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Omega-3 fatty acids, lipid rafts, and T cell signaling

Tim Y. Hou[a,b,c], **David N. McMurray**[b,c,e,f,g], and **Robert S. Chapkin**[a,b,c,d,e,f]

[a]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas, USA

[b]Department of Nutrition and Food Science, Texas A&M University, College Station, Texas, USA

[c]Program in Integrative Nutrition and Complex Diseases, Texas A&M University, College Station, Texas, USA

[d]Center for Translational Environmental Health Research, Texas A&M University, College Station, Texas, USA

[e]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas, USA

[f]Department of Microbial Pathogenesis and Immunology, Texas A&M University System Health Science Center, College Station, Texas, 77843, USA

Abstract

n-3 PUFA have been shown in many clinical studies to attenuate inflammatory responses. Although inflammatory responses are orchestrated by a wide spectrum of cells, $CD4^+$ T cells play an important role in the etiology of many chronic inflammatory diseases such as inflammatory bowel disease and obesity. In light of recent concerns over the safety profiles of non-steroidal anti-inflammatory drugs (NSAIDs), alternatives such as bioactive nutraceuticals are becoming more attractive. In order for these agents to be accepted into mainstream medicine, however, the mechanisms by which nutraceuticals such as n-3 polyunsaturated fatty acids (PUFA) exert their anti-inflammatory effects must be fully elucidated. Lipid rafts are nanoscale, dynamic domains in the plasma membrane that are formed through favorable lipid-lipid (cholesterol, sphingolipids, and saturated fatty acids) and lipid-protein (membrane-actin cytoskeleton) interactions. These domains optimize the clustering of signaling proteins at the membrane to facilitate efficient cell signaling which is required for $CD4^+$ T cell activation and differentiation. This review summarizes novel emerging data documenting the ability of n-3 PUFA to perturb membrane-cytoskeletal structure and function in $CD4^+$ T cells. An understanding of these underlying mechanisms will provide a rationale for the use of n-3 PUFA in the treatment of chronic inflammation.

[g]Address correspondence to: Robert S. Chapkin, 111 Cater-Mattil, MS 2253, Texas A&M University, College Station, TX, 77843. Phone: 1-979-845-0419. r-chapkin@tamu.edu.

Keywords

Omega-3 fatty acids; Lipid rafts; T cell activation; T cell differentiation

1. Introduction

The mammalian immune system is critical in defending the host against foreign pathogens and malignant cells. Under normal conditions, T lymphocytes circulate throughout the body to survey for foreign antigens and transformed cells and target them for destruction. However, under certain pathophysiological conditions, the adaptive immune system may lose the ability to differentiate between self and foreign antigens, resulting in self-reactive T lymphocyte activation and effector function. The result of the loss of self-tolerance could be autoimmune diseases such as inflammatory bowel disease (IBD), e.g., Crohn's disease and ulcerative colitis (Zenewicz et al., 2009). Alternatively, the adaptive immune system may become over-reactive against self-antigens and unable to resolve appropriately, resulting in chronic inflammatory diseases such as rheumatoid arthritis. The mammalian immune system is comprised of the innate and the adaptive system; this review will focus on the adaptive arm, specifically $CD4^+$ T lymphocytes. These cells typically further differentiate into other effector cell types (T_H1, T_H2, T_{reg}, T_H17), which have opposing roles in autoimmune diseases such as IBD (Zenewicz et al., 2009).

The cell membrane, composed of a phospholipid bilayer and a myriad of proteins, constitutes the outer boundary of the cell. Not only does the cell membrane control molecular transport, but it also regulates communication between the cell and its environment by transducing signals. . The first model of the plasma membrane, the fluid mosaic model, was proposed in 1972 by Singer and Nicolson (Singer and Nicolson, 1972). In this model, the phospholipid bilayer is thought of as a fluid, dynamic, passive solvent in which proteins are either embedded in and span the membrane (i.e. integral proteins), or loosely associate (i.e. peripheral proteins), with the phospholipid bilayer. The plasma membrane contains three classes of amphiphilic lipids: phospholipids, glycolipids, and sterols. Phospholipids and glycolipids are further subdivided into various fatty acids and headgroups at the *sn-1, sn-2*, and *sn-3* positions (Fahy et al., 2009). For example, a major species of phosphatidylinositol-(4,5)-bisphosphate [$PI(4,5)P_2$] is composed of a saturated C18:0 fatty acid at the *sn-1* position, an unsaturated C20:4 ^{5,8,11,14} fatty acid at the *sn-2* position, and *myo*-inositol 4,5-bisphosphate at the *sn-3* position. The heterogeneity of the lipids in the plasma membrane is not well studied, with the potential to generate 9,000 – 100,000 different molecular species (Shevchenko and Simons, 2010; van Meer, 2005; Yetukuri et al., 2008). With all these layers of complexity, could lipids in the plasma membrane form local structures that can function to regulate cell signaling?

