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Polyunsaturated fatty acids and membrane organization: The balance between immunotherapy and susceptibility to infection

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Abstract

Polyunsaturated fatty acids (PUFAs), notably of the n-3 series, have immunosuppressive effects which make these molecules candidates for treating inflammatory symptoms associated with cardiovascular disease, obesity, arthritis, and asthma. However, immunosuppression by PUFAs could increase susceptibility to bacterial and viral infection. A detailed molecular picture is required in order to understand the balance between the benefits and risks of utilizing PUFAs as adjuvant immunosuppressants. Here we review evidence that incorporation of PUFAs into membrane lipids of antigen-presenting cells (APCs) downregulates APC function. We propose that PUFAs modulate antigen presentation by altering the organization of lipid and protein molecules of the plasma membrane and endomembranes; this alters recognition and responses by T cells. The foundation of our hypothesis is built on data from artificial bilayer experiments which provide the physical principles by which PUFA acyl chains affect membrane architecture. This review also reconciles conflicting results in the literature by discussing the advantages and disadvantages of differing methods of PUFA treatment of cells. We suggest that membrane modulation of immune cells may be an important and overlooked mechanism of immunomodulation. In addition, we propose that mechanistic studies with defined experimental protocols will speed the translation of laboratory studies on PUFAs to the clinic.

Keywords

antigen presentation; major histocompatability complex; membrane structure; polyunsaturated fatty acids

Introduction

The diet of the Western population is devoid of n-3 polyunsaturated fatty acids (PUFAs) which correlates with high rates of inflammation compared to populations that consume high levels of n-3 PUFAs and display low incidence of inflammation (Simopoulos 2002). Hence, the increasing incidence of inflammation, related to diabetes, cardiovascular disease, obesity, arthritis, asthma and the metabolic syndrome, may be linked to a lack of dietary consumption of n-3 polyunsaturated fatty acids (PUFAs) (Simopoulos, 2002). Based largely on epidemiological research, a major hypothesis has emerged that increasing n-3 PUFA consumption may be a physiological method to prevent or reduce inflammation. Cell culture and animal studies have generally substantiated this hypothesis by showing that n-3 and even some n-6 PUFAs exert immunosuppressive effects (Belch and Hill, 2000; Stulnig, 2003).

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Human clinical trials on the other hand have been less conclusive. In some cases, human clinical trials have verified the basic hypothesis, further advancing the idea that PUFAs could serve as adjuvant immunosuppressants in the clinic, but in other studies no beneficial effects of n-3 PUFAs have been found (Calder, 2006; Fritsche, 2006). A general limitation of all PUFA studies has been the lack of understanding of the targets and mechanisms of PUFAs at the molecular level (Shaikh and Edidin, 2006).

Here we review the role of PUFAs in modulating antigen presentation and effector T cell responses, a vital function of the adaptive arm of immunity in combating infection. We focus on the role of membrane modulation by PUFAs as a mechanism by which these lipids exert their effects, an overlooked but important target of immunomodulation. We draw on data from artificial bilayer experiments that show PUFAs as excellent modulators of membrane lateral organization. We conclude by discussing the different methodologies employed for studying PUFAs, which may be a source of conflicting results in the literature. We propose more unified and consistent methods of study in order to understand how these important, but misunderstood molecules function.

PUFAs exert immunosuppressive effects

PUFAs of the n-3 and some n-6 classes exert immunosuppressive effects on a variety of lymphocyte functions including generation of eicosanoids, cytokine production, phagocytosis, proliferation, and surface molecule expression (Hughes and Pinder, 1997; Kew et al., 2003; Costabile et al., 2005; Meydani, 1988). These effects, notably of the n-3 PUFAs may be beneficial for a variety of inflammatory disorders including arthritis, Crohns disease, atherosclerosis, psoriasis, graft-versus-host disease, systemic lupus erythematosus, multiple sclerosis, and asthma (Belluzzi et al., 1996; Broughton et al., 1997; Mayser et al., 2002; Takatsuka et al., 2002; Thies et al., 2003; Dwyer et al., 2004; Mills et al., 2005). For some of these diseases, the evidence for dietary n-3 PUFA supplementation to combat inflammation is strong whereas in other cases the data are weak. The best evidence for the beneficial effects of n-3 PUFAs on inflammation has come from studies on rheumatoid arthritis (RA) (Goldberg and Katz, 2007; Calder, 2006). Clinical trials show that dietary intake of n-3 PUFAs or fish oil provide some benefits to patients suffering from RA. A recent review concluded based on 17 clinical trials, 2 meta-analyses, and an Agency for Healthcare Research and Quality report that fish oil supplementation in the diet can lower the need for non-steroidal anti-inflammatory drugs, reduce tender joints, and morning stiffness (Calder, 2006). Still, there are clinical studies which show little effect of n-3 PUFAs on inflammation. One reason for this discrepancy may be due to limited sample size. As suggested by Calder, larger and better designed clinical trials are needed in order to make statistically significant conclusions about PUFAs and inflammation (Calder, 2006).

PUFAs and susceptibility to infection

The human body responds to diverse infections from bacteria, viruses, parasites, and fungi. The first line of defense against such pathogens is mediated by the innate arm of the immune system. A combination of chemical (e.g. inflammatory mediators) and humoral responses (e.g. complement activation) are instigated by a variety of cells (e.g. dendritic cells, macrophages, monocytes, γδ T cells, etc.). With time, the adaptive arm of the immune system is activated in which an antigen specific response is mounted by effector cells. This allows elimination of the pathogen through the production of antibodies (humoral response) or through the activation of other effector cells that are intimately connected with the innate immune system through cytokine production. A notable feature of adaptive immunity is the establishment of memory against a given pathogen.

