that can result in the formation of long-chain PUFAs. Common dietary sources of n−6 PUFAs are corn, safflower, soybean, and sunflower oils (4). LA is converted to arachidonic acid (AA, 20:4 n−6), which along with other PUFAs (including some n−3 fatty acids) can serve as the precursor for eicosanoid mediators of inflammation (prostaglandins, thromboxanes, and leukotrienes). ALA, which is obtained from green leafy vegetables, walnuts, and rapeseed and flaxseed oils, can be further elongated, albeit not very efficiently, to the long-chain eicosapentaenoic acid (EPA, 20:5n−3) (5). Most of the long chain n−3 PUFAs, EPA, and docosahexaenoic acid (DHA, 22:6n−3), are obtained directly from dietary intake of marine fish oils.

Immunomodulatory and immunosuppressive effects of PUFAs

It has become increasingly clear that PUFAs have immunomodulatory effects in cell culture and in vivo (6, 7). PUFAs modulate cytokine production, lymphocyte proliferation, surface molecule expression, phagocytosis, and apoptosis and inhibit natural killer (NK) cell activity (8–16). n−3 PUFAs are generally considered good for human health, whereas n−6

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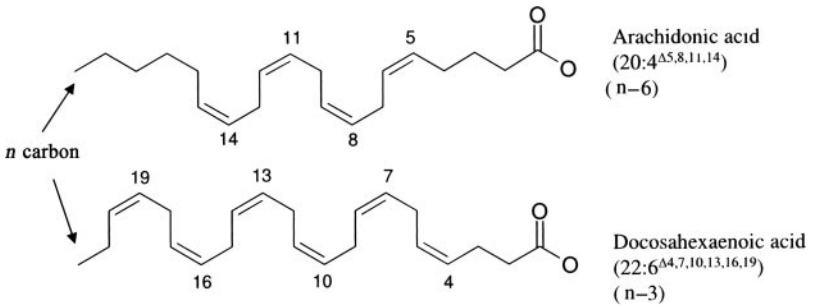

FIGURE 1. Classification of polyunsaturated fatty acids as n−3 or n−6 based on the location of the double bond nearest to the terminal methyl carbon. The numbers before and after the colon, respectively, designate the number of carbon atoms per chain and the number of double bonds in the fatty acid molecule.

PUFAs are less beneficial (4). Although the studies are not conclusive and there are conflicting reports (17), the n−3 PUFAs decrease specific disease symptoms and the need for antiinflammatory drugs for patients with chronic inflammatory diseases (6, 18). n−3 Fatty acids may be immunosuppressive agents for a variety of inflammatory ailments, including Crohn disease, atherosclerosis, colitis, graft-versus-host disease, rheumatoid arthritis, psoriasis, multiple sclerosis, asthma, and systemic lupus erythematosus (19–28). n−3 PUFAs are also thought to have protective effects in a variety of other afflictions, such as aging and depression, which have been associated with elevated concentrations of proinflammatory cytokines (6). Apart from inflammation, a tremendous focus has been placed on elucidating the anticancer properties of select PUFAs, although the very recent data question this possibility (29, 30).

Some of the best evidence for the clinical efficacy of n−3 PUFAs involves rheumatoid arthritis (31), which is characterized by chronic joint inflammation. There have been many clinical trials in which dietary supplementation with fish oil resulted in modest benefits for patients with rheumatoid arthritis (31). The general conclusion from 17 published trials, 2 meta-analyses, and a report from the Agency for Healthcare Research and Quality is that dietary fish-oil supplementation lowers the need for nonsteroidal antiinflammatory drugs and reduces morning stiffness and the number of tender joints; however, pain, swelling, and global assessments are unaffected by n−3 PUFA intake (32–34).

One major postulate is that long-chain n−3 PUFAs, on dietary supplementation, compete with n−6 PUFAs, mostly AA, for incorporation into cellular membranes, which results in a decrease in the production of proinflammatory eicosanoids and cytokines (7). Twenty-carbon n−3 PUFAs generally inhibit AA metabolism by serving as poor substrates for cyclooxygenase and lipoxygenase enzymes; this prevents immune cells from producing proinflammatory eicosanoids (7). Recent work by Serhan et al (28, 35, 36) has also identified novel eicosanoids generated from n−3 PUFAs, termed resolvins and protectins, that have antiinflammatory properties. The most potent n−3 antiinflammatory PUFAs are EPA and DHA. According to one estimate based on studies in humans, long-chain n−3 PUFAs are 9 times as potent as their precursor ALA (31); however, studies with ALA are limited and require further investigation. In addition, dietary supplementation with the n−6 PUFA γ-linolenic acid (GLA, 18:3n−6) leads to the n−6 elongation product, dihomo-γ-linolenic acid (DGLA, 20:3n−6), also found in evening primrose oil, which exerts some antiinflammatory effects (37). It is thought that DGLA acts as a competitive inhibitor of AA eicosanoid metabolism.

It is speculated by some researchers that the ratio of n−3 to n−6 may be an important factor in modulating inflammation and autoimmunity (38). Increasing the ratio of n−3 to n−6 relieves distress associated with some inflammatory diseases (27), although this may not hold true for all cases, especially with atopic diseases (39). According to one hypothesis, the change in the ratio of n−3 to n−6, due to increasing consumption of n−6 PUFAs in the Western diet, may be responsible for the increasing incidence of inflammatory diseases relative to populations where the n−6 intake is low and n−3 intake is high (eg, the Mediterranean diet) (4, 6). In the Western diet, the ratio of n−3 to n−6 is ≈1:20–30; in populations that consume fish-based diets, the ratio is ≈1:1–2 (4). One clinical solution for altering the dietary ratio of n−3 to n−6, or simply increasing the total n−3 content, has been supplementation with fish oil capsules, which are enriched in DHA and EPA. Although the molecular mechanisms by which fish-oil capsules exert their effects remain unclear, these capsules are readily available over the counter and are often advertised as a cure for a variety of ailments, especially for inflammatory disorders. There are drawbacks to using fish-oil supplements, including bad taste, compliance, gastrointestinal discomfort, and potential impairment of immune response to pathogens (2, 20, 40).