2. n-3 PUFA and lipid rafts in the $CD4^+$ T cell plasma membrane

2.1 Lipid rafts

In a simple model system where two lipids (one high melting temperature, one low melting temperature) and cholesterol are mixed together, micron-scale domains phase separate and

are easily visualized using conventional fluorescence microscopy (Nicolau et al., 2006). These micron-sized microdomains, one example of local structures in the plasma membrane, can be observed in epithelial cells, where the apical plasma membrane is enriched in sphingolipids, while the basolateral plasma membrane is enriched in phosphatidylcholine (Zidovetzki and Levitan, 2007). Small invaginations in the plasma membrane, enriched with cholesterol, sphingolipids, and the protein caveolin, can also be found in many cells such as endothelial and intestinal epithelial cells and adipocytes (Ma et al., 2004; Toulmay and Prinz, 2013). Smaller, highly dynamic, nanoscale lipid rafts enriched in sphingolipids, cholesterol, and saturated fatty acids, have been proposed to play a role in signal transduction (Fig. 1). In fact, stable nanodomains can be visualized in yeast vacuole membranes in response to various stresses such as nutrient deprivation and pH change; proteins that sort to these vacuolar membranes also segregate to one of two domains, similar to what would be predicted by the simple system composed of two lipids and cholesterol (Toulmay and Prinz, 2013). These nanodomains are thought to organize select proteins to optimize their signaling capacity upon ligand engagement. Computer simulations suggest that in order for lipid rafts to promote protein-protein interactions, these nanoscale domains must be small (6 to 14 nm in diameter) in order to operate as protein concentrators in the plasma membrane (Nicolau et al., 2006).

Although lipid rafts can associate and dissociate as a mechanism to regulate the formation of raft phases in the plasma membrane, one way to achieve a stabilized raft phase (i.e., stabilize the size and/or lifetime of the raft) is the presence of the actin cytoskeleton. Monomeric actin (G-actin) protein is capable of polymerization to form long, complex filamentous actin (F-actin) that can provide the force required for organelle movement, and the scaffold required for stabilization of membrane raft phases. F-actin is connected to the plasma membrane by interacting with integral and membrane-associated proteins; e.g., various protein-actin cytoskeleton interactions in erythrocytes (Luna and Hitt, 1992). One current model is that these “membrane skeletons” form the “fences” in the plasma membrane, impeding the diffusion of membrane proteins and lipids (Kusumi et al., 2012).

The participation of the actin cytoskeleton in the formation of nanoscale domains was first postulated when it was observed that the coefficient of diffusion of phospholipid probes were significantly lower in live cells (Lee et al., 1993; Swaisgood and Schindler, 1989), than those estimated in artificial membranes (Ladha et al., 1996; Sonnleitner et al., 1999). This difference was also observed for transmembrane protein markers and glycosylphosphatidylinositol-anchored protein markers (Kusumi et al., 2012). One suggestion to explain the difference in the diffusion coefficient was the presence of the membrane cytoskeleton in live cells compared to artificial membranes. The requirement for the actin cytoskeleton in maintaining the properties of nanoscale domains was also observed using secondary ion mass spectrometry, in which treatment of NIH 3T3 mouse fibroblasts with latrunculin A (prevents G-actin polymerization) resulted in a random distribution of sphingomyelin instead of sphingomyelin-enriched microdomains in control fibroblasts (Frisz et al., 2013).

The actin cytoskeleton is bridged to membranes through interactions with integral proteins, or through proteins recruited to the plasma membrane by specific phospholipids. One such

phospholipid is phosphatidylinositol-(4,5)-bisphosphate [$PI(4,5)P_2$], which comprises 5% of the total phosphatidylinositol species, and 1% of the total plasma membrane phospholipid in mammalian cells (Di Paolo and De Camilli, 2006; Kwiatkowska, 2010). Classically, $PI(4,5)P_2$ is important in signal transduction because of the hydrolysis products diacylglycerol (DAG), and inositol(1,4,5)-trisphosphate (IP_3) produced by phospholipase C. However, an additional function of $PI(4,5)P_2$ is to recruit actin remodeling proteins to localized sites at the plasma membrane. $PI(4,5)P_2$ is thought to activate actin-regulatory proteins that induce actin polymerization, while concurrently inhibiting those proteins that promote actin disassembly. Indeed, the increase in local $PI(4,5)P_2$ results in stress-fiber formation, indicative of increased actin filament formation (Kwiatkowska, 2010). Due to multiple acidic phosphate groups on $PI(4,5)P_2$, basic protein domains are thought to play a role in recruiting proteins to the plasma membrane. For example, the Wiskott-Aldrich syndrome protein (WASP) is released from its autoinhibitory conformation when the basic domain binds to $PI(4,5)P_2$ (Padrick et al., 2008). Similarly, $PI(4,5)P_2$ can recruit and activate Rho family GTPase through its polybasic region. Some small GTPases play a role in cytoskeletal remodeling (Saarikangas et al., 2010).

A third interaction to consider in the formation and organization of optimal nanoscale lipid rafts is the role of higher-ordered protein complexes (i.e., protein-protein interactions), as proposed by Kai Simons and Akihiro Kusumi (Kusumi et al., 2011; Simons and Sampaio, 2011). As an example, epidermal growth factor receptor (EGFR) undergoes conformational change and dimerization upon binding to its ligand (EGF), resulting in downstream signaling (Turk et al., 2012; Turk and Chapkin, 2013). Optimal EGFR signaling, however, takes place in lipid rafts (Turk and Chapkin, 2013; 2015), highlighting the role of protein-protein interactions in driving the formation and organization of optimal nanoscale domains for cellular signaling.