Given that PUFAs, especially the n-3 class, exert immunosuppressive effects, it is reasonable to expect that dietary intake of these fatty acids would compromise an individual's response to infection. It is beyond the scope of this review to cover all of the studies on PUFAs and viral, bacterial, and parasitic infections. The readers should turn to an excellent review by Anderson and Fritsche on this matter for additional information (Anderson and Fritsche, 2002). Here we summarize by noting that most of the studies on PUFAs and infection have simply measured pathogen clearance or host survival (Anderson and Fritsche, 2002). The results from these studies are inconclusive. While many studies show that PUFAs diminish the ability to clear a pathogen or host survival, others show beneficial effects or no effects of PUFAs on fighting infection (Anderson and Fritsche, 2002). Possible factors which may influence the discrepant results are the limited number of studies and the variability in the diets administered between studies. In addition, mechanistic details are sparse. It is of extreme importance to learn these details given that n-3 PUFA-rich fish oil capsules are readily available over the counter and the inclusion of n-3 PUFAs in prepared foods is on the rise. Better dietary recommendations can be made once we have a detailed mechanistic and molecular understanding of PUFA modulation of innate and adaptive immunity.

Molecular mechanisms by which PUFAs modulate immune function

N-3 and n-6 PUFAs are thought to exert immuomodulatory effects through four major mechanisms which are not mutually exclusive. These are:

a. Membrane modulation – Of the four major mechanisms by which PUFAs can alter cellular function, membrane modulation has been the least studied and is the focus of this review. Dietary intake of PUFAs results in their incorporation into the membranes of essentially all cells in the body. PUFAs are primarily esterefied into the *sn*-2 acyl chain position of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) phospholipids (Stubbs and Smith, 1984). The only exception to this rule is in neuronal tissues where PUFAs are esterefied to phosphatidylserines (PS) (Stubbs and Smith, 1984). In tissues devoid of PUFAs, uptake into the membrane can result in dramatic changes in the acyl chain profile of membrane lipids. For instance, incorporation of docosahexaenoic acid (DHA, 22:6) into certain tissues can increase the membrane composition of this n-3 PUFA by 8-fold (Salem Jr., 1986.). It is likely that such changes will affect lipid-protein interactions and membrane lateral organization (Stillwell and Wassall, 2003).

b. Eicosanoid metabolism – eicosanoids are twenty carbon lipid mediators of inflammation. The major eicosanoid precursor is the n-6 PUFA arachidonic acid (AA, 20:4), which upon cleavage from phospholipids with phospholipase A2 serves as a substrate for cycloxygenase (COX) and lipoxygenase (LOX) enzymes leading to the production of proinflammatory cytokines (Calder, 2002). One mechanism by which n-3 PUFAs exert immunosuppressive effects is that by competing with AA as substrates for COX and LOX enzymes, AA metabolism is inhibited, thereby lowering the production of proinflammatory eicosanoids. More recent studies have shown that n-3 PUFAs can generate novel eicosanoids that have anti-inflammatory properties (Serhan and Savill, 2005). We note that n-6 PUFAs can also exert anti-inflammatory effects (Sacks and Campos, 2006). For example, the n-6 PUFA dihomo-γ-linolenic acid (DGLA, 20:3) can act as a competitive inhibitor of eicosanoid metabolism and inhibit the production of proinflammatory cytokines (Belch and Hill, 2000).

c. Gene expression – A considerable amount of information is available about the way PUFAs alter gene expression, either by affecting signaling pathways or directly by interacting with nuclear receptors (Nakamura et al., 2004). PUFAs can directly modify transcription by interacting with sterol regulatory element binding proteins,

liver X receptor, and peroxisome proliferator activated receptors (PPARs). This in turn can influence cellular signaling.

d. Cellular signaling – Alterations in cellular signaling are tied to all three of the other mechanisms. Some of the best evidence for the role of PUFAs in immune cell signaling comes from studies in which PUFAs downregulate T cells signal and proliferation by altering the organization of membrane bilayers (Switzer et al., 2004; Zeyda and Stulnig, 2006).

Modulation of antigen presentation with PUFAs

Overview of antigen presentation by MHC glycoproteins

The adaptive arm of the immune system uses major histocompatability complex (MHC) glycoproteins on the surface of antigen presenting cells (APCs) to trigger cognate T cells resulting in the elimination of pathogens. Peptide fragments derived from most bacteria and parasites residing in vesicular compartments (lysosomes and endosomes) are presented by MHC class II molecules. These molecules bind to the T cell receptor (TCR) of $CD4^+$ T lymphocytes which in turn activate other effector molecules to mount an immune response. Peptide fragments derived from viruses and some bacteria residing in the cytosol are presented by MHC class I molecules to $CD8^+$ cytotoxic T lymphocytes (CTLs). Successful engagement of MHC class I molecules with the TCR of $CD8^+$ CTLs and co-stimulation through co-receptors insures destruction of the infected APC. The two classes of MHC molecules differ in their trafficking mechanisms to the cell surface but are structurally similar. We review below the effects of PUFAs on MHC class I and II glycoproteins. We first cover MHC class II since far more is know about it in this area than about MHC class I.

