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

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ABSTRACT

n-3 polyunsaturated fatty acids (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 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.

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, Treg, 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 by 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 $C20{:}4^{\Delta 5,8,11,14}$ fatty acid at the

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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 9000–100,000 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 (6–14 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

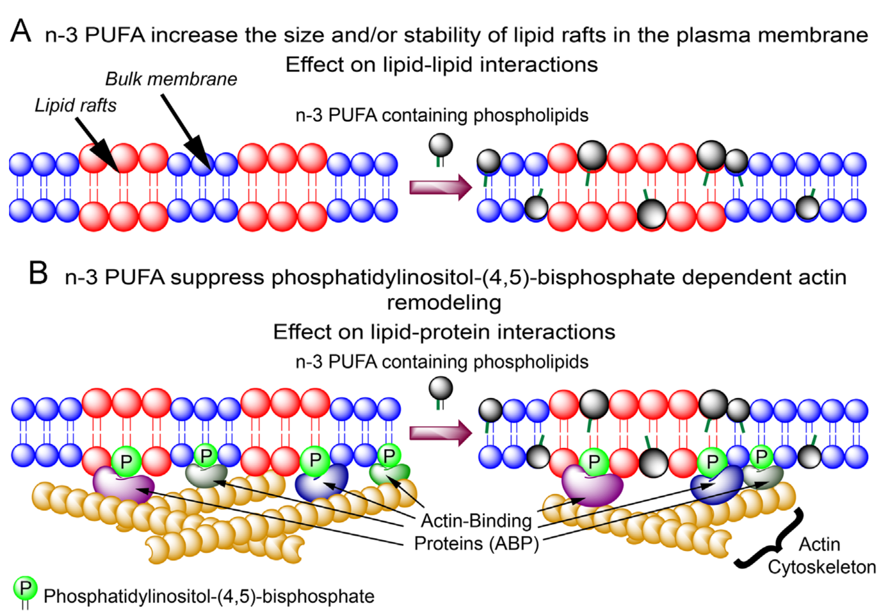

Fig. 1. Proposed mechanisms by which n-3 PUFA modulate adaptive immune responses by (A) modulating lipid–lipid interactions in the plasma membrane; and (B) altering plasma membrane lipid–protein interactions by decreasing PI(4,5)P_2 level, thereby lowering the recruitment of actin-binding proteins and 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)