Anti-Inflammatory Effects of Omega-3 Fatty Acids in the Brain: Physiological Mechanisms and Relevance to Pharmacology

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Abstract—Classically, polyunsaturated fatty acids (PUFA) were largely thought to be relatively inert structural components of brain, largely important for the formation of cellular membranes. Over the past 10 years, a host of bioactive lipid mediators that are enzymatically derived from arachidonic acid, the main n-6 PUFA, and docosahexaenoic acid, the main n-3 PUFA in the brain, known to regulate peripheral immune function, have been detected in the brain and shown to regulate microglia activation. Recent advances have focused on how PUFA regulate the molecular signaling of microglia, especially in the context of neuroinflammation and behavior. Several active drugs regulate brain lipid signaling and provide proof of concept for targeting the brain. Because brain lipid metabolism relies on a complex integration of diet, peripheral metabolism, including the liver and blood, which supply the brain with PUFAs that can be altered by genetics, sex, and aging, there are many pathways that can be disrupted, leading to altered brain lipid homeostasis. Brain lipid signaling pathways are altered in neurologic disorders and may be viable targets for the development of novel therapeutics. In this study, we discuss in particular how n-3 PUFAs and their metabolites regulate microglia phenotype and function to exert their anti-inflammatory and proresolving activities in the brain.

I. Introduction

Polyunsaturated fatty acids (PUFAs) are generally considered to be essential fatty acids, meaning they are necessary for maintaining normal physiology, but cannot be produced by mammals and need to be provided by the diet (Bazinet and Layé, 2014). There are two main families of PUFAs, the n-6 and n-3 PUFAs (also referred as omega 6 and omega 3). Linoleic acid (LA; 18:2n-6) is the dietary-essential shorter-chain n-6 PUFA precursor of arachidonic acid (AA), whereas α-linolenic acid (ALA; 18:3n-3) is the dietary-essential shorter chain n-3 PUFA precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). AA, DHA, and EPA are also consumed in the diet, although as distinct sources. The major dietary sources of ALA are green plant tissues, nuts, flaxseed, and rapeseed oil, whereas oily fish is the main source of EPA and DHA.

PUFAs from the diet are absorbed from the gut to the blood and are available for storage (in the adipose tissue), conversion into longer-chain PUFA (mainly in the liver), or energy production through β-oxidation. LA and ALA biosynthetic pathway to AA and EPA and DHA, respectively, involves a series of desaturation, elongation occurring in the endoplasmic reticulum (Fig. 1). The last step in DHA formation involves β-oxidation, occurring in peroxisomes. As ALA and LA use the same metabolic pathways to generate long-chain (LC) PUFA, there is a competition between these two pathways, with end products generated, at least somewhat, proportional to their precursors.

Generally speaking, LA and ALA poorly accumulate in tissues, as compared with AA and DHA, which is in line with their role as precursors to longer-chain PUFA. The rate of synthesis of ALA into EPA and DHA occurring mainly in the liver is considered to be low, with about 8% of ALA being converted to EPA and 1% to DHA. The enzymes necessary to metabolize ALA are present in the brain; however, the brain’s major source of DHA is coming from the blood, as discussed later.

The brain is highly enriched in AA and DHA (Bazinet and Layé, 2014). Both n-3 and n-6 PUFAs are esterified in the sn-2 position into phospholipids, which are well known to play critical role in the structures and functions of brain cell membranes. Brain cell membrane contains mainly phosphatidylcholine, phosphatidylethanolamine, phosphatidyserine, phosphoinositides, and plasmalogens with specific PUFA profiles. At the level of the membrane, PUFAs undergo turnover due to the activity of phospholipase A2 (PLA2) and acyl-CoA lysophospholipid transferases. Two distinct groups of PLA2 are involved in the release of PUFA, namely the