PUFAs and antigen presentation through MHC class II

A number of laboratories have tested the effects of PUFAs on MHC class II surface expression and antigen presentation (Hughes and Pinder, 1996; Khair-el-Din et al., 1996; Weatherill et al., 2005; Schweitzer et al., 2006). Since MHC class II molecules are upregulated in a variety of autoimmune and inflammatory disorders, downregulation with PUFAs may be of clinical importance (Calder, 2006). The majority of studies indeed show that addition of PUFAs to cell culture or feeding PUFAs to animals and humans lower MHC class II surface expression (Table 1). As an example, treatment of INF-γ stimulated monocytes with eicosapentenoic acid (EPA, 20:5)/DHA (3:2) at a ratio found in fish oil capsules lowered MHC class II surface levels and lowered the ability of APCs to present tetanus toxoid antigen to autologous lymphocytes (Hughes and Pinder, 1997). In another study, animals fed an n-3 rich diet had lowered MHC class II expression on the surface of dendritic cells (DCs). These DCs could not present keyhole limpet hemocyanin (KLH) antigen to KLH-sensitized spleen cells relative to controls (Sanderson et al., 1997). In many of these studies, it has also been observed that adhesion molecules are downregulated by PUFAs. In a study in which humans were provided fish oil supplements for 21 days, it was found that in addition to a reduction in MHC class II levels, ICAM-1 and LFA-1 expression were lowered on peripheral blood monocytes (Hughes and Pinder, 1997).

One study showed that treatment of macrophages with 100 μM DHA, EPA, and AA lowered MHC class II expression by reducing the number of mRNA transcripts of $I\alpha\beta^b$ molecules (Khair-el-Din et al., 1995). Except for this, the molecular mechanisms by which PUFAs lower MHC class II surface expression have not been defined. In addition, the relationship between change in surface MHC class II levels and antigen presentation has not been examined. In one of the studies on PUFAs and DCs, it was stated by the authors that the reduction in MHC class II surface levels appeared to be too low to account for the reduction in the ability to present

antigen (Sanderson et al., 1997). We found the same discrepancy for MHC class I (Shaikh and Edidin, 2007).

There are a few studies where PUFA treatment had no effect on MHC class II surface expression or even increased surface levels (Table I). HLA-DR expression was not affected in a recent study that showed EPA and AA treatment could block DC activation (Zeyda et al., 2005). In another study, cells from fish oil-fed mice stimulated with platelet-activating factor showed an increase in MHC class II surface expression (Erickson et al., 1997). We think that differences in MHC class II surface expression between studies may be due to differing methods of lipid treatment of cells. This will be discussed at length later in the review.

PUFAs and antigen presentation by MHC class I

Although the vast majority of studies on PUFA modulation of antigen presentation have focused on surface expression of MHC II glycoproteins, there are some data available on MHC class I glycoproteins (Table 1). The earliest work on lipid modulation of MHC class I surface expression focused on membrane structure (Muller et al., 1983). It was shown by Muller et al. (1983) that decreasing membrane microviscosity with egg PC increased MHC class I surface expression while increasing membrane microviscosity with cholesterol decreased MHC class I surface expression (Muller et al., 1983). Surface expression of a variety of other proteins (e.g. Thy 1.2, Fc receptors, insulin receptors) was also affected by membrane microviscosity (Muller and Krueger, 1986). This phenomenon was collectively termed vertical phase separation (Muller and Krueger, 1986). However, there were serious limitations of the vertical phase separation model. First, no proof was presented that changes in surface expression were directly linked to changes in membrane microviscosity. Second, there was no evidence that changes in surface expression would have any functional consequences.

We recently revisited the vertical phase separation model to test if phospholipids containing PUFAs would have any effects on MHC class I surface levels and consequent functional effects (Table 1). We expanded the membranes of B lymphoblasts using the polymer polyvinypyrollidone (PVP) and incorporated heteroacid PCs with a *sn*-1 saturated acyl chain and a *sn*-2 PUFA acyl chain. We found that AA and DHA acyl chains of PCs increased human MHC class I surface expression by ~10–15% and this correlated with a decrease in membrane microviscosity (Shaikh and Edidin, 2007). The increase in MHC class I surface expression was not sufficient to modulate susceptibility of the lipid modified APCs to $CD8^+$ T cell lysis (Shaikh and Edidin, 2007). Although our findings support the original concept that lipids can modulate the epitopes of MHC class I molecules, this method of lipid treatment has limited physiological relevance. More will be discussed about this issue later in the review.

Nearly a decade after the vertical phase separation model was proposed, the Jenski laboratory showed that feeding PUFAs to murine cells had functional consequences for MHC class I. They found that murine MHC class I conformation could be altered by fusing mouse T27A tumor cells with phospholipids vesicles containing DHA (Jenski et al., 1993; Pascale et al., 1993). Changes in conformation were measured with antibody binding assays. PC vesicles containing DHA but not oleic acid (OA) resulted in the increase in expression of one MHC class I epitope while the expression of another decreased. More importantly, fusion of tumor cells with DHA-containing but not OA-containing vesicles increased their susceptibility to CTL lysis. Although the relationship between MHC class I conformation and increased CTL activity was not demonstrated, it established a role for DHA in modifying antigen presentation through MHC class I. More recently, our laboratory has shown the treatment of human B lymphoblasts with PUFAs can modify antigen presentation (Shaikh and Edidin, 2007). Treatment of B lymphoblasts with either AA or DHA as free fatty acids complexed to BSA reduced APC lysis by alloreactive CTLs. Unlike Jenski's study, we could not detect changes in conformation of MHC class I as assessed by antibody binding. Rather, we observed a small