2.2 Effects of n-3 PUFA on lipid rafts in $CD4^+$ T cell plasma membrane

Eicosapentaenoic acid (EPA, 20:5 $^{\Delta 5,8,11,14,17}$) and docosahexaenoic acid (DHA, 22:6 $^{\Delta 4,7,10,13,16,19}$) are thought to be the major bioactive n-3 PUFA in fish oil. EPA and DHA are incorporated into the two major classes of phospholipids in the plasma membrane, phosphatidylenthanolamine and phosphatidylcholine at the *sn-2* position (Fan et al., 2004; Fan et al., 2003), consistent with the observation that saturated fatty acids occupy the *sn-1* position of phospholipids, while unsaturated fatty acids are inserted at the *sn-2* position. DHA is highly disordered and adopts various conformations on the subnanosecond time scale (Gawrisch and Soubias, 2008; Soubias and Gawrisch, 2007). By eliminating the double bond at the n-3 position to generate docosapentaenoic acid (DPA, 22:5 $^{\Delta 4,7,10,13,16}$), chain dynamics of the fatty acid are reduced (Gawrisch and Soubias, 2008), suggesting that the unsaturation at the n-3 position affords unique properties to EPA and DHA in the plasma membrane.

Studies on ion channels have demonstrated that EPA and DHA can modulate membrane protein properties. The incorporation of DHA into the lipid bilayer resulted in a decrease in bilayer stiffness without changing the negative curvature of the bilayer (Bruno et al., 2007). DHA increased the appearance and lifetime of gramicidin channels, and decreased the free

energy for channel formation. Oleic acid (OA, 18:1 $^{\Delta 9}$) that intercalated into the plasma membrane at a greater rate, had no effect on gramicidin channel formation, suggesting that the effect of DHA was due to changes in bilayer properties and not due to specific binding (Bruno et al., 2007; Bruno et al., 2013). EPA and DHA inhibit cardiac Na^+ and L-type Ca^{2+} channels (Xiao et al., 1997; Xiao et al., 1995), activate TRAAK-1 and TRPV1 channels (Fink et al., 1998; Matta et al., 2007), and increase desensitization of nAChR and $GABA_a$ channels (Bouzat and Barrantes, 1993; Nabekura et al., 1998).

DHA is thought to play a major role in altering the size and/or stability of nanoscale lipid rafts in the plasma membrane. As a result of its highly disordered nature, DHA may modify the lateral organization of the plasma membrane by forming a distinct non-raft DHA domain (Shaikh et al., 2014; Wassall and Stillwell, 2008). This DHA domain is distinct from the sphingolipid and cholesterol enriched lipid rafts due to the highly flexible DHA, which is incompatible with the rigidity of sphingolipids and cholesterol. Incorporation of DHA into the plasma membrane results in the distinct non-raft DHA domain inserting itself into lipid rafts, increasing the size of lipid rafts. This mechanism would explain nuclear magnetic resonance (NMR) data which indicated that EPA and DHA are incorporated into both raft and non-raft domains in a phosphatidylethanolamine/sphingomyelin/cholesterol membrane mixture (Williams et al., 2012). Similar results have also been observed *in vivo* by analyzing phospholipids of $CD4^+$ T cells after isolating the detergent-resistant and –soluble membrane fractions (Fan et al., 2004; Fan et al., 2003), as well as utilizing lipid-sensitive fluorescent probes (Kim et al., 2014; Kim et al., 2008).

An alternative mechanism proposed to explain the effects of n-3 PUFA on lipid raft organization comes from the flexibility of n-3 PUFA which is incompatible with the rigid cholesterol. This effect promotes the aggregation of cholesterol in the plasma membrane, resulting in the coalescence of sphingolipid and cholesterol lipid rafts from the bulk membrane (Wassall and Stillwell, 2009). Indeed, it has been shown that EPA and DHA can increase the size of lipid rafts in HEK cells, $CD4^+$ T cells, and B cells (Chapkin et al., 2008; Kim et al., 2008; Rockett et al., 2012). The resulting alteration in the optimal size of nanoscale lipid rafts could perturb the cellular signaling required for $CD4^+$ T cell activation and differentiation.

3. $CD4^+$ T cell activation

The engagement of the $CD4^+$ T cell receptor (TCR) by an antigenic peptide presented by the major histocompatibility complex II (MHCII) molecule results in the formation of the immunological synapse (IS, Fig. 2). Proteins essential for propagating the signal are enriched at the IS to form the central supramolecular activation cluster (cSMAC). Adhesion molecules necessary to stabilize the IS form the peripheral supramolecular activation cluster (pSMAC) leading to the “bull’s eye” pattern of the IS (Lee et al., 2003; Monks et al., 1998). Proteins such as phosphatases that can abrogate signaling are excluded from the cSMAC and pSMAC in order for T cell activation to take place. At the IS, tyrosine kinases Lck and ZAP70 are activated and subsequently phosphorylate the adaptor protein linker for activation of T cells (LAT), leading to the assembly of the signalsome comprised of many proteins, including GADS, SLP76, NCK, ITK, VAV1, PAK, and PLC-γ1 (Tybulewicz and Henderson,

2009). The proper formation of the IS is required for sustained T cell activation and is stabilized by the actin cytoskeleton (Gomez and Billadeau, 2008; Huang and Burkhardt, 2007; Meiri, 2005). When the adhesion proteins coalesce to form the pSMAC, the actin cytoskeleton is connected to the IS by proteins such as talin, vinculin, and WASp. The dynamics of the actin cytoskeleton, however, are also important for sustained T cell activation. It is thought that the centripetal flow of F-actin to the initial engagement site between TCR and MHCII is important for the strength and duration of T cell activation. Indeed, T cell activation can be disrupted by inhibiting actin polymerization (Campi et al., 2005; DeMond et al., 2008; Kaizuka et al., 2007; Yokosuka et al., 2005).