It is important to point out that there are some limitations to concluding that PUFAs, mostly of the n−3 series, exert immunosuppressive effects, as described in this review for T cell and antigen-presenting cell (APC) function. First, in vitro studies have mainly involved the feeding of cells single lipid species; this fails to mimic the combination of PUFAs acquired through the diet. Second, animal studies use physiologically relevant fish

TABLE 1
Polyunsaturated fatty acids (PUFAs) and their abbreviations

PUFA	Chain length:double bonds	PUFA class	Common abbreviation
Linoleic acid	$18:2^{\Delta 9,12}$	n−6	LA
α-Linolenic acid	$18:3^{\Delta 9,12,15}$	n−3	ALA
γ-Linolenic acid	$18:3^{\Delta 6,9,12}$	n−6	GLA
Dihomo-γ-linolenic acid	$20:3^{\Delta 8,11,14}$	n−6	DGLA
Arachidonic acid	$20:4^{\Delta 5,8,11,14}$	n−6	AA
Eicosapentaenoic acid	$20:5^{\Delta 5,8,11,14,17}$	n−3	EPA
Docosapentaenoic acid	$22:5^{\Delta 4,7,10,13,16}$	n−6	DPA
Docosahexaenoic acid	$22:6^{\Delta 4,7,10,13,16,19}$	n−3	DHA

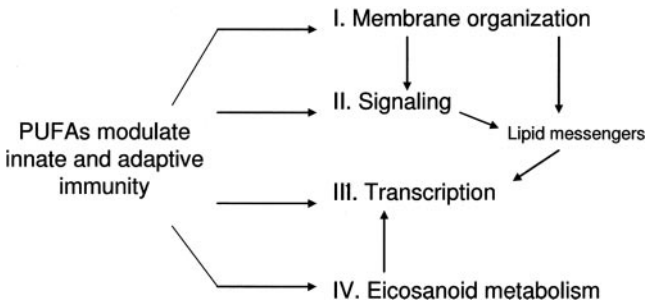

FIGURE 2. Four general mechanisms by which polyunsaturated fatty acids (PUFAs) can exert their effects on cellular function.

oils, but their effects are magnified when compared with control groups that received no PUFAs (34). Finally, it is difficult to assess the immunosuppressive effects of non-PUFA lipophilic compounds found in the purified diets of animals that may contribute to observed phenotypes. Despite these limitations, it has become increasingly accepted that PUFAs exert immunomodulatory effects in cell culture and in animal studies, which makes them potential therapeutic agents for human afflictions.

Molecular mechanisms by which PUFAs modulate immune function

To use PUFAs as adjuvant immunosuppressants, their molecular modes of action must be understood. There are 4 broad targets for PUFAs, none of which excludes the others (3): gene expression, cellular signaling, eicosanoid metabolism, and membrane organization (**Figure 2**) (7, 41–43). There is a reasonable understanding of how n−3 and n−6 PUFAs affect eicosanoid production and subsequent downstream effects, which have consequences for pro inflammatory and antiinflammatory responses, as described above (18). Extensive data exist that PUFAs alter gene expression, by either modifying signaling pathways that lead to changes in transcription or through direct interactions of PUFAs with nuclear receptors. The best characterized transcription factors, sterol regulatory element binding protein-1c and peroxisome proliferator activated receptor α, have emerged as the key mediators of gene regulation by PUFAs (41).

With the recent interest in the lipid raft hypothesis, the area of PUFA modification of membranes and consequent changes in cellular signaling are emerging as a new frontier of research (44). Historically, most of the research on lipids and their role in membrane structure and immune function focused on changes in membrane fluidity (45). As the literature for membrane lipid domains, such as rafts, has expanded, the attention has shifted to understanding how PUFAs may modulate immune function through an alteration or disruption in membrane microdomain organization (46).

PUFAs and membrane microdomains

On dietary intake, PUFAs are distributed to virtually every cell of the body (3). Although the n−6 content is relatively high, the n−3 PUFA content of the plasma membrane, in most tissues, is <5 mol percent. In tissues devoid of n−3 PUFAs, dietary intake of DHA, for instance, can increase the membrane content up to 8-fold (47). PUFAs are predominately esterified into the *sn*-2 position of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) phospholipids, although a notable exception to this rule is in neuronal tissues, where PUFAs are primarily esterified in phosphatidylserines (PSs) (48). It has been suggested that PUFA incorporation into membrane phospholipids would have an effect on the formation and stability of membrane microdomains (49).

Although various types of membrane domains have been characterized, the most recent and controversial have been membrane lipid raft microdomains (50, 51). Lipid rafts are defined as membrane microdomains that are enriched in (glyco)sphingolipids and cholesterol, which accumulate lipidated proteins and play a role in cellular signaling events, endocytosis, and trafficking (52). The operational definition of rafts varies depending on the method of detection or isolation. Also, rafts in vivo may be modulated or stabilized by receptor cross-linking (52, 53). The association of particular proteins with rafts has relied heavily on the idea that raft microdomains are detergent resistant at low temperatures and can therefore be isolated through sucrose density gradient fractionation. However, it is likely that the detergent itself induces the formation of large raft microdomains (54). We make note of this because much of the data reviewed below on PUFA modification of rafts and T cell signaling events is based on detergent extraction at low temperature. It must be kept in mind that the size, stability, and lifetime of rafts remain uncertain (52).

Membrane rafts require lipids with saturated acyl chains and cholesterol, which pack tightly to form a liquid ordered phase. The tight packing density of lipids in liquid ordered microdomains is conferred by favorable van der Waals forces between saturated acyl chains in addition to hydrogen bonding between neighboring sphingolipids and between the variable sphingolipid amide and the 3-OH of cholesterol. Unsaturated acyl chains, including PUFAs, do not pack well with cholesterol molecules and therefore form a liquid disordered phase (52). This is an important point that we will return to later in the review when we reconcile differences between the physical principles by which PUFAs interact with cholesterol molecules and data from cellular experiments (*see*"Effects of PUFAs on bilayers and proteins from model membrane studies" below).

PUFA MODULATION OF T CELL ACTIVATION AND SIGNALING THROUGH CHANGES IN MEMBRANE ORGANIZATION

Overview of interactions between T cells and APCs

T cells are a subset of lymphocytes that play a large role in the cell-mediated immune response of adaptive immunity. T lymphocytes are divided into 2 major classes distinguished by the expression of the cell surface molecules CD4 or CD8. $CD8^+$ cytotoxic T lymphocytes (CTLs) primarily destroy virus-infected cells, whereas $CD4^+$ helper T lymphocytes are involved in activating B cells and macrophages. The response of both classes of T lymphocytes is dependent on interactions through the T cell receptor (TCR) and coreceptors (CD4 or CD8) with target APCs that present peptides in the context of MHC class I or class II molecules. MHC class I and class II molecules are structurally similar but differ in their source of antigenic peptide and in the transport mechanisms for their peptides. Class I MHC molecules present self peptides or viral products from the cytosol to $CD8^+$ T lymphocytes, whereas MHC class II molecules

present peptides from pathogens that either reside or were endocytosed into intracellular compartments. T cells are activated on encountering antigen, which results in either lysis of target cells by $CD8^+$ T lymphocytes or recruitment of other effector cells by $CD4^+$ cells.