reduction in MHC class I surface expression and a substantial decrease in the rate of APC-T cell conjugation upon incorporation of PUFAs (Shaikh and Edidin, 2007). These findings suggest that PUFAs are modifying the adhesion properties of APC-T cell conjugates and perhaps altering the formation of the immunological synapse. Our findings are very similar to data on MHC class II surface expression and antigen presentation, suggesting commonalities in the mechanisms by which PUFAs exert their effects. However, the immunological implications of our data are somewhat different from the MHC class II model system. While downregulation of MHC class II antigen presentation could reduce inflammation, inhibition of MHC class I antigen presentation could make an individual more susceptible to viral infection. The benefits of lowering MHC class I levels could be meaningful for some autoimmune disorders. For instance, type 1 diabetes is an autoimmune disease in which autoreactive cytotoxic T cells destroy pancreatic β-cells expressing peptide fragments presented by MHC class I molecules (Panina-Bordignon et al., 1995; Panagiotopoulos et al., 2003). Lowering of MHC class I levels would be advantageous for pancreatic β-cells.

PUFAs and antigen presentation to NK cells

NK cells are cytotoxic lymphocytes activated by interferons or macrophage-derived cytokines during the innate response to infection. They function to contain viral infections while the adaptive immune system generates a more specific antigen response to clear the infection. NK cells also distinguish infected from uninfected cells based on activation of ‘activating’ or ‘inhibiting’ receptors. The effects of PUFAs on NK cell activity are conflicting and lack mechanistic details. Rat hepatoma cells modified with oleic or linolenic acid had an increased susceptibility to NK cell mediated lysis (Yoo et al, 1982). Another study showed that incubation of cells with egg PCs containing some unsaturation had no effect on NK cell activity (Roozemond and Bonavida, 1985). The most physiologically relevant studies show that feeding animals n-3 PUFAs lowers NK cell activity. In one study, a high fat diet rich in either olive oil, n-6 PUFAs, or n-3 PUFAs lowered NK cell activity relative to a low fat diet (Yaqoob et al., 1994). The n-3 PUFA diet showed the greatest reduction of NK cell activity. In another study, only n-3 PUFAs but not n-6 PUFAs fed to animals lowered NK cell activity (Sasaki et al., 2000). Again, the differences between studies may lie in methodology.

PUFAs and membrane organization

As described above, PUFAs can affect the surface levels of both MHC class I and class II glycoproteins and modulate antigen presentation. However, the mechanisms by which PUFAs exert their effects are poorly characterized. We propose that PUFAs can modify membrane organization and thereby influence a variety of cellular events including the expression of surface proteins. We first describe below the physical principles which make PUFAs unique to modify bilayer organization. Subsequently, we propose novel hypotheses on how PUFAs may affect antigen presentation.

PUFA acyl chains have a unique orientation

The multiple double bonds in PUFA acyl chains have many consequences for bilayer organization (Stillwell and Wassall, 2003; Feller and Gawrisch, 2005). PUFAs acyl chains have been shown to affect bilayer properties including lateral pressure, microviscosity, curvature, permeability, elasticity, microdomain formation, hydrophobic match, which can all affect protein function (Stubbs and Smith, 1984; Lavoie et al., 1991; Cantor, 1999; Stillwell and Wassall, 2003; Carrillo-Tripp and Feller, 2005; Feller and Gawrisch, 2005). Relative to saturated or monounsaturated acyl chains, PUFA acyl chains are highly disordered (Feller et al., 2002; Shaikh et al., 2003). Biophysical measurements have shown that PUFA acyl chains have high conformational flexibility despite the rigidity of the double bonds (Feller and Gawrisch, 2005). The conformational flexibility arises from the low potential energy barriers

to rotation about the single carbon-carbon bonds. The degree of flexibility is not uniform among all PUFA acyl chains and varies depending on the acyl chain length and the number of double bonds. For instance, AA-containing phospholipid bilayers are more disordered and deformable than DHA-containing phospholipid bilayers (Rajamoorthi et al., 2005). Differences in order between n-3 and n-6 acyl chains may influence membrane physical properties which could explain some of the differing functional consequences for these two subclasses of PUFAs (Eldho et al., 2003).

PUFAs, phase separation, and protein function

PUFA-containing phospholipids can modulate the activity of a number of membrane proteins by either direct protein-lipid interactions or through changes in membrane organization. The role of PUFAs in modulating lateral organization is well established in artificial bilayer systems (Stillwell and Wassall, 2003). It has been demonstrated that PUFA acyl chains phase segregate from membrane microdomains enriched in sphingolipids and cholesterol. Phase separation is driven by steric incompatibility between PUFA acyl chains and cholesterol molecules. This has led to the hypothesis that PUFA acyl chains even promote the formation of sphingolipid cholesterol-rich “raft” microdomains (Shaikh et al., 2001; Shaikh et al., 2002; Shaikh et al. 2003; Shaikh et al., 2004). However, the evidence for this is limited in cellular systems where the nature of raft microdomains remains in question (Edidin, 2003). Nevertheless, steric incompatibility between PUFAs and cholesterol molecules may cause proteins to redistribute in the membrane (Shaikh et al., 2004). Protein redistribution between ordered domains and unsaturated acyl chain-containing disordered domains has been demonstrated in artificial bilayers (Hammond et al., 2005).

It is also possible that PUFA acyl chains directly interact with proteins and thereby alter their activity. This hypothesis is supported by crystal structures which show that PUFA acyl chains directly interact with protein surfaces (Malkowski et al., 2000). The best chacterization of direct PUFA-protein interactions comes from studies on rhodopsin, which show that DHA acyl chains can modulate its conformational state (Grossfield et al., 2006).