3.1 Role of lipid rafts in CD4$^+$ T cell activation

Lipid rafts in the plasma membrane are thought to be critical in CD4$^+$ T cell activation The interaction between Ag:MHCII and TCR results in a major reorganization of the nanoscale lipid rafts and signaling proteins to form the IS (Jury et al., 2007; Lillemeier et al., 2010; Meiri, 2005). IS formation involves changes in lipid-lipid interactions in the membranes. Liquid ordered (Lo) lipids such as cholesterol and sphingolipids accumulate at the IS (Burack et al., 2002; Fan et al., 2003; Kim et al., 2008; Zech et al., 2009). The involvement of lipid rafts in CD4$^+$ T cell activation is conclusive; disruption of lipid rafts with various agents such as methyl-β-cyclodextrin (Xavier et al., 1998), 7-ketocholesterol (Rentero et al., 2008), or n-3 PUFA such as EPA (discussed below, Zech et al., 2009) resulted in suppressed T cell activation. Importantly, these data suggest that an alteration in the size of rafts can impact IS formation, and thus regulate T cell activation (Rentero et al., 2008; Zech et al., 2009).

3.2 Effects of n-3 PUFA on CD4$^+$ T cell activation

Since it has been shown that n-3 PUFA can alter the size and/or stability of lipid rafts in CD4$^+$ T cells (Fan et al., 2004; Fan et al., 2003; Kim et al., 2014; Kim et al., 2008; Zech et al., 2009), it is not surprising that many studies have shown downstream perturbations in T cell activation when the CD4$^+$ T cell plasma membrane is enriched with n-3 PUFA. Studies using the immortalized Jurkat T cell line demonstrated that n-3 PUFA displaced many of the signaling proteins necessary for T cell activation, including the Src family kinases Lck and Fyn (Stulnig et al., 1998) and LAT (Stulnig et al., 2001) from detergent-resistant membrane fractions. Recruitment and activation of signaling proteins such as PKC , LAT, Fas, PLC-γ1, and F-actin were also altered (Fan et al., 2004; Kim et al., 2008; Sanderson and Calder, 1998). n-3 PUFA, however, did not universally inhibit protein localization; one study observed an enhancement of surface CTLA-4 expression, a negative regulator of T cell activation, in CD4$^+$ T cells isolated from mice fed an EPA-enriched diet (Ly et al., 2006). It is now appreciated that n-3 PUFA broadly suppress downstream activation signaling in CD4$^+$ T cells, including mitochondrial translocation (Yog et al., 2010), IL-2 secretion (Arrington et al., 2001; Chapkin et al., 2002; Fan et al., 2004; Jolly et al., 1997; Ly et al., 2006; McMurray et al., 2000), and lymphoproliferation (Fan et al., 2008; Kim et al., 2008; McMurray et al., 2000).

Both lipid-lipid interactions and lipid-protein interactions (i.e., membrane-actin cytoskeleton interactions) are important for the formation of the IS. In a breakthrough finding, we

recently demonstrated that n-3 PUFA directly perturb the membrane-actin cytoskeleton interactions by depleting the overall level of $PI(4,5)P_2$ in $CD4^+$ T cells, resulting in the suppression/normalization of actin cytoskeletal rearrangement upon T cell activation (Hou et al., 2012). This effect was rescued by exogenous incubation of n-3 PUFA-enriched $CD4^+$ T cells with $PI(4, 5)P_2$, demonstrating the direct perturbation of membrane-actin cytoskeleton interactions by n-3 PUFA. Perturbation of membrane-actin cytoskeleton interactions by n-3 PUFA may occur in other cell types. Colonocytes enriched with n-3 PUFA exhibited suppressed activation of cytoksksletal remodeling proteins such as PLC-γ1, Rc1, and Cdc42 (Turk et al., 2013).

4. $CD4^+$ T cell differentiation

Activated $CD4^+$ T cells differentiate into pro-inflammatory effector subsets (T_H1, T_H17) in the presence of the cytokines such as interferon- (IFN-) and interleukin-12 (IL-12) for T_H1 cells, and Transforming growth factor-β (TGFβ), interleukin-6 (IL-6), and interleukin 21 (IL-21) for T_H17 cells (Zhu et al., 2010). $CD4^+$ T cells can differentiate into anti-inflammatory T_H2 cells in the presence of IL-4 and IL-2, and into regulatory Treg cells (Zhu et al., 2010). Typically these $CD4^+$ T effector cell subsets are defined by their transcriptional signatures. However, the role of the plasma membrane in directing the differentiation of $CD4^+$ T cells is underappreciated. Although the anti-inflammatory effects of EPA and DHA have been well documented, their precise biochemical mechanisms of action with respect to cell polarization/differentiation have yet to be clearly defined. We propose that the ability of n-3 PUFA to alter plasma membrane and cytoskeleton-dependent signaling may impact $CD4^+$ T cell differentiation. These molecular targets may provide a novel therapeutic strategy for modulating the pathologic effector T cell subsets in autoimmune and chronic inflammatory diseases.