PUFA modulation of the plasma membrane alters the T cell Ca^{2+} response

A series of studies from Kleinfeld's laboratory (55) showed that unsaturated free fatty acids (FFAs) could inhibit specific aspects of cytotoxic T cell function by perturbing membranes. Initially, it was shown that short-term exposure of murine allogeneic effector T cells to low levels of unsaturated FFAs (<10 μmol/L), including PUFAs, inhibited lysis of target APCs. The change in lysis of target cells was a direct consequence of the FFA added to the CTLs, because lysis could be inhibited by extracting the unsaturated FFA with bovine serum albumin before CTL-target conjugation (55). Specific aspects of T cell function inhibited by unsaturated lipids included the initial rise in intracellular $[Ca^{2+}]$ on conjugate formation, protein phosphorylation events, and subsequent CTL esterase release. On the other hand, release of inositol phosphates and binding to target cells were unaffected (55–59). Because the inhibition in CTL calcium release linearly correlated with the decrease in membrane acyl chain order induced by the presence of increasing unsaturation in the plasma membrane (56), it was hypothesized that modulation of membrane structure affected T cell Ca^{2+} signaling.

Information on the effects of PUFAs on the T cell calcium response has continued to grow (60, 61). For example, Stulnig et al (61) showed that administration of PUFAs reduced the calcium response dose dependently in vitro on stimulation of Jurkat T lymphocytes. Although no change was observed with the saturated palmitic acid, maximal effects were observed with GLA and DGLA and modest effects with EPA and AA. These findings were clinically relevant since in vivo data from both animal and human studies have shown that GLA can suppress inflammation, swelling, and tissue damage (62).

Models by which PUFAs alter T cell signaling

One of the initial steps in T cell–mediated immunity is the engagement of the TCR with antigens presented by MHC molecules on the surface of APCs. Activation of the TCR and coreceptors initiates many intracellular signaling pathways (**Figure 3**). Inhibitory phosphate groups are removed from the TCR complex, which allows for the activation of the proteins Lck and Fyn. Phosphorylation of Lck and Fyn activates the cytosolic protein ZAP-70, which subsequently activates other adapter and linker proteins involved in initiating the signaling cascade. Several pathways are activated through G proteins and phospholipase C (PLC) γ, which eventually converge on the nucleus and modulate gene expression.

In the past few years, 2 models, which are not mutually exclusive, have emerged to explain specific changes in T cell signaling in response to feeding cells PUFAs. These models have grown from the correlation observed between the T cell calcium response and the displacement of specific proteins from rafts, defined as detergent-resistant membrane (DRM) fractions, on PUFA treatment (63, 64). According to the first model, EPA and AA directly modify T cell signaling proteins. Webb et al (65) showed that EPA and AA blocked localization of the src kinase

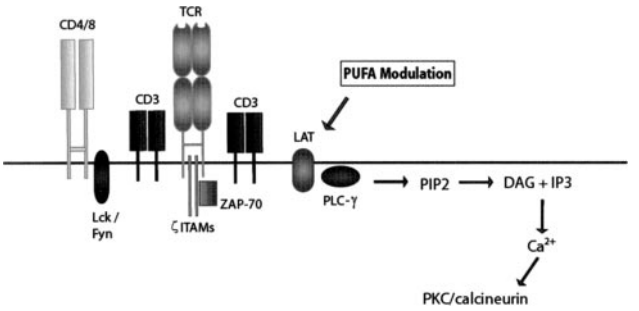

FIGURE 3. Simplified illustration of T cell signaling. Engagement of the T cell receptor (TCR) complex results in changes in phosphorylation, which allow for the activation of the proteins Lck and Fyn. Phosphorylation of Lck and Fyn activates the cytosolic protein ZAP-70, which subsequently activates other adapter and linker proteins such as linker of activation of T cells (LAT), which is involved in initiating the signaling cascade for activation of specific transcription factors. Proteins such as LAT are localized in lipid rafts that undergo changes in localization from detergent-resistant membrane to detergent-soluble membrane (DSM) fractions on enrichment with polyunsaturated fatty acids (PUFAs). Signaling events and downstream transcription factor activation are affected when PUFAs interact with raft molecules and inhibit LAT-induced activation of phospholipase C γ (PLC-γ). PUFAs may also affect other aspects of signaling as described in the text. Relative sizes of protein and lipid molecules are not drawn to scale. ITAM, immunoreceptor tyrosine-based activation motif; PIP2, phosphatidylinositol bisphosphate; DAG, diacylglycerol; PKC, protein kinase C; IP3, inositol triphosphate.

Fyn to DRM fractions by inhibiting its palmitoylation in COS-1 cells, which suggests that PUFAs could be acylated to src kinases. Indeed, a subsequent report by the same laboratory showed that cysteine residues on Fyn could be *S*-acylated with monounsaturated or polyunsaturated lipids; this not only displaced these molecules from DRM fractions but inhibited subsequent T cell signal transduction (66). These findings are consistent with previous work that showed that palmitoyl transferases are nonselective and can covalently attach PUFAs to proteins (67).

According to a second model, which has gained wider acceptance, PUFAs inhibit T cell signaling by displacing Lck, Fyn, and LAT from DRMs to nonraft detergent-soluble membranes (DSMs). Incubation of Jurkat T cells with EPA resulted in significant incorporation into lipids of the inner (PE-rich) and outer (sphingolipid, PC-rich) leaflets of the plasma membrane (68). Significant EPA enrichment was found in DRM fractions, which suggests that the addition of exogenous PUFA could make the raft fractions an unsaturated environment and cause changes in protein distribution between DRMs and DSMs. In fact, AA content was found to be higher in isolated DRM fractions than in bulk membranes, whereas the cholesterol content of these fractions was unaltered by PUFA treatment (68). However, it is important to point out that the molecular mechanisms by which PUFAs affect sphingolipids and cholesterol with subsequent changes in protein localization are unclear. As described above, raft formation and stability is based on favorable interactions between saturated acyl chains and cholesterol. Therefore, if DRM fractions are any representation of rafts, then the finding that PUFAs incorporate into these fractions is contrary to expectations from molecular studies of PUFAs and raft lipids in bilayer models. This discrepancy is discussed in detail in the final section of the review.

PUFA incorporation into DRMs and signaling events

Displacement of LAT from DRMs appeared to be one of the most important events that inhibited calcium signaling after PUFA treatment (Figure 3) (64). EPA treatment prevented phosphorylation of LAT and this was followed by a reduction in the phosphorylation of PLC, which is directly involved in elevating calcium levels on T cell activation. A genetically modified LAT (additional 16 amino acid extracellular domain), which was not displaced from DRMs on PUFA treatment, restored tyrosine phosphorylation of PLC-γ and the calcium response (64). On the other hand, PUFAs had no effect on Lck-mediated tyrosine phosphorylation of CD3ζ chains, binding of ZAP-70, and subsequent phosphorylation of ZAP-70. Further work from the same group showed downstream effects at the transcriptional level (69).