There are a variety of ways in which PUFAs could affect antigen presentation by either changing membrane bilayer organization or by interactions with proteins. We discuss each of these below:

a. PUFAs, membrane microviscosity, and lateral organization—Many studies over the years have suggested that lipids including PUFAs can alter membrane microviscosity and so affect cellular function. Changes in microviscosity do appear to be important for antigen presentation. For instance, a recent study showed that infection of APCs with *Leishmania donovani* resulted in an increase in membrane fluidity and a reduction in antigen presentation by macrophages to T cells by disrupting lipid rafts (Chakraborty et al., 2005) The effects could be reversed with the addition of exogeneous cholesterol. However, the link between membrane microviscosity and protein function has not been established.

We suggest that a good starting point in studying PUFAs and membrane organization is to understand how PUFAs affect the lateral organization of MHC glycoproteins, costimulatory molecules, and adhesion proteins, all of which are required for an effective T cell response upon conjugation with APCs. Our laboratory and others have shown that MHC class I and class II molecules are clustered on the surfaces of APCs and the degree of clustering can modulate function (Jenei et al., 1997; Fooksman et al., 2006). We recently showed that cholesterol depletion increases MHC class I clustering by modulating the organization of the actin cytoskeleton (Kwik et al., 2003; Fooksman et al., 2006). In turn, clustering of class I molecules enhances presentation of antigen to $CD8^+$ T cells (Fooksman et al., 2006). In this case, membrane lipid modulation mediated functional changes through the signaling lipid

phosphatidylinositol 4,5-bisphosphate (PIP2) and not directly by changes in membrane viscosity.

PUFA modulation of adhesion molecules also needs to be examined. We recently showed that APCs treated with AA and DHA do not conjugate as effectively with T cells as controls (Shaikh and Edidin, 2007). One possibility is that PUFAs modulate the lateral organization of adhesion molecules. A recent study showed that different receptor conformations of LFA-1 on T cells had different diffusion coefficients for different receptor conformations which correlated to the activation state of the T cell (Cairo et al., 2006). Lateral organization is also linked to the cytoskeletal organization of the cell. Therefore, like cholesterol depletion, PUFAs may be regulating cytoskeletal interactions in unison with changes in lateral organization. The Stulnig laboratory has shown that treatment of T cells with PUFAs but not saturated fatty acids inhibits formation of the immunological synapse, which is defined as the synapse between APCs and T cells (Geyeregger et al., 2005). Inhibition of adhesion by PUFAs was shown to have effects through the nucleotide exchange factor VAV, which controls cytoskeletal rearrangements during the formation of the synapse. So, a plausible hypothesis is that PUFAs may exert changes in the lateral organization of membrane proteins by altering the membrane skeleton.

b. PUFAs and membrane microdomains—Lipid microdomains are another aspect of membrane lateral organization and membrane microviscosity. An important question is can PUFAs modulate antigen presentation by altering the formation of membrane microdomains? In artificial membranes, PUFAs laterally phase segregate from cholesterol-rich domains, driven by the steric incompatibility between cholesterol molecules and PUFA acyl chain (Huster et al., 1998; Shaikh et al., 2001; Brzustowicz et al., 2002; Shaikh et al., 2002; Shaikh et al., 2003; Shaikh et al., 2004). However, evidence for these types of domains has remained elusive in cellular systems. The artificial membrane results raise the possibility that PUFA-domains, as it were anti-rafts, could exist in cells. We have recently suggested that we need to probe for other membrane microdomains and a good starting point is to look for PUFA-enriched domains in membranes such as the ER where cholesterol levels are low and PUFA levels are high (Shaikh and Edidin, 2006).

MHC class I and II molecules are associated with lipid rafts defined by detergent extraction (Anderson et al., 2000; Goebel et al., 2002; Bene et al., 2004). However, detergent extraction may itself create artifacts of protein lipid interactions (Heerklotz, 2002). In any case, PUFAs can laterally segregate from cholesterol-rich domains as the bilayer studies suggest, then this could have functional consequences for MHC proteins, adhesion molecules, and co-stimulatory molecules. One of the best examples of the role of PUFAs in modulating cellular function through organization of membrane microdomains is in T cell signaling, which is initiated in response to effective MHC-TCR interactions (Ma et al., 2004). A central player in T cell signaling is the protein LAT (linker for T cell activation), which is localized to detergent-resistant membranes (Zeyda et al., 2002). Addition of exogenous PUFAs in cell culture displaces LAT from detergent resistant microdomains (DRMs) and correlates with downregulation of T cell signaling (Stulnig et al., 2001; Zeyda et al., 2002). This has been proposed to be one mechanism by which PUFAs affect T cell signaling and perhaps lower T cell activation and proliferation. It is important to note that some of the evidence on PUFAs and DRMs is not in agreement with the membrane biophysical studies and we recently proposed a hypothesis to reconcile the differences (Shaikh and Edidin, 2006). Nonetheless, the results of PUFA modulation of T cells through membrane microdomains suggest that further work is needed in this area.

c. PUFA modulation of protein trafficking—There is little information available about the effects of PUFAs on endomembranes. We recently showed that both AA and DHA uptake inhibits the trafficking of nascent MHC class I molecules from the ER to Golgi complex,

suggesting PUFA incorporation affects endomembrane function (Shaikh and Edidin, 2007). Another recent study by Seo and co-workers showed that DHA can inhibit Ras trafficking through the secretory pathway (Seo et al., 2006). Inhibition of trafficking could be a result of PUFAs modifying the structure of membrane vesicles in the secretory pathway. As an example, PUFAs may modify the membrane structure of the Golgi complex. Phase separation between sphingolipids/cholesterol and PUFA-rich regions could alter the rate of COPI mediated vesicle transport to the plasma membrane.