4.1 Role of lipid rafts in $CD4^+$ T cell differentiation

Early reports suggested that different $CD4^+$ effector T cell populations depend upon distinct plasma membrane characteristics. For example, treatment of T cells with methyl-β-cyclodextrin to disturb lipid rafts demonstrated that T_H1, but not T_H2,cells were more sensitive as detected by suppressed Ca^{2+} influx upon antigen stimulation (Balamuth et al., 2001). Indeed, later studies demonstrated that T_H1 and T_H2 cells have distinct IS. The T_H1 cell IS is characterized by the bull's eye pattern where the cSMAC, comprised of Ag:MHCII/TCR interactions, is surrounded by the pSMAC populated with adhesion molecules. In contrast, the T_H2 cell IS is described as multifocal and dependent on the concentration of antigens (Thauland et al., 2008). Furthermore, individual human $CD4^+$ T cells can be categorized into three classes based on the lipid order of their plasma membrane (low, intermediate, and high), as determined by the generalized polarization of di-4-ANEPPDHQ, a probe that changes fluorescence intensities at 570 nm and 620 nm based on the lipid order of its surrounding microenvironment upon incorporation into the plasma membrane (Miguel et al., 2011). $CD4^+$ T cells with intermediate membrane order were associated with IFN- production (i.e. T_H1 phenotype), while high membrane order was associated with IL-4 production (i.e. T_H2 phenotype); low membrane order $CD4^+$ T cells were associated with increased apoptosis. When $CD4^+$ T cells were cultured in either T_H1 or

T_H2-polarizing conditions, the membrane order of the $CD4^+$ T cells changed to the corresponding T cell subset; intermediate membrane order was observed under T_H1 conditions, while high membrane order was observed under T_H2 conditions. To further link the role of membrane order with $CD4^+$ T cell differentiation, the reduction of membrane order with 7-ketoxycholesterol resulted in an increase in the number of $CD4^+$ T cells producing IFN-, indicative of a switch to an intermediate membrane order associated with the T_H1 phenotype. Clinically, $CD4^+$ T cells isolated from human patients with systemic lupus erythematosus, Sjogrens Syndrome, or rheumatoid arthritis have an increased population of intermediate membrane order $CD4^+$ T cells, associated with IFN- production and pro-inflammatory phenotype (McDonald et al., 2014; Miguel et al., 2011). T_H17 cells are also known to be affected by membrane order. Decreasing levels of glycosphingolipid, a lipid known to be associated with lipid rafts in $CD4^+$ T cells, resulted in a reduction in T_H17 differentiation (Zhu et al., 2011). These studies demonstrate the structural importance of membrane nanoscale domains in directing $CD4^+$ T cell differentiation.

4.2 Effects of n-3 PUFA on $CD4^+$ T cell differentiation

n-3 PUFA suppress $CD4^+$ T cell activation and differentiation. Only T_H1-like cells exhibited enhanced activation-induced cell death (AICD) upon enrichment with n-3 PUFA, whereas T_H2-like cells were unaffected by n-3 PUFA (Switzer et al., 2004; Switzer et al., 2003). In addition, $CD4^+$ T cells enriched with n-3 PUFA failed to polarize into T_H1 or T_H17 cells as efficiently when compared to control $CD4^+$ T cells (Monk et al., 2013; Monk et al., 2012a; Zhang et al., 2005). These phenotypes were accompanied by changes in downstream signaling, such as reduced activation of T_H17-related STAT3, reduced expression of T_H17 signature cytokines (i.e., IL-17A), reduced expression of the T_H17 transcription factor (i.e., RORγt), and reduced surface expressions of critical surface receptors for T_H17 polarization (i.e., IL-6R and IL-23R) (Monk et al., 2013; Monk et al., 2012a; Zhang et al., 2005).

Interestingly, n-3 PUFA do not seem to affect the polarization of $CD4^+$ T cells into T_H2 or T_{reg} subsets (Monk et al., 2013; Monk et al., 2012a; Monk et al., 2012b). This may be due to the different membrane properties optimized for polarization into these subsets (Miguel et al., 2011; Thauland et al., 2008). However, a recent study reported a decrease in $CD4^+$ T cell differentiation under T_H2 polarization conditions in cells isolated from *Fat-1* mice, which incorporate n-3 PUFA into cell membranes endogenously (Jang et al., 2014). This highlights the need for more study into how n-3 PUFA affect $CD4^+$ T cell differentiation, such as examining the dose of n-3 PUFA, and the differentiation environment the $CD4^+$ T cells are selected under.

One mechanism by which long chain n-3 PUFA (i.e., EPA and DHA) suppress $CD4^+$ T cell polarization into T_H17 cells involves the IL-6-gp-130-STAT3 signaling axis which regulates the earliest events in T_H17 cell differentiation (Bettelli et al., 2006; Jones et al., 2013; Jones et al., 2010; Nishihara et al., 2007; Veldhoen et al., 2006). A hexameric signaling complex composed of two IL-6 binding to two membrane bound IL-6 receptors (mIL-6R) and two molecules of glycoprotein 130 (gp130) is formed, leading to the phosphorylation of STAT3, translocation to the nucleus, and subsequent activation of RORγt, the master regulator of T_H17 transcription (Briso et al., 2008; Jones et al., 2010). As mentioned above,