Additional evidence that direct modulation of DRMs by PUFAs inhibited T cell signaling came from a recent study that examined the effects of DHA on interleukin 2 receptor (IL-2R) signaling in Jurkat T cells (70). Binding of IL-2R by IL-2 phosphorylate Janus kinases, which activate signal transducer and activators of transcription (STAT) proteins. Activated STATs are then recruited to the nucleus, where they are involved in gene transcription. PUFA incorporation was found to significantly alter the fatty acyl composition of DRM fractions, which correlated with a reduction in surface expression of IL-2Rα and partial displacement of IL-2α, IL-2β, and IL-2γ_c to DSM fractions. Consistent with this, small amounts of STAT5a and STAT5b of the Janus kinase pathway found in DRM fractions in controls shifted to the nonraft DSM fractions on DHA incorporation. Changes in IL-2R signaling could account for reduced proliferation of T cells fed PUFAs.

PUFAs have also been shown to have effects on the CD3-induced phosphorylation of the nucleotide exchange factor (VAV), which controls cytoskeletal rearrangements that are critical for the formation of the immunologic synapse (IS). Treatment of T cells with EPA inhibited VAV phosphorylation, which resulted in an incomplete formation of the IS and defective APC–T cell conjugates (71). Cytoskeletal rearrangements depended on the distribution of LAT between DRM and DSM fractions, which suggests that PUFA modification of raft domains may control IS formation (71).

Finally, PUFA alteration of DRMs may have a role in the activation of phospholipase D (PLD), which can transmit antiproliferative signals (72). After feeding DHA to peripheral mononuclear blood cells, DHA was found to be significantly incorporated into DRM fractions. PLD shifted from DRM to DSM fraction and was activated by ADP-ribosylation factor (73).

PUFA modulation of T cell signaling in vivo

Some of the in vivo or ex vivo data on PUFAs and T cell function are consistent with findings from cell culture. For example, healthy persons infused with lipids to elevate serum PUFAs had a reduced calcium response of their $CD4^+$ and $CD8^+$ T cells (61). Also, feeding mice n−3 PUFAs, predominately DHA, lowered the proliferative response of splenic T lymphocytes on in vitro stimulation through CD28 and the TCR/CD3 complex (74). On the other hand, clinical trials have shown no effect of PUFA modulation on T lymphocyte proliferation (75, 76), perhaps because they are statistically underpowered and fail to account for genetic variability in the population (34).

Inner leaflet lipids (eg, PEs and PSs) isolated from mice fed diets rich in fish oils were enriched with PUFAs to a greater extent than were outer leaflet lipids (77, 78). Surprisingly, it was also observed that the sphingomyelin content of DRM fractions was significantly (≈30%) lower in fish oil–fed mice than in control corn oil–fed animals (77, 78). In the same study, recruitment of PKCθ into rafts of T cells from mice fed diets supplemented with fish oils or purified DHA was inhibited relative to cells isolated from mice fed corn oil. In addition, receptor-induced activation of the transcription factors AP-1 and nuclear transcription factor κB was lowered and, consequently, IL-2 production and T cell proliferation were inhibited in cells isolated from fish oil–fed mice (78). Fish oil–fed mice also show elevated concentrations of Fas colocalization with raft molecules in naive T cells (78). In light of data that showed that n−3 PUFAs enhanced activation induced cell death in T cells (79), it was speculated that Fas relocalization by PUFAs may be yet another mechanism by which n−3 PUFAs inhibit T cell activity (78, 80).

PUFA MODULATION OF MHC SURFACE EXPRESSION AND ANTIGEN PRESENTATION ACTIVITY

T cell signaling is driven by engagement of the TCR/CD3 complex by MHC molecules and associated peptide on APCs. Relative to what is known about PUFA modulation of T cell membranes, far less is known about how APC function may be altered by incorporation of PUFAs into membrane lipids. Many studies have shown that PUFA supplementation through the diet or in cell culture can result in changes in expression of surface proteins, including costimulators, adhesion molecules, and MHC glycoproteins (81). It is beyond the scope of this review to cover all of the surface molecules affected by PUFAs; rather, we focus on MHC class I and II molecules (**Table 2**) (10, 82–90). An understanding of how PUFAs modulate surface expression of MHC molecules has physiologic relevance because some immune disorders are characterized by overactive immune responses that include overexpression of MHC class I or class II molecules (91, 92).

Some data on how PUFAs modulate the surface expression of MHC class I molecules are available (Table 2). Fusion of PC lipid vesicles containing DHA (DHA-PC), esterified to the *sn*-2 acyl chain position, affected the expression levels of murine MHC class I (83). An important finding of this work was that the expression of one MHC class I epitope increased while the expression of another epitope decreased, which suggested a change in conformation of class I in response to changes in bilayer organization. This hypothesis was further substantiated by reconstitution studies in which the greatest binding of a monoclonal antibody, which detected conformationally sensitive epitopes of H-$2K^b$ molecules, was in MHC proteoliposomes containing DHA phospholipids (93). The change in the H-$2K^b$ epitope may have functional consequences. Mouse T27A tumor cells modified by DHA-PCs were more sensitive to CTLs than were controls (94). However, a link between MHC class I conformation and increase in APC lysis on PUFA modification was not established in these studies. On the other hand, our laboratory has found that incubation of B cells with PUFAs complexed to bovine serum albumin lowers MHC class I surface levels independent of changes in conformation. In addition, we found that AA and DHA treatment of B cells equally lowers susceptibility

TABLE 2
Polyunsaturated fatty acid (PUFA) modulation of major histocompatibility complex (MHC) class I and II surface expression[1]

Treatment	Model system	Effect	Reference
MHC class I			
Cholesteryl hemisuccinate or egg PC (contains unsaturated acyl chains)	BALB/c spleen cells	Reduced expression of H-2L, H-2K, and H-2D with cholesterol hemisuccinate and increased expression with egg PC treatment	(82)
AA, DHA	JY B lymphoblasts	Decreased HLA-I expression	Shaikh and Edidin (in press)
Phospholipids containing DHA	EL4 lymphomas	Increased expression of one class I epitope, decreased expression of another class I epitope	(83)
MHC class II			
DHA	Peritoneal exudate macrophages	Decreased MHC class II expression	(84)
DHA	Murine dendritic cells	Inhibited LPS-induced upregulation of MHC class II molecules	(85)
DHA, AA, EPA	Murine macrophages	Decreased IFN-γ–induced Ia expression	(86)
AA, EPA	Human dendritic cells	Unaffected HLA-DR expression	(87)
EPA, DHA	Unstimulated monocytes	EPA inhibited HLA-DR expression, DHA increased HLA-DR expression	(10)
EPA, DHA	IFN-γ–stimulated monocytes	Decreased HLA-DR expression	(10)
EPA:DHA (3:2)	IFN-γ–stimulated monocytes	Decreased HLA-DR expression	(88)
Fish oil	Rat dendritic cells	Decreased MHC II expression	(89)
Fish oil	Stimulated exudate macrophages	Increased MHC II expression	(90)

[1] PC, phosphatidylcholine; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IFN, interferon; LPS, lipopolysaccharide.

to T cell lysis (SR Shaikh and M Edidin, unpublished observations, 2006).