d. PUFA modulation and protein conformation—PUFAs can affect the conformation of multispan membrane proteins such as rhodopsin, the photo-inducible G-protein coupled receptor (GPCR) of the rod outer segment (Brown, 1994; Feller and Gawrisch, 2005). Rod outer segment membranes isolated from animals fed n-3 deficient diets showed a drastic reduction in rhodopsin signaling due to replacement of DHA with the n-6 docosapentenoic acid (DPA, 22:5) which correlated with changes in acyl chain order (Niu et al., 2004). Recent biophysical measurements and molecular dynamic simulations show that DHA specifically binds in the grooves between the helixes of rhodopsin (Eldho et al., 2003; Grossfield et al., 2006).

It is reasonable to expect then that single transmembrane proteins such as MHC proteins are also affected by changes in acyl chain order. As described above, conformation of MHC class I is influenced by DHA in the diet and in cell culture (Jenski et al., 1993; Pascale et al., 1993). MHC class II proteins have also been shown to be influenced by their lipid environment. A study by Roof et al., showed that specific acyl chains (including a PUFA) and phospholipid headgroups altered MHC class II conformation which led to an increase in extracellular peptide binding by 10 to 50 fold (Roof et al., 1990). Perhaps the changes in peptide binding to MHC class II are due to a change in orientation of the protein as a result of changes in lipid composition. Interactions between the ionic headgroups of MHC class I can establish a new orientation of the protein (Mitra et al., 2004).

e. PUFA modulation of T cell responses by membrane transfer from APCs—T cells upon conjugation with APCs can extract membrane fragments and surface molecules of the APCs (Joly and Hudrisier, 2003). It is unknown if PUFAs influence the rate of this so called trogocytosis. MHC class I molecules on the surface of APCs can be transferred to cognate T cells (Hwang et al., 2000). Additional molecule such as B7 and ICAM-1 are also taken up (Hwang et al., 2000). T cells that take up MHC class I molecules are then subject to fracticide, or lysis by neighboring CTLs (Hudrisier et al., 2001). Similar observations have been made with MHC class II molecules transferring molecules to $CD4^+$ T cells in addition to transfer of molecules from T cells to APCs (Patel et al., 1999; Nolte-'t Hoen, 2004). The functional relevance of trogocytosis remains unclear, but several suggestions have been made. One possibility is that uptake of membranes from APCs contributes to the metabolism of the T cell (Joly and Hudrisier, 2003). Clearly, if there is an increase in the PUFA content of the APC, then uptake of PUFA molecules by T cells could influence a variety of events such as TCR organization and signaling. In addition, transfer of molecules could influence T cell signaling or may be a mechanism by which cell-cell contact is terminated (Hwang et al., 2000). Transfer of molecules between cells via trogocytosis has also been suggested as a means by which pathogens could spread (Joly and Hudrisier, 2003). This would be consistent with findings that upon viral infection, CTLs proliferate rapidly followed by a sudden disappearance or “exhaustion” (Hanon et al., 2000).

Reconciling different methods of lipid treatment

It is clear from our summary that the data on PUFA modifcation of APC function are often conflicting. Conflicting data can arise from differences in methods, cell lines, concentrations

of lipids, and oxidation state of lipids. In particular, more consistent methods of lipid treatment between laboratories will allow us to make more significant conclusions on how PUFAs modulate immune function. Of course, consensus methods of lipid treatment are not easy to establish since different questions require different conditions. However, an awareness of this issue is warranted in light of the some of the conflicting data we described above on PUFA modulation of NK cell activity and MHC class I or II mediated antigen presentation.

A broad spectrum of methods to modulate lipids is utilized in cell culture, animals and humans. In Table 2, we list different methods used to alter cellular lipids and with each method we point out the major advantages and disadvantages. Cell culture studies can employ differing methods to isolate the effects on lipids on membrane structure using polyvinylpyrollidone (PVP) membrane expansion or vesicle fusion to incorporate phospholipids into the plasma membrane. Both methods have physiological limitations and do not yield similar results. We have found that when we treat B lymphoblasts with 16:0-20:4PC and 16:0-22:6PC phospholipids for 2 hours in the presence of PVP, MHC class I surface levels increased (Fig. 1A). In contrast, fusing small unilamellar vesicles (SUVs) with the same phospholipids had no effects on MHC class I surface levels (Fig. 1A).

The more common methods of cell culture focus on metabolism by treating cells with varying concentrations of lipids as FFAs or FFAs complexed to BSA in the absence or presence of serum in the medium (Table 2). A few major factors that affect cellular metabolism and hence the outcome of feeding can be better controlled. First, the FFA/BSA ratio is important. In the absence of BSA, only low concentrations of lipid can be tested, which is relevant if one is trying to understand the effects of unbound FFAs on cell function. Serum levels of unbound FFAs are in the nanomolar range while bound FFA levels are in the micromolar range. Selecting the right ratio of FFA to BSA should take into account the model system. For instance, for models of obesity, one should utilize high ratios of FFAs to BSA (e.g. 6/1) to mimic in vivo conditions. In the literature, there is a large range of FFA/BSA ratios to mimic normal physiological conditions (e.g. from 1/1 to 5/1). Second, the effects of serum must be taken into account. Some laboratories treat cells with PUFAs in the absence of serum while others use 5–10% serum. In our laboratory we have found that inclusion of serum can drastically affect the outcome of PUFA feeding. When measuring MHC class I surface levels of B lymphoblasts, we found a significant decrease in expression with PUFA treatment in serum-free conditions but no significant effects were observed in the presence of 10% FBS (Fig. 1B). Third, the type of BSA utilized can be better controlled. Some laboratories use fatty acid-free BSA while others use Fraction V BSA which contains fatty acids. Another confounding problem is the length of treatment which in turn is limited by culture conditions and method of lipid addition. For instance, one can treat cells with PUFAs for 72 hours in the presence of serum, but this is not possible in serum-free culture.