ABBREVIATIONS: AA, arachidonic acid; AD, Alzheimer’s disease; AEA, anandamide; ALA, α-linolenic acid; AT, aspirin triggered; Aβ, amyloid-β; BBB, blood-brain barrier; CNS, central nervous system; COX, cyclooxygenase; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamide; DPA, docosapentaenoic acid; eCB, endocannabinoid; EFOX, electrophilic oxo-derivatives; EPA, eicosapentaenoic acid; EPEA, eicosapentaenoyl ethanolamide; ER, estrogen receptor; EV, extracellular vesicle; FABP, fatty acid–binding protein; FATP, fatty acid transport protein; GPR, G-coupled receptor; IL, interleukin; iNOS, inducible NO synthase; KO, knockout; LA, linoleic acid; LB, lipid bodies; LC, long chain; LOX, lipoxigenase; LPS, lipopolysaccharide; LT, leukotriene; lymphocyte; lysPFC, lysophosphatidylcholine; Lxa4, lipoxin A4; M2R, maresin; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NF, nuclear factor; NO, nitric oxide; NPD1, neutrophil protease D1; NSAID, nonsteroidal anti-inflammatory drug; PBMC, peripheral blood mononuclear cell; PG, prostaglandin; PLA2, phospholipase A2; PPAR, peroxisome proliferator-activated receptor; PUF A, polyunsaturated fatty acid; Rv, resolving; SPM, specialized proresolving mediator; TLR, Toll-like receptor; TNF, tumor necrosis factor; TX, thromboxane.
group IV cytosolic PLA2, which releases AA, and group VI calcium-independent phospholipase, which releases DHA. The free forms of PUFA are metabolized into specific derivatives [eicosanoids, specialized proresolving mediators, specialized proresolving mediator (SPMs), and endocannabinoids (eCBs)], which are key regulators of inflammation (Lukiw and Bazan, 2000; Buckley et al., 2013; Serhan, 2014; Calder, 2015; DiMarzo et al., 2015; Witkamp, 2016). AA derivatives mainly display proinflammatory activities, albeit there are some exceptions, whereas DHA derivatives are anti-inflammatory and proresolving.

More recently, particular attention has been paid to these derivatives in the regulation of neuroinflammation. Neuroinflammation is a double-edged sword that exerts both beneficial and detrimental effects on neurons. Microglia, the brain-resident innate immune cells, are thought to be protective when properly activated. However, inadequate activation worsens neuropathological processes and increases neuronal death, as observed in neurodegenerative diseases. The complexity of the microglia phenotype and its regulation may account for its protective and detrimental effects toward neurons, as discussed elsewhere. An increasing body of evidence suggests that PUFA and their derivatives may be involved in microglia regulation and the control of neuroinflammation (Layé, 2010; Bazinet and Layé, 2014). Furthermore, because of the high quantity of PUFA in the brain, specific alterations in PUFA metabolism in the brain may play an important role in neuroinflammatory events.

In this review, we will present recent updates on the metabolism and role of endogenous AA, DHA, and their bioactive derivatives involved in the resolution of neuroinflammation, with a specific focus on microglial cells. In particular, we will discuss how PUFAs can be used to target microglia and how drugs targeting PUFA metabolism regulate neuroinflammation. We will highlight recent controversies and examine adverse events.

II. Definition of PUFAs

Lipids represent 33%–40% of the energy intake in France and the United States (Malvy et al., 1999; Simopoulos, 2011). They are essentially found (90%–95%) in the form of triacylglycerides, a structure consisting of a glycerol backbone and three fatty acids. They are also found in the form of phospholipids, in which the fatty acid in the three position on the glycerol is replaced by a phosphorylated functional group. The structure of a triacylglyceride and a phospholipid is shown in Fig. 2. Fatty acids have many physiologic roles. They are the primary source of energy storage for tissues and, as components of membrane phospholipids, play a structural role. Fatty acids of phospholipids are also mobilized by the cells as precursors of lipid mediators, which regulate many physiologic processes, such as inflammation. There are three families of fatty acids classified according to the number of double bonds on their carbon chain they contain: the saturated ones (no double bond), the monounsaturated ones (one double bond), and the polyunsaturated ones (two or more double bonds). In rodents, the brain contains 36%–46% saturated fatty acids, 18%–33% monounsaturated fatty acids, and 18%–28% PUFAs (Joffre et al., 2016).

A. PUFA Metabolism

As previously described, PUFAs are classified into two main categories, the n-6 PUFAs and the n-3 PUFAs. LA (18:2 n-6) and ALA (18:3 n-3) are, respectively, the precursors of these two series. They are called essential fatty acids because mammals cannot synthesize them. In vivo, these precursors can be metabolized by series of elongation, desaturation, and a β-oxidation–producing PUFA with additional unsaturations and/or carbon atoms sometimes referred to as LC-PUFA (Joffre et al., 2016). EPA (20:5 n-3) is also an important n-3 PUFA metabolite, despite its low level in the brain because of its rapid β-oxidation (Chen and Bazinet, 2015). Docosapentaenoic acid (DPA; 22:5 n-6) for the n-6
family is also relevant because it replaces DHA during dietary n-3 PUFA deficiency.