Although T cells receive activating signals on recognition of antigen presented on MHC molecules, natural killer (NK) cells receive inhibitory signals through killer inhibitory receptors on recognition of MHC molecules. Yoo et al (95) showed that modulation of rat hepatoma cells with either oleic or linoleic acid resulted in increased susceptibility of these cells to NK cell–mediated cytotoxicity. On the other hand, another group found that lipid modulation of target cells with egg PC, which contains a mixture of PUFAs, caused a small increase in their lysis by NK cells, although the increase was not statistically significant (96). Clearly, further work is required to understand whether PUFA modification of APCs can alter susceptibility to T cell– or NK cell–mediated lysis.

Considerable data are available on PUFA modulation of MHC class II surface expression in vitro and in vivo (Table 2). In general, cells cultured with PUFAs, notably n−3 fatty acids, show a reduction in class II surface expression and adhesion molecule expression (84, 86, 88). Histocompatibility antigen (HLA) DR (HLA-DR) and intracellular adhesion molecule 1 (ICAM-1) surface expression in monocytes was lowered by EPA, whereas HLA-DR and HLA-DP expression increased by DHA (10). Treatment of interferon γ (IFN-γ)–stimulated monocytes with EPA and DHA for 48 h at a ratio of 3:2, mimicking the composition of fish-oil capsules, reduced the surface expression levels of HLA-DR, HLA-DP, and ICAM-1. Associated with this was a significant reduction in the ability of IFN-γ–stimulated monocytes treated with EPA and DHA at a ratio of 3:2 to present tetanus toxoid antigen to autologous lymphocytes (3:2) (88). In another study, treatment with 100 μmol DHA/L for 24 h lowered MHC class II expression in macrophages, and smaller, but significant, changes were observed with EPA and AA (86). This study suggests a molecular mechanism by which DHA reduced expression. The number of messenger RNA transcripts of Iaβ^b molecules was lower in DHA-treated cells than in controls, independent of cycloxygenase metabolism (86). Otherwise, the mechanism by which PUFAs modulate MHC class II expression has remained elusive (97).

Dietary fish-oil supplementation for 21 d in humans resulted in a significant decrease in the surface expression of MHC class II molecules (HLA-DP, HLA-DQ, and HLA-DR) and ICAM-1 and of LFA-1 expression in peripheral blood monocytes (PBMs), whereas no change in the number of cells expressing these molecules was observed (88). In the same study, ex vivo stimulation of PUFA-treated PBMs with IFN-γ lowered HLA-DR and HLA-DP expression and the number of cells expressing MHC class II molecules (98). Similar results were observed in mice fed diets supplemented with n−3 PUFAs. In these animals, MHC class II expression on peritoneal cells (predominately B cells and macrophages) was lowered by 40% relative to animals fed a saturated-, monounsaturated-, or n−6–rich diet (99). Dendritic cells (DCs) isolated from rats fed an n−3–rich diet similarly showed a reduction in MHC class II expression; CD2, CD18, and CD11a decreased as well (89). These DCs did not present keyhole limpet hemocyanin (KLH) antigen to KLH-sensitized responder spleen cells as effectively as did controls; this finding is consistent with a previous report that spleen cells from mice fed EPA-rich diets had diminished the ability to present KLH to T cell clones (100); however, the reduction in MHC class II expression of DCs was thought to be too small to account for the reduction in antigen presentation activity (89). We found that changes in MHC class I surface expression levels on PUFA modification are too low to account for changes in antigen presentation through the MHC class I pathway (SR Shaikh and M Edidin, unpublished observations, 2006). Perhaps concomitant changes in MHC, costimulatory, and adhesion protein surface