phosphorylation of STAT3 and activation of RORγt are both suppressed by n-3 PUFA in $CD4^+$ T cells (Monk et al., 2013; Monk et al., 2012a). While the IL-6 receptor (IL-6R) is an 80 kDa surface protein expressed on naive $CD4^+$ T cells (Betz and Muller, 1998), gp130 is ubiquitously expressed and mediates signal transduction for IL-6 and other cytokines (Silver and Hunter, 2010). Both IL-6R and gp130 are associated with lipid rafts; the localization of IL-6R at the plasma membrane is regulated by cholesterol (Matthews et al., 2003), while gp130 has been found to reside in lipid rafts of kidney (Buk et al., 2004) and neuroepithelial (Yanagisawa et al., 2004) cell plasma membranes. Based upon the essential nature of the IL-6-gp130-STAT3 signaling axis in T_H17 differentiation (Veldhoen et al., 2006) and the fact that membrane modulation disrupts T_H17 development (Zhu et al., 2011), Allen and colleagues hypothesized that n-3 PUFA reduce T_H17 differentiation by interfering with IL-6 signaling in a lipid raft-dependent fashion (Allen et al., 2014). The presence of n-3 PUFA decreased surface expression of IL-6R and the association of gp130 with lipid rafts (Fig. 3). Specifically, the homodimerization of gp130 was reduced in activated $CD4^+$ T cells enriched with n-3 PUFA. The lipid raft perturbations caused by n-3 PUFA led to downstream STAT3 phosphorylation. These results demonstrate that membrane alterations induced by n-3 PUFA suppress T_H17 differentiation by altering the IL6/gp130/Stat3 pathway.

5. Future Directions

A third potential target for n-3 PUFA in the formation and organization of optimal mesoscale lipid rafts are higher-ordered protein complexes (i.e., protein-protein interactions), as proposed by Kai Simons and Akihiro Kusumi (Kusumi et al., 2011; Simons and Sampaio, 2011). Similar to EGFR, Fas receptor (FasR) localizes in lipid rafts, and upon engagement with its ligand, Fas ligand (FasL), FasR undergoes oligomerization to induce favorable interactions between lipid rafts, again highlighting how protein-protein interactions can mediate lipid raft formation and organization (Muppidi and Siegel, 2004; Wang et al., 2010; Wilson et al., 2009). The TCR itself forms nanoclusters in the plasma membrane in a cholesterol-dependent fashion, requiring both cholesterol and sphingomyelin for the formation of TCR dimers (Molnar et al., 2012). Thus, the hierarchical organization of the plasma membrane is aided by lipid-lipid, lipid-protein, and protein-protein interactions. With advances in super-resolution microscopy to visualize nanoclusters at the plasma membrane, it will be possible to determine whether n-3 PUFA can also affect protein-protein interactions in forming TCR nanoclusters at the plasma membrane of $CD4^+$ T cells.

Not all n-3 PUFA are effective at perturbing plasma membrane lipid rafts. Phenotypes are not interchangeable when cells are treated with EPA or DHA (Corsetto et al., 2012; Turk et al., 2013), and biophysical studies have demonstrated that EPA and DHA do not disrupt lipid rafts with equal efficiency (Williams et al., 2012). Adding to the complication, 1-palmitoyl-2-docosahexaenoylphosphatidylethanolamine preferentially segregates into the non-raft plasma membrane, while 1-palmitoyl-2-docosahexaenoylphosphatidylcholine prefers the mesoscale raft domains of the plasma membrane (Shaikh et al., 2014), demonstrating that the headgroups of phospholipids can also affect the mechanisms by which these n-3 PUFA modulate plasma membrane properties.

Although it is appreciated that n-3 PUFA are pleotropic and can act upon targets outside of the plasma membrane, cogent data demonstrate that incorporation of n-3 PUFA can alter the biochemical and biophysical properties of $CD4^+$ T cell plasma membranes, favorably modulating cytoskeletal dependent $CD4^+$ T cell activation and differentiation (Fig. 1). In conclusion, n-3 PUFA target the plasma membrane of immune cells, and may contribute in the attenuation of autoimmune and chronic inflammatory diseases. However, in order to provide further rationale for their potential use as therapeutics, additional research is required to further elucidate how n-3 PUFA affect the nanoscale organization of the plasma membrane, and how this mediates the suppression of $CD4^+$ T cell activation and differentiation into pathologic effector subsets.