**B. Dietary Origin of PUFAs**

AA and DHA come mainly from the diet. Although humans can synthesize them from LA and ALA, respectively, that are found in vegetables, the conversion efficiency is very low (<1%) even in healthy adults (Kidd, 2007; Plourde and Cunnane, 2007). In the western diet, there is thought to be an imbalance between n-6 and n-3 PUFAs, leading to a n-3 PUFA consumption 12–20 times lower than n-6 PUFA consumption (Simopoulos, 2002, 2011). This is due to the increased industrialization in the developed nations accompanied by changes in dietary habits. It is particularly characterized by an increase in LA, abundant in many vegetable oils (60%–65% in sunflower oil for example) (Orsavova et al., 2015) and AA, found in meats (5%–10%) and eggs (15%) (Taber et al., 1998; Meyer et al., 2003), together with relatively low intakes of ALA, found in some green vegetables, rapeseed oil (10%) (Lewinska et al., 2015), and nuts, and EPA and DHA abundant in fatty fish (18.7% EPA plus DHA in salmon, 32.9% EPA plus DHA in tuna) (Strobel et al., 2012). A high intake of LA associated with a low intake of ALA leads to the accumulation of n-6 PUFAs, including AA. In the case of severe n-3 PUFA deficiency, the expression of desaturases and elongases is upregulated in the liver to compensate and provide DHA to the brain (Igarashi et al., 2007). In addition, under dietary n-3 PUFA deficiency, the half-life of brain DHA is increased by twofold (Demar et al., 2004).

Although not universally accepted, several dietary recommendations state a ratio LA/ALA close to 4–5 and a ~500 mg/d supply in EPA and DHA sufficient to meet the n-3 PUFA needs of the body and to protect against cardiovascular disease risk (Burdge, 2004; Lucas et al., 2009). Preclinical and clinical studies indicate that increasing dietary ALA and reducing LA are beneficial in increasing n-3 LC-PUFA bioavailability (Blanchard et al., 2013; Taha et al., 2014). Concerning the bioavailability of dietary EPA/DHA in the form of phospholipids (krill oil source) or triacylglycerides (fish oil source), no clear evidence actually identifies a better source (Salem and Kuratko, 2014; Yurko-Mauro et al., 2015) to date.

**C. Accumulation and Regional Distribution in the Brain**

AA and DHA accumulate during brain development, especially during the perinatal period: in humans between the beginning of the third trimester and 2 years of age and in rodents between the 7th and the 21st postnatal day (Clandinin et al., 1980). These periods correspond to the rapid neuronal maturation, synaptogenesis, and gray matter expansion (Morgane et al., 1993; Giedd et al., 1999).

LC-PUFAs vary across brain regions (Delion et al., 1994; Carrié et al., 2000; McNamara et al., 2009; Joffre et al., 2016). For example, in the adult C57BL6/J mice, the highest level of AA is found in the hippocampus (10.2%), followed by the prefrontal cortex (9.7%), the hypothalamus (8.5%), the cortex (7.7%), the cerebellum (6.5%), and the brain stem (5.5%) (Joffre et al., 2016). The highest level of DHA is found in the prefrontal cortex (14.3%) and in the hippocampus (13.7%), followed by cerebellum (12.2%), cortex (11.9%), hypothalamus (10.1%), and brain stem (8.2%) (Joffre et al., 2016). Then the AA/DHA ratio varies from 0.75 to 0.85 in the hypothalamus and hippocampus to 0.54 in the cerebellum. These variations may be due to different LC-PUFA entry mechanisms into the brain or to different incorporation into membranes of cells composing the structure considered.

Brain DHA levels are comparable in human and mice: between 12.3% and 15.9% in the prefrontal cortex of rats and mice (Moriguichi et al., 2001; Xiao et al., 2005; Joffre et al., 2016) and between 14.1% and 15.9% in post-mortem frontal cortex in human (Hamazaki et al., 2015, 2016). However, Cortie et al. (2015) reported that mouse mitochondria contain higher levels of PUFA as compared with those from humans.
Differences in brain DHA levels depending on brain structures, dietary intake, gender, and aging may have consequences on inflammatory processes because n-3 LC-PUFAs have immunomodulatory properties (Layé, 2010).