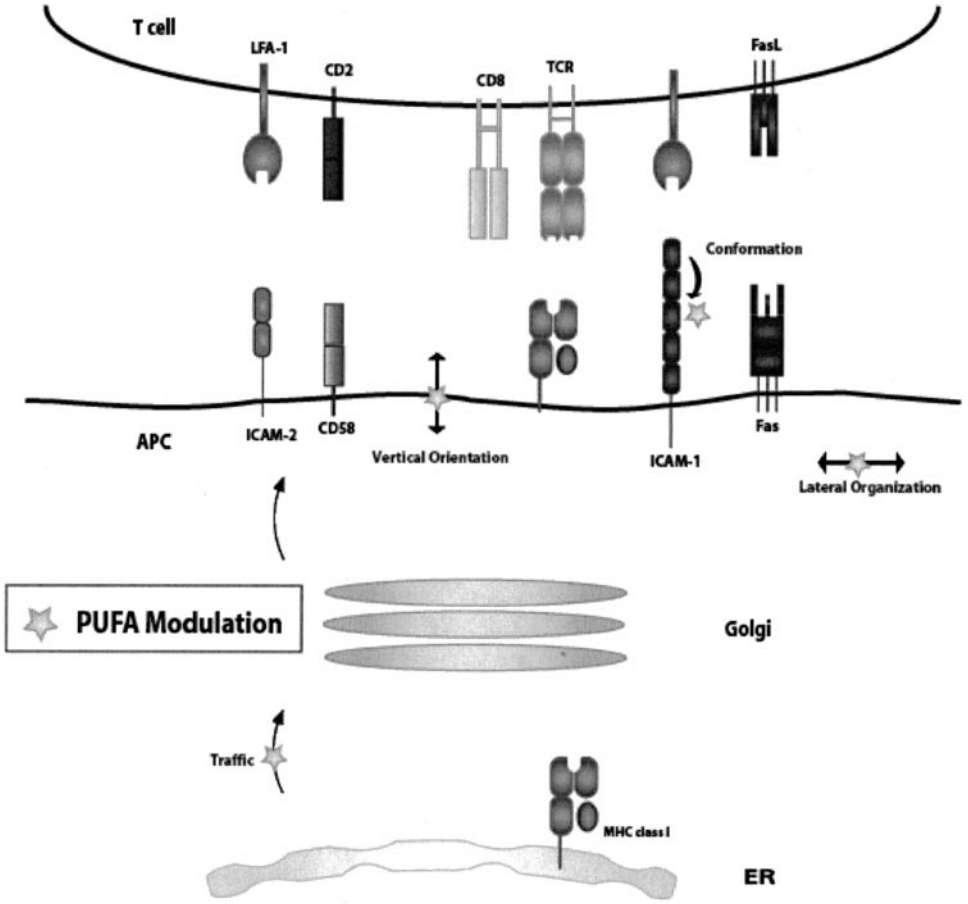

FIGURE 4. Polyunsaturated fatty acids (PUFAs) exert their immunomodulatory effects, indicated by stars, by modulating surface protein expression. On incorporation into the plasma membrane, PUFAs may alter the conformation, lateral organization, and vertical displacement or orientation of molecules involved in interactions with cognate T cells. In addition, uptake of PUFAs into endomembranes may alter the rate of trafficking of molecules such as major histocompatibility complex (MHC) class I to the plasma membrane and may explain the reduction in surface molecule expression often observed with n−3 PUFAs. Lipid modulation of antigen presenting cell (APC) membranes may result in changes in recognition by the T cell. The membrane and proteins are not drawn to scale relative to one another. TCR, T cell receptor; ICAM, intracellular adhesion molecule; ER, endoplasmic reticulum; LFA-1, lymphocyte function associated antigen-1.

expression contribute to the observed inhibition in antigen presentation activity.

There are a few exceptions to the observation that PUFAs lower MHC class II expression (Table 2). In a recent study showing that PUFAs block lipopolysaccharide-induced DC activation, HLA-DR expression was unaffected by EPA or AA treatment, although surface expression of CD40, CD80, and the mannose receptor were lowered by ≈50% relative to controls (87). Catalytic hydrogenation of LA, AA, and DHA chains in leukemia cells and in liposomes had little or no effect on both MHC class I and II surface expression (101). Finally, when mice were fed fish oil, MHC class II expression of macrophages increased when the cells were stimulated with platelet activation factor (90). These disparate results may have been due to different cell types, methods, conditions, levels of oxidation, and mechanisms by which PUFAs exert their effects.

Mechanisms by which PUFAs may alter surface expression and T cell recognition through membrane modulation

The molecular mechanisms by which PUFAs alter surface expression are unexplored and must be understood if fish-oil supplementation is to be used effectively in treating inflammatory disorders while minimizing drawbacks. We approach the mechanisms by proposing that surface molecule expression is, in part, influenced by changes in membrane architecture of both the plasma membrane and endomembranes after PUFA incorporation. A diagram of interactions between APC-expressing MHC class I and costimulatory molecules and a $CD8^+$ cytotoxic effector T cell is presented in **Figure 4**. The diagram is also applicable to MHC class II antigen presentation to $CD4^+$ T cells, although the trafficking of class II molecules would be different from shown in Figure 4. The specific points at which PUFAs could affect MHC class I surface expression through membrane modulation are indicated by stars. We address each star below.

Conformation of MHC

At the surface, incorporation of PUFAs may alter the conformation of MHC class I and class II glycoproteins. As mentioned previously, class I conformation is influenced by DHA as assessed by antibody binding. Similar observations were also made with purified MHC class II molecules reconstituted in synthetic bilayers of different compositions. Specific phospholipid headgroups and acyl chains favored conformational states of MHC

class II that allowed an enhancement in lysozyme and ovalbumin peptide binding by 10–50 –fold, although the off-rate was unaffected (102). Although the only PUFA tested was LA, a change in conformation was observed with this lipid, which suggested that acyl chain composition could modify MHC class II conformation. In addition, it is also conceivable that PUFA acylation of proteins could also influence conformation of proteins. Further work is required to learn how membrane acyl chain composition can modify the conformation of both MHC class I and class II molecules and of costimulatory and adhesion molecules, all of which are vital for effective recognition and response from T cells.

Vertical orientation of MHC

PUFAs could change the surface expression of MHC molecules by modulating their orientation in the plane of the membrane. This hypothesis is an extension of the vertical phase separation model proposed by Muller and Krueger (45) and Borochov and Shinitzky (103). Their model was based on experiments in which modulation of membrane fluidity altered surface expression levels of membrane proteins. Cells incubated with egg PC (which is a mixture of saturated and unsaturated acyl chains) for ≈2 h in the presence of the plasma membrane expander polyvinylpyrollidone increased MHC class I expression levels, assessed by antibody binding, with parallel changes in membrane fluidity (Table 2). The opposite effect was observed on incubation with cholesterol hemisuccinate, which made the membrane more rigid and lowered MHC class I expression (82). However, there was no direct experimental evidence for their idea that the changes in surface expression reflected changes in membrane microviscosity. We suggest that the changes in membrane fluidity may alter MHC orientation rather than vertical displacement. Indeed, a recent study showed that interactions between ionic headgroups and MHC could establish a new orientation of the protein (104). Changes in the orientation of MHC molecules, adhesion proteins, and costimulatory molecules would influence their interactions with the TCR and other T cell surface receptors.

Lateral organization of MHC

The lateral organization of surface molecules, including MHC class I and class II antigens, could be modulated by incorporation of PUFAs in the plasma membrane. Data from fluorescence resonance energy transfer and scanning force microscopy have shown that both MHC class I and II molecules are clustered (105). Our laboratory found that acute and chronic depletion of cholesterol from B lymphoblasts and fibroblasts lowered the lateral mobility of MHC class I molecules through changes in the organization of the actin cytoskeleton and this increased clustering of class I molecules (106). In turn, MHC I clustering enhanced agonist peptide presentation to CTLs at low peptide doses (107). We speculate that PUFAs can also influence cluster formation through changes in membrane organization, which consequently leads to changes in effector function. MHC class II and possibly class I molecules are also thought to be raft localized based primarily on detergent extraction data (108–110). If PUFAs modify membrane microdomains, this could alter interactions between MHC molecules and the TCR. Studies have shown that disruption of membrane rafts of APCs by *Leishmania donovani*, associated with an increase in membrane fluidity, lowered antigen presentation to cognate T cells. Rigidification of the membrane with exogenous cholesterol restored antigen presentation (111, 112).