Animal and human lipid feeding studies are more physiologically relevant than cell culture studies although there are problems with these methods too (Table 2). We can only highlight these issues here. For further depth on problems associated with animal and human feeding, the reader should turn to a review by Fritsche (Fritsche, 2006). Both animal and human studies often result in only modest changes in lipid composition of tissues after feeding unlike cell culture where much larger effects are observed. The major limitation of feeding studies is selecting the right amount of lipids in the food source for experimentation and modeling a human diet. Defining control background diets is also difficult, especially in humans where there can be considerable variability in dietary consumption of PUFAs. A recent breakthrough in animal models has been the fat-1 transgenic mouse which can convert n-6 to n-3 fatty acids and thereby eliminate the need for external n-3 PUFA enriched diets (Kang et al., 2004). Clearly, researchers in the field have a variety of methods to choose from to address their questions. Perhaps a greater range of methods should be employed to address specific questions

from whole animals to cell culture studies. This will provide us with a broader picture of the molecular mechanisms by which PUFAs modulate cell-mediated immunity.

Conclusions

Studies on PUFAs and antigen presentation are in their infancy. Much of what we know about the immunosuppressive effects of PUFAs on treatment of inflammatory symptoms and modulation of susceptibility to infection has come from nutritional studies with highly variable outcomes. We lack a detailed molecular level understanding that will allow us to devise better dietary strategies for both the prevention and treatment of illnesses with PUFAs. One good starting point is to evaluate how PUFAs, upon incorporation into the membranes of immune cells, affect the organization bilayers and proteins. Analysis at the interface of nutritional studies, biochemistry, membrane biophysics and cell immunology may provide answers on how to most effectively utilize PUFAs in the clinic.

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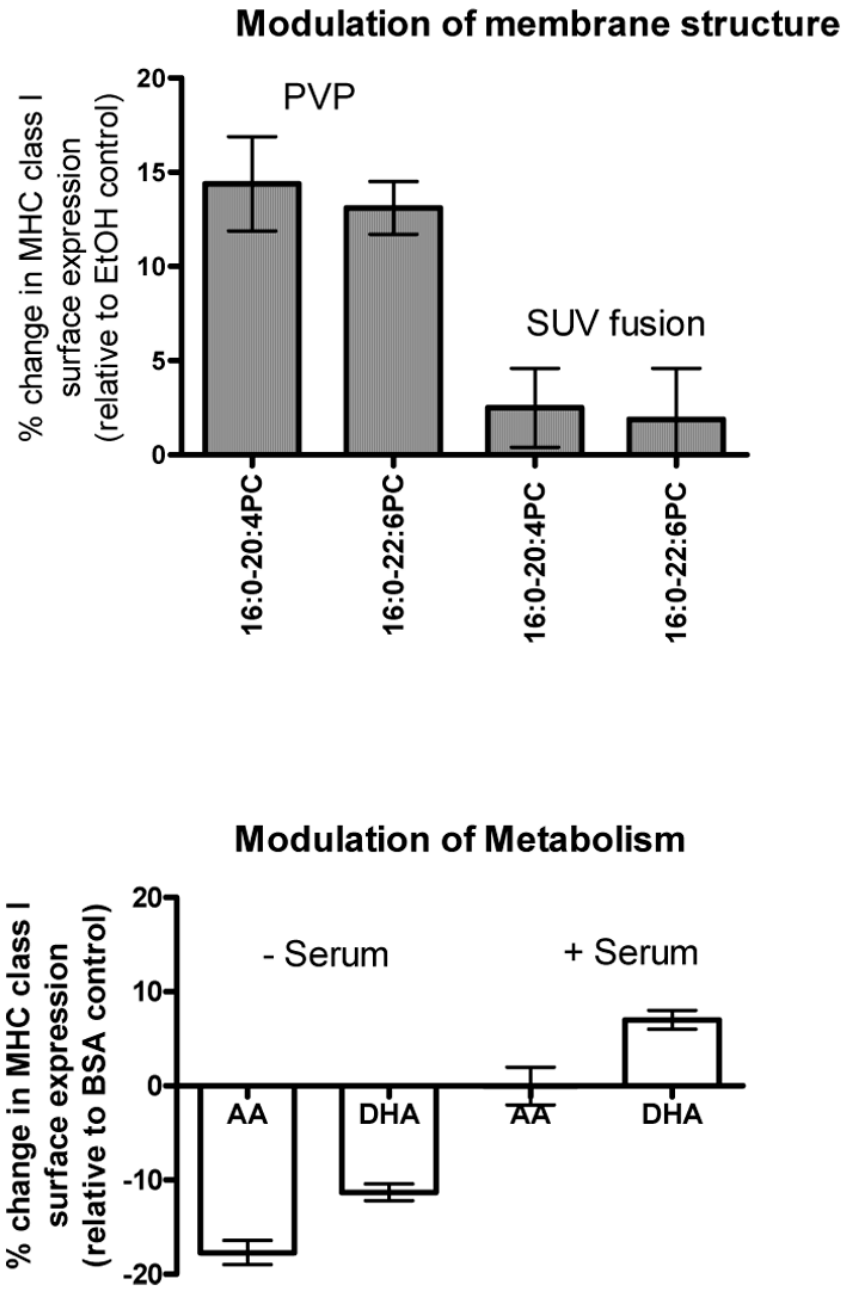

Figure 1.