**D. Intrinsic (Age, Sex) and Extrinsic (Diet) Factors Influencing Brain PUFA Content**

The brain LC-PUFA levels fluctuate with differential extrinsic and intrinsic factors. Brain LC-PUFA levels are modified by the fatty acid composition of the diet (Calder, 2007). Indeed, low consumption in n-3 PUFAs induces a decrease in brain DHA levels (Connor et al., 1990; Carrié et al., 2000; Larrieu et al., 2012; Joffre et al., 2016) and an increase in brain DPA (22:5n-6) and often AA levels (Connor et al., 1990; Larrieu et al., 2012), whereas genetic-driven enrichments in n-3 PUFAs induce the opposite (He et al., 2009; Boudrault et al., 2010; Orr et al., 2010; Bousquet et al., 2011; Joffre et al., 2016). These modifications impact all brain structures, but some of them are more affected than others: the prefrontal cortex and the hippocampus, which contain the highest DHA content and are the most sensitive, whereas the hypothalamus, which contains the lowest DHA, is the least sensitive. These differences may be attributed to the evolution of brain performance (Crawford et al., 1999; Broadhurst et al., 2002).

Furthermore, several studies conducted in humans and rodents suggest that LC-PUFA levels vary with gender. Indeed, DHA is higher in females than in males, independently of the status of dietary n-3 PUFA (Lin et al., 2016). This gender difference is attributed to the levels of hormones that increase the mRNA expression of fatty acid desaturase 2, the gene encoding 6 desaturase (Giltay et al., 2004; Magnusardottir et al., 2009). It was specifically found that DHA was higher in phosphatidylcholine and phosphatidylethanolamine of platelets in women (Geppert et al., 2010). Differences between males and females are also reported in rat liver and cerebral cortex (Extier et al., 2010).

In addition, age influences brain LC-PUFA levels, as aging is often characterized by a decrease in LC-PUFAs (Calderini et al., 1983; Lopez et al., 1995; Zhang et al., 1996; Favreliere et al., 2003; Little et al., 2007; McNamara et al., 2008; Labrousse et al., 2012; Ledesma et al., 2012; Moranis et al., 2012). The decrease in brain DHA is accentuated in aged animals fed a n-3 PUFA-deficient diet (Joffre et al., 2016).

**III. Mechanisms of Entry of PUFAs into the Brain**

**A. Plasma Pools for Brain DHA Supply**

As previously mentioned, the brain is enriched with PUFAs, particularly DHA and the n-6 PUFA AA. Although the brain can synthesize saturated and mono-unsaturated fatty acids, it must rely on uptake of either the preformed DHA and AA or their dietary precursors, ALA and LA, respectively, which can be converted to DHA and AA within the brain. Whereas the brain does have the capacity to synthesize DHA and AA, their rate of synthesis relative to uptake from the plasma is low, suggesting that uptake from plasma and not synthesis within the brain is the major source (DeMar et al., 2006; Igarashi et al., 2007). Furthermore, although the liver can upregulate its ability to synthesize DHA, especially under conditions of low dietary n-3 PUFA intake, the brain does not upregulate DHA synthesis under these conditions, further demonstrating the need for a constant plasma supply to the brain (Igarashi et al., 2007).

Within the blood, DHA can be free (sometimes referred to as unesterified) or esterified to triglycerides, phospholipids, and cholesteryl esters. Whereas red blood cells contain esterified DHA, this pool is generally not thought to contribute DHA, at least not directly, to the brain. The vast majority of DHA that is esterified in the blood occurs as circulating lipoproteins, but there are small pools of free esterified DHA, especially lysophosphatidylcholine containing DHA (Rapoport et al., 2001). The plasma pools that contribute DHA to the brain have been and remain somewhat controversial (for review, see Mitchell and Hatch, 2011), and, below, we will attempt to highlight potential reasons for disagreement. Although it was originally hypothesized that lipoprotein containing DHA was the major source supplying the brain with DHA, knockout (KO) of either the low-density or very-low-density lipoprotein receptors does not decrease brain DHA (or AA) concentrations (Chen et al., 2008; Rahman et al., 2010). However, caution must be taken with interpreting these lifelong KO studies as compensation via another mechanism could maintain brain DHA concentrations. However, it is clear that these lipoprotein receptors are not necessary for maintaining brain DHA levels and other mechanisms must exist. As an attempt to identify the major plasma pools for supplying DHA to the brain, we used a kinetic model in combination with labeled DHA, in rats, where unesterified DHA is infused i.v. to achieve a steady state, and calculated the rate of uptake of unesterified DHA to the brain (Chen et al., 2015). We then administered radiolabeled DHA by gavage, which labels multiple plasma pools as well as the brain. We found that the coefficient of uptake or the rate of uptake from the unesterified pool, alone, was sufficient to explain the rate of uptake of all the labeled plasma pools upon oral administration. Or more, simply put, it appeared as if the unesterified pool was the major source, if not the only source, supplying the brain upon oral administration. We then used another kinetic model to calculate the rate at which DHA enter the brain and found the rate of DHA exiting the brain to be similar to the uptake rate from the plasma unesterified pool. Because DHA is not accumulating in the adult rodent brain, this suggested that unesterified DHA is, again, the major pool supplying the brain. Importantly,
it had been reported that upon acute i.v. administration of labeled unesterified DHA or DHA esterified to lysophosphatidylcholine (lysoPC), more radioactivity, presumably from DHA, was present in the brain of rodents receiving lysoPC-containing DHA upon several hours (Thiess et al., 1994; Lagarde et al., 2001). Furthermore, evidence that Mfsd2a, a protein that facilitates the uptake of lysoPC containing DHA into the brain, KO had lower brain DHA levels compared with wild-type controls combined with observation of more radiolabeled DHA entering the brain suggested that lysoPC containing DHA was the major plasma source supplying the brain (Nguyen et al., 2014). However, upon close examination, it was determined that i.v. administered lysoPC containing DHA has a longer plasma half-life than unesterified DHA; thus, more of it is directed to the brain, albeit at a much slower rate than unesterified DHA (Chen et al., 2015). This is of significance as, although the net rate of plasma lysoPC containing DHA is lower than unesterified DHA in vivo, i.v. lysoPC containing DHA would be useful for targeting the brain with DHA and possibly other lipids (Chauveau et al., 2011; Lo Van et al., 2016).

B. Mechanisms of DHA Entry into the Brain

Similar to the plasma pools that supply the brain, there has been considerable debate on the mechanisms by which DHA is uptaken into the brain. Some of the confusion may be the result of studies that have failed to differentiate direct transport from uptake, which is often coupled to metabolism. Although it is clear that fatty acids, including DHA, do not need a protein to cross the cell membrane, several proteins have been implicated in facilitating the uptake of DHA. These include members of the fatty acid transport protein (FATP) family, CD36, Mfsd2a, and fatty acid–binding proteins (FABP).

Whereas earlier work suggested a role of FATPs in the uptake of fatty acids, and hence their naming, it was later realized that they possessed acyl CoA synthetase activity and were, likely, quenching fatty acids and/or facilitating their metabolism, which led to an increase in fatty acid uptake, but not transport per se (DiRusso et al., 2005; Jia et al., 2007; Mashek et al., 2007). An analogy can be drawn from hexokinase or glucokinase, which phosphorylates glucose, increasing glucose uptake, whereas glucose transport is mediated by the GLUT proteins. Furthermore, it is important to note that the transport of fatty acids across the membrane occurs at rates commonly measured in the low msec range, and studies lasting several seconds, let alone minutes, do not have the temporal resolution to separate transport from uptake and metabolism, especially in in vivo studies (Hamilton 1998). Nevertheless, it has become clear that members of the FATP family, especially FAPT1, are important for DHA uptake (Ochiai et al., 2017). CD36 has remained more elusive in the transport of fatty acids, but recent studies suggest that CD36 is not a fatty acid transporter and, likely, also facilitates the uptake of fatty acids secondary to changes in metabolism (Xu et al., 2013a; Jay and Hamilton 2016). KO of Mfsd2a leads to approximately 50% less neurons and brain DHA as compared with wild-type controls, and Mfsd2a appears critical for the brain uptake of LPC, including those esterified with DHA (Nguyen et al., 2014). However, similar to the previously mentioned proteins, caution must be taken, as the studies do not have the temporal resolution to distinguish between transport and uptake secondary to changes in metabolism. Members of the FABP family facilitate the uptake of fatty acid secondary to their metabolism and are important in the trafficking of fatty acids, the regulation of eCB signaling among others, and we refer the reader to several recent reviews (Moule et al., 2012; Elsherbiny et al., 2013; Schroeder et al., 2016). Of particular interest are recent studies on FAPB-5, which facilitates the uptake of DHA into the brain, and KO reduces brain DHA by approximately 15% and is associated with impaired working and short-term memory (Pan et al., 2015, 2016). Although the precise mechanisms by which DHA is transported and uptaken into the brain have been and still are of considerable debate, it is evident that numerous candidate mechanisms have been identified that could be targeted to alter the uptake of DHA into the brain and ultimately brain levels affecting neuronal survival and behavior.

IV. Anti-Inflammatory Activities of n-3 PUFAs