Trafficking of MHC

PUFAs incorporate not only into the plasma membrane but also into internal membranes, and changes in these membranes affect MHC trafficking. This is an area of PUFA modulation that is highly unexplored. Only 2 studies have shown a role for PUFAs in protein trafficking events. Chapkin's laboratory (113) showed that DHA can inhibit trafficking of Ras and other lipidated proteins to the plasma membrane through the secretory pathway. We too have found that PUFA treatment alters trafficking events; in our case, PUFAs inhibit transport of MHC class I molecules from the endoplasmic reticulum to the Golgi complex (SR Shaikh and M Edidin, unpublished observations, 2006). Inhibition in trafficking could be due to an alteration in the biophysical properties of transport vesicles (*see*"Effects of PUFAs on bilayers and proteins from model membrane studies" below). PUFAs have been shown to modify GTP-dependent vesicle fusion (114) which could be relevant for the transport of molecules in COPII or COPI vesicles.

EFFECTS OF PUFAS ON BILAYERS AND PROTEINS FROM MODEL MEMBRANE STUDIES

In the final section of this review, we cover data from emerging molecular level studies on PUFAs in artificial membranes. The model bilayer data point to the underlying physical principles by which PUFA-containing phospholipids may affect LAT or MHC proteins, as described in the previous sections.

PUFA acyl chains adopt unique molecular orientations

It was initially proposed that the presence of multiple rigid double bonds in PUFA acyl chains rendered them highly inflexible (115). Computer modeling studies predicted 2 conformations of DHA, referred to as the "angle iron" and "helix," which favored extended acyl chains aligned with the membrane normal that allowed for tight acyl chain packing (115). However, more recent studies have shown that PUFA acyl chains are highly disordered relative to saturated or monounsaturated acyl chains (116, 117). Data from nuclear magnetic resonance measurements and molecular dynamics calculations show that PUFA acyl chains are flexible and can rapidly convert between various conformational states (118). High conformational flexibility and disorder is conferred by low potential energy barriers to rotation about the single carbon-carbon bonds that compensate for the rigidity of the double bonds (117). Acyl chain flexibility differs substantially between n−3 and n−6 PUFAs, which may have functional consequences. As an example, insufficient supply of DHA in developing mammalian brains results in substantial replacement of DHA with the n−6 fatty acid docosapentaenoic acid (DPA, 22:5n−6). Biophysical measurements show that the addition of a single double bond from DPA to DHA significantly increases its flexibility (119). Similarly, Rajamoorthi et al (120) showed that bilayers containing AA were more disordered and deformable than were those containing 2 more double bonds (eg, DHA). High conformational flexibility of both n−3 and n−6 PUFA acyl chains alters the physical properties of the membrane, including lateral organization, hydrophobic match, curvature stress, and the lateral pressure density profile, which can modify

protein function, trafficking, vesicle budding, and fusion (48, 49, 119–126).

PUFAs and membrane lateral organization

Biophysical measurements in artificial membranes suggest that PUFA-containing phospholipids form microdomains that could influence the lateral organization of the bilayer and even protein function. Huster et al (1998) showed that cholesterol preferentially associates with the saturated chains of mixed heteroacid *sn*-1 saturated *sn*-2 polyunsaturated phospholipids modeling the lipid composition of the rod outer segment (121). Sequestering cholesterol from PUFA acyl chains resulted in the formation of microdomains with radii ≈250 Å (121). Stillwell and coworkers (116, 127–129) have shown in artificial membranes that PUFA-containing PEs laterally segregate from the lipid raft molecules sphingomyelin and cholesterol. The lateral segregation between PUFAs and raft lipids is driven by steric incompatibility of cholesterol with PUFA acyl chains (130). Cholesterol solubility decreases with increasing levels of unsaturation with both PC and PE headgroups (130). The lowest levels of cholesterol solubility are observed for homoacid dipolyunsaturated PEs (130). This has led to a model in which PUFA incorporation into membrane phospholipids results in the formation of liquid ordered–rich, PUFA-poor (liquid disordered), and liquid ordered–poor, PUFA-rich (liquid disordered) microdomains (**Figure 5**) (129, 130). These findings are also consistent with studies with less unsaturated phospholipids that also segregate from sphingolipid-cholesterol mixtures (131). According to this model, lipid separation would cause a redistribution of protein between liquid ordered and liquid disordered microdomains (Figure 5). Indeed, a study by Hammond et al (132) showed that on cross-linking of membranes containing gangliosides, uniform membranes separated into liquid ordered and liquid disordered microdomains, which caused a redistribution of LAT.

PUFAs alter protein function

PUFA-containing phospholipids influence the function of numerous proteins, channels, and transporters (133). PUFAs may modify protein function by influencing membrane lateral organization or through direct protein-lipid interactions. The evidence for direct PUFA-protein interactions is scarce, perhaps because of technical limitations (118). A few crystal structures suggest that PUFA acyl chains can directly interact with the fatty acid amide hydrolase, prostaglandin synthase, and the human brain fatty acid–binding protein (134–136).

There is one excellent example of a protein that interacts directly with a PUFA, rhodopsin, the photo-inducible G protein coupled receptor of the rod outer segment. Brown et al (137) showed that rhodopsin conformation depends on the lipid environment. Phospholipids with high elastic curvature (eg, small headgroups with PUFA acyl chains) influenced the energetics of the conformational states of rhodopsin. Functional studies of rod outer segment membranes isolated from rats fed n−3–deficient diets showed a drastic reduction in G protein coupled receptor signaling of the rhodopsin photocycle due to replacement of DHA with DPA, which correlated with significant changes in acyl chain order (138, 139). More recent nuclear magnetic resonance experiments show that DHA acyl chains can bind to specific sites on rhodopsin (140). Corroborating molecular dynamics simulations provide evidence for DHA binding, primarily in the grooves between the helixes of rhodopsin, unlike saturated acyl chains or cholesterol (140). This has led to the hypothesis that DHA binding may weaken interhelical packing and thereby influence rhodopsin conformation and function.