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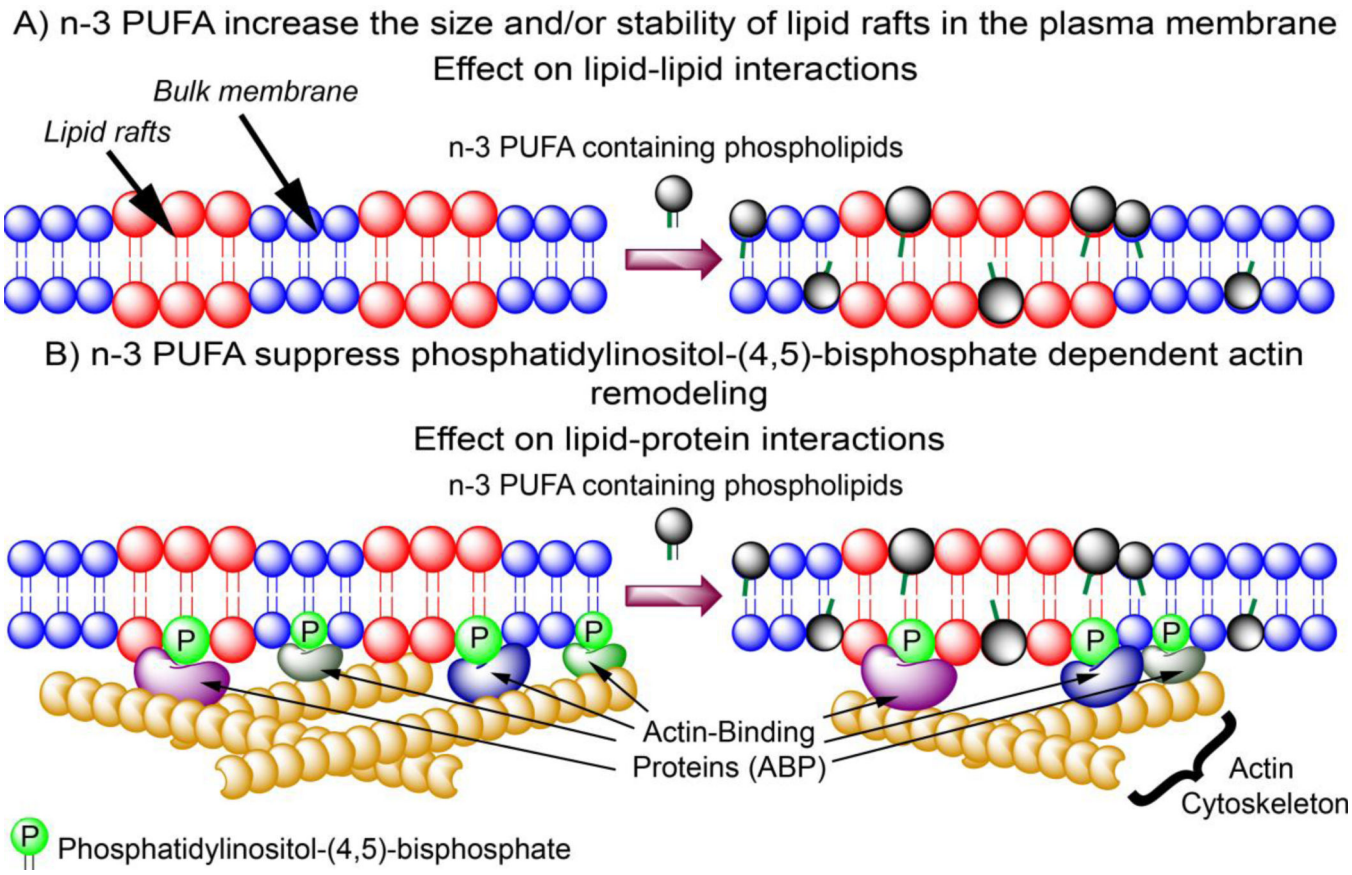

Figure 1.

Proposed mechanisms by which n-3 PUFA modulate adaptive immune responses by **A**) modulating lipid-lipid interactions in the plasma membrane; and **B**) altering plasma membrane lipid-protein interactions by decreasing $PI(4,5)P_2$ level, thereby lowering the recruitment of actin-binding proteins and suppressing actin cytoskeleton remodeling. Consequently, incorporation of n-3 PUFA into the plasma membrane increase the size and/or stability of the mesoscale lipid rafts and physiologically. This translates into suppressed $CD4^+$ T cell activation and differentiation. Red highlight indicates liquid ordered lipid rafts; Blue indicates bulk membrane.

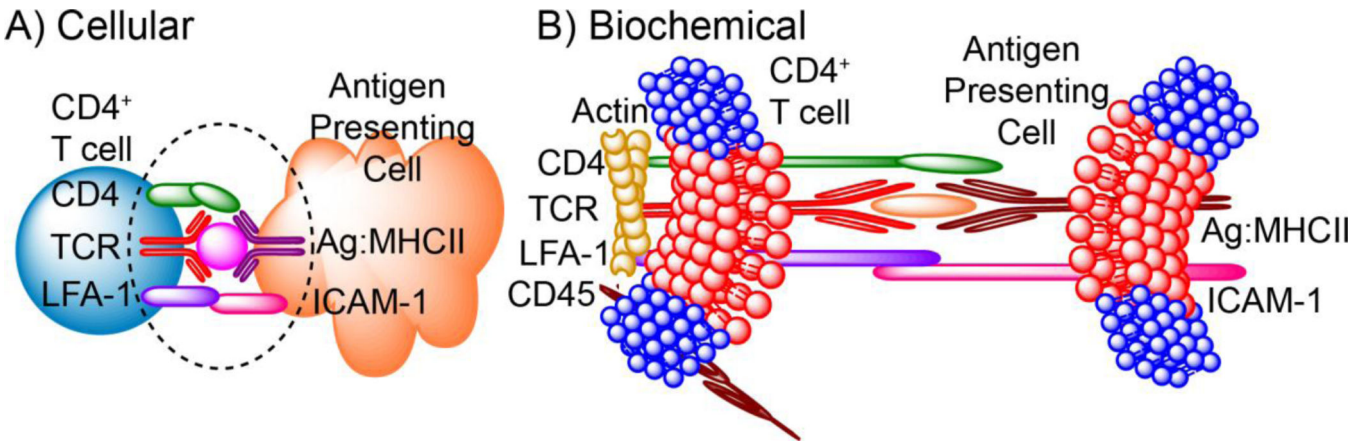

Figure 2.