The method of lipid delivery influences MHC class I surface expression levels. (Top) Modifcation of membrane structure with AA- and DHA-containing heteroacid PCs in the presence of the plasma membrane expander PVP for 2 hours increases MHC class I surface levels of human B lymphoblasts. Modification with the same heteroacid PC SUVs has no effect on MHC class I expression. (Bottom) Feeding human B lymphoblasts 100 μM AA and DHA complexed to fatty acid-free BSA for 12 hours in serum-free conditions lowers MHC class I surface levels whereas no effects are observed in the presence of serum. Surface expression levels of MHC class I were obtained and quantified with FACS analysis of Ke2-Cy5 antibody binding to HLA-A2 and –B7 alleles.

Table 1

Major histocompatability complex (MHC) class I and II surface expression is differentially modulated by lipid treatment.

Lipids	System	Effect on MHC Expression	Mechanism	Reference
		MHC CLASS I		
Delipidated serum	Human HL60 and U-937 cells	↓ expression	Unknown	(Schweitzer et al., 2006)
PVP membrane expansion and cholesteryl hemisuccinate or egg PC	Mouse BALB/c spleen cells	↓ expression with cholesteryl hemisuccinate ↑ with egg PC treatment	Vertical phase separation (or vertical orientation)	(Muller et al., 1983)
PVP membrane expansion with AA- or DHA-containing PCs	Human B lymphoblasts	↑ expression	Unknown (presumably vertical orientation)	(Shaikh and Edidin, 2007)
AA and DHA	Human B lymphoblasts	↓ expression	Inhibition in MHCI trafficking	(Shaikh and Edidin, 2007)
Vesicle fusion with DHA-containing PCs	Mouse EL4 lymphomas	↑ expression of one epitope ↓ expression of another	Conformation	(Pascale et al., 1993)
Cholesterol depletion	Human B lymphoblasts	No change in expression	Alteration in MHC I clustering	(Fooksman et al., 2006)
		MHC CLASS II		
Delipidated serum	Human HL60, U-937, mouse L-1210 cells	↓ expression	Alterations in lysosomal acidity	(Schweitzer et al., 2006)
DHA	Mouse macrophages	↓ expression	Unknown but independent of eicosanoid metabolism	(Khair-el-Din et al., 1996)
DHA	Mouse dendritic cells	↓ LPS-induced upregulation	Mediated in part through toll-like receptors	(Weatherill et al., 2005)
DHA, AA, EPA	Mouse macrophages	↓ IFN-γ induced expression.	Influence on Ia mRNA transcripts	(Khair-el-Din et al., 1995)
AA, EPA	Human dendritic cells	No change	unknown	(Zeyda et al., 2005)
EPA, DHA	Unstimulated human monocytes	↓ expression with EPA ↑ expression with DHA	unknown	(Hughes and Pinder, 1996)
EPA, DHA and EPA/DHA (3:2)	IFN-γ stimulated human monocytes	↓ expression	unknown	(Hughes and Pinder, 1997)
Fish oil	Rat dendritic cells	↓ expression	unknown	(Sanderson et al., 1997)
Fish oil	Stimulated exudate mouse macrophages	↑ expression	unknown	(Erickson et al., 1997)

Table 2

Advantages and disadvantages of differing methods of PUFA treatment.

Method of lipid treatment	Advantages	Disadvantages
Fusion with lipid vesicles	• Easy, cheap • Good method for altering plasma membrane structure/organization	• not physiologically relevant • cannot measure effects with saturated phospholipids at 37±C
Treatment with phospholipids in the presence of PVP	• Easy, cheap • Good method for altering membrane structure/organization	• not physiologically relevant • must rule out any effects of PVP
FFAs in serum free medium	• Easy, cheap • Avoid possible effects of serum fatty acids • Limited to low concentrations of free fatty acids • Assess effects of unbound FFAs • Achieve large changes in lipid composition	• Not physiologically relevant • Cell metabolism in serum free medium may be different than in serum • Physiological relevance limited compared to animal models
FFAs complexed to fatty acid free BSA in serum free medium	• Easy, cheap • Avoid possible effects of serum fatty acids • Test large range of fatty acid concentrations • Achieve large changes in lipid composition	• Cell metabolism in serum free medium may be different than in serum • Physiological relevance limited compared to animal models • More difficult to isolate effects of unbound FFAs
FFAs complexed to fatty acid-free BSA in medium with serum	• Easy, cheap • Physiologically relevant method • Cells can accommodate high levels of FFAs (>100μM) • Achieve large changes in lipid composition	• Physiological relevance limited compared to animal models • Difficult to isolate effects of unbound FFAs
Animal feeding	• Physiologically relevant • Avoid artifacts of cell culture • Control genetic variability • Control normal diet	• Expensive • Control diets do not adequately represent normal diets • Difficult to achieve large changes in membrane lipid composition
Human feeding	• The most physiologically relevant method	• Expensive • Difficult to design control diet • Need large study groups to make statistically significant conclusions • Difficult to achieve large changes in lipid composition • Large genetic heterogeneity in the population
Fat-1 transgenic mouse	• Alter n-6/n-3 ratio in vivo • No need for manipulations of the animal diet	• Expensive • Difficult to obtain