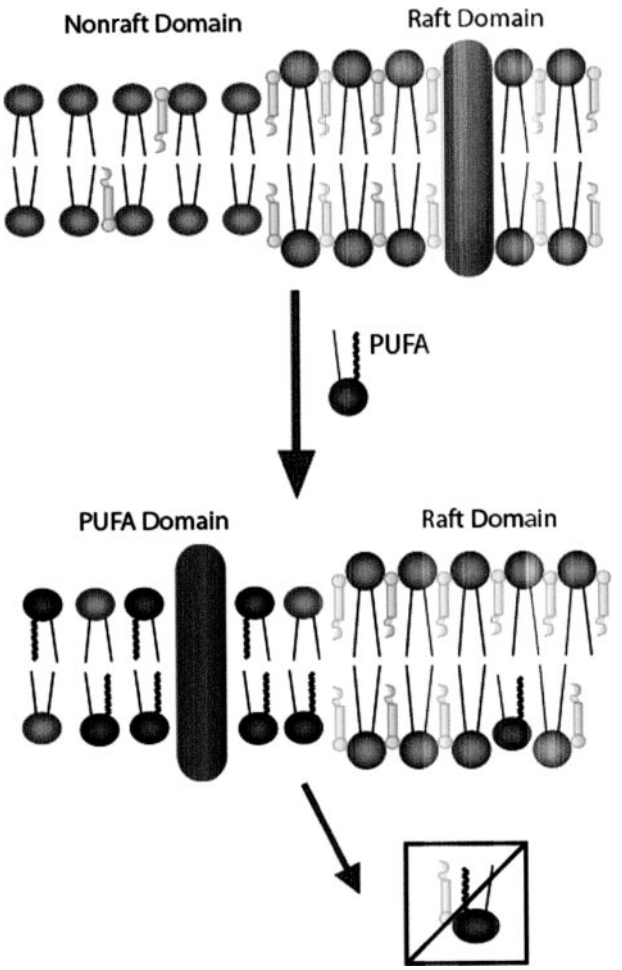

FIGURE 5. Model for polyunsaturated fatty acid (PUFA) modulation of the plasma membrane based on bilayer experiments. Biophysical measurements show that PUFA-containing phospholipids phase segregate from raft microdomains based on steric incompatibility between rigid cholesterol molecules and disordered PUFA acyl chains. The model predicts that PUFA incorporation into membrane phospholipids (eg, through dietary intake) could drive proteins from their resident raft-rich environment into raft-poor, PUFA-rich phases or vice versa, which may influence cellular activity. Relative sizes of protein and lipid molecules are not drawn to scale. Model adapted from reference 129 with permission.

Reconciling model membrane studies and cellular data

We believe that the unique biophysical properties of PUFA acyl chains, high conformational flexibility and low solubility with cholesterol, can alter the function of T cell and APC proteins. On incorporation of PUFAs into membranes, a modification in T cell or APC function could occur through either direct PUFA-protein interactions or PUFA-mediated changes in membrane lateral organization. Relatively little is known about how PUFAs can directly interact with key proteins in T cells and APCs and could be an emerging area of PUFA research. However, as exemplified in this review, a considerable number of

cellular studies have examined the effects of PUFAs on proteins involved in T cell activation and signaling through changes in membrane lateral organization. These studies show that PUFAs are incorporated into DRM fractions, which correlates with displacement of proteins (eg, LAT, PLD, or IL-2R) from DRM to DSM fractions and changes in T cell function. The model membrane data are discrepant to the cellular studies, which predict that PUFA acyl chains will not favorably interact with the components of the DRM fractions. A major source of the discrepancy between synthetic bilayer experiments and cellular studies may lie in the use of detergent extraction, which can induce artifacts (54).

It is clear that the addition of PUFAs to either cells or artificial membranes can respectively displace proteins between DRM and DSM or liquid ordered and liquid disordered domains. What is unclear are the mechanisms by which PUFA acyl chains drive the displacement of proteins between domains. We reevaluated the model on protein displacement in T cell activation and signaling by focusing on the fact that steric incompatibility between cholesterol and PUFAs can play a central role in altering the lateral organization of the plasma membrane. The differences between the cell and model membrane data can be reconciled to explain how PUFAs may influence protein displacement. We propose the following:

1) PUFA incorporation disrupts sphingomyelin-cholesterol microdomain formation and alters signal transduction. A perturbation in the stability of liquid ordered microdomains by PUFAs changes the distribution of proteins between domains as readout by localization to either DRMs or DSMs (assuming that detergent extraction is some crude representation of differing membrane heterogeneities). The physical disruption would be driven by the low affinity of cholesterol for PUFA acyl chains. Disruption of liquid ordered domains by PUFAs may also explain the reduction in sphingomyelin concentrations in DRM fractions in ex vivo studies (77). Indeed, raft disruption by cholesterol depletion has been shown to effect cellular signaling (141).

2) DRMs may not accurately depict the differing types of lipid microdomains that may sequester into these fractions. It has become increasingly clear from cell studies that raft microdomains may exist as nanoclusters <5 nm, with a few glycosylphosphatidylinositol-anchored proteins in each cluster (142). It has been suggested that there is tremendous heterogeneity in DRM microdomains (51) and it is reasonable to speculate that PUFA microdomains may exist on a nanometer scale (143). Therefore, PUFA-rich microdomains may show up in DRM fractions but may still segregate from liquid ordered domains based on steric incompatibility between cholesterol and PUFAs. These interactions may trigger protein displacement and loss of function.

3) On dietary intake, most PUFAs are esterified to phospholipids in the *sn*-2 position, with saturated acyl chains in the *sn*-1 position. Therefore, the *sn*-1 chains may participate in microdomain formation with cholesterol and exclude PUFAs, as suggested by the work of Huster et al (1998). However, during detergent extraction, the high affinity between the *sn*-1 chain and cholesterol may result in PUFA enrichment in the DRM fraction. Even though biochemical analysis of DRMs shows substantial amounts of PUFAs, they are not directly involved in the formation of liquid ordered domains.

4) PUFA incorporation into cells drives cholesterol from the outer to the inner leaflet (144, 145). The efflux of cholesterol may drive changes in outer and inner leaflet microdomain formation. The signaling complexes of the inner leaflet may respond to a reduction in cholesterol concentrations and alter T cell signaling. Our laboratory has shown that cholesterol depletion can result in a reorganization of the actin cytoskeleton (106). Perhaps PUFA-induced changes in cholesterol also alter the organization of the cytoskeleton, which could modify cellular signaling.

CONCLUSIONS

As the data grow on how select PUFAs may modulate inflammatory and autoimmune diseases, there is a growing need for elucidating the underlying molecular mechanisms. An understanding of the effect of PUFAs at the level of T cell membrane organization is only starting to emerge, whereas relatively little is known about membrane modulation of APCs. The data from cellular and animal studies tell us that PUFAs induce changes in localization of proteins from DRM to DSM fractions, which has measurable consequences for T cell signaling and proliferation. However, the mechanisms by which PUFAs induce changes in protein localization are not known, and it is here that model membrane experiments suggest testable hypotheses. We know that PUFA-containing phospholipids impart unique structural effects on bilayers, but what is required is better knowledge of how these effects translate into functional consequences at the cellular level. Further investigation at the interface between model bilayers and cellular systems may answer some questions regarding PUFA-raft interactions. How PUFAs modulate MHC conformation, vertical orientation, lateral organization, and trafficking—on the basis of literature reports and some data from our own laboratory—also requires extensive investigation at all levels, from synthetic bilayers to animal experiments. Because dietary intake of PUFAs will result in a distribution to virtually every cell of the body, the changes described above for APCs are also applicable to T cells. PUFA modification of cellular membranes may be an important target for immunosuppression.

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