Immunological synapse at the A) cellular; and B) biochemical level. When $CD4^+$ T cells recognize cognate antigen presented by the antigen presenting cell, an immunological synapse is formed at the cellular level. At the biochemical level, major lipidomic and proteomic rearrangement occurs to propagate T cell activation. At the lipidomic level, lipids associated with lipid rafts are found to coalesce at the immunological synapse (represented in red). Adhesion molecules, such as LFA-1, are important for the stabilization of the immunological synapse, while CD45, which is required to terminate T cell activation, is excluded from the immunological synapse. Additionally, the actin cytoskeleton is important for T cell activation by stabilizing the immunological synapse.

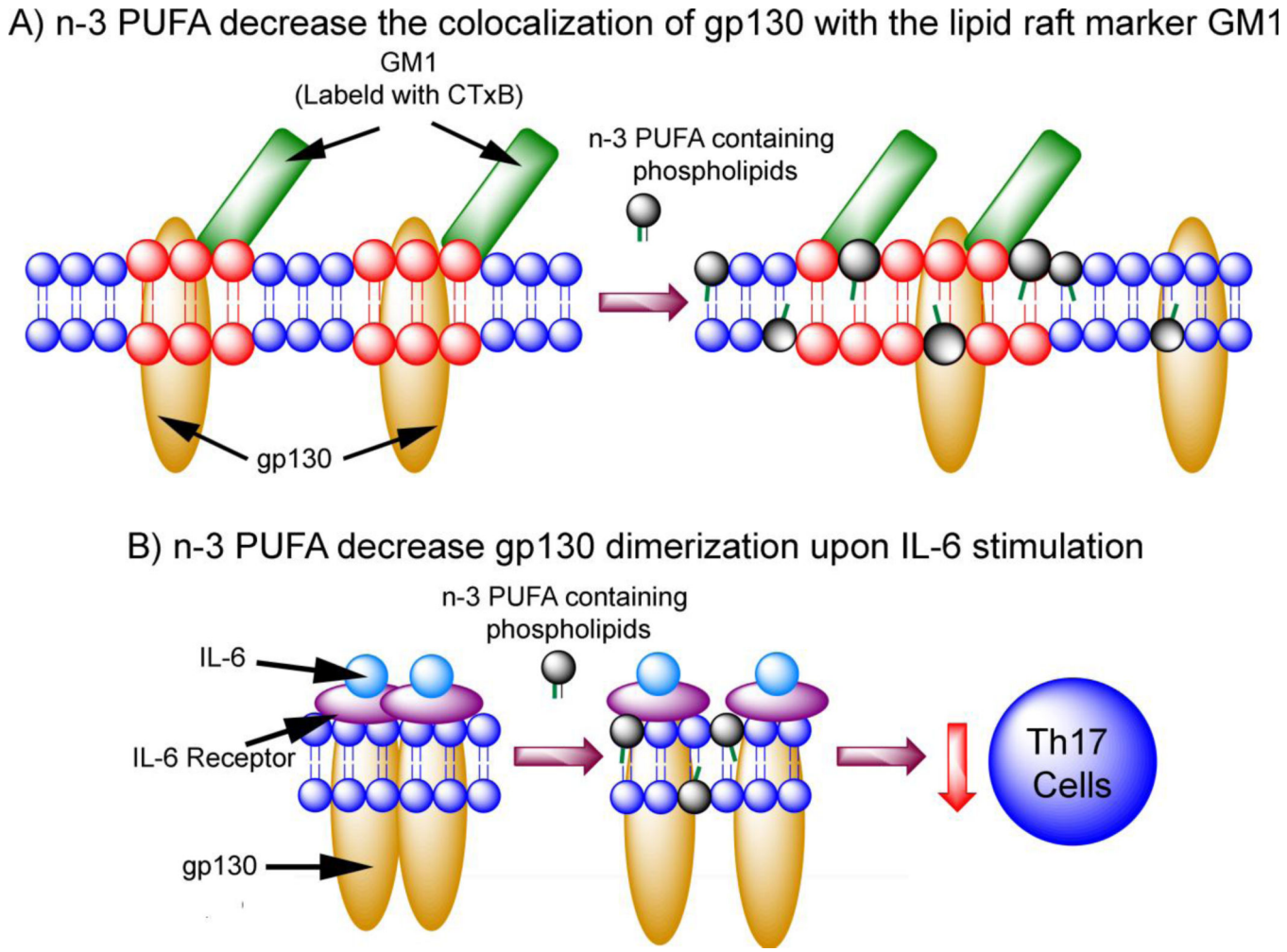

Figure 3.

Proposed mechanism by which n-3 PUFA suppress T_H17 cell differentiation. n-3 PUFA directly modulate IL-6/gp130 signaling at the plasma membrane, thus decreasing T_H17 differentiation. **A**) n-3 PUFA decrease colocalization of gp130 in lipid rafts, as assessed by colocalization with cholera toxin (CTxB), a marker of lipid rafts. Red highlight indicates liquid ordered lipid rafts; Blue indicates bulk membrane. **B**) n-3 PUFA decrease the dimerization of gp130 upon IL-6 stimulation, suppressing downstream T_H17 differentiation (Allen et al., 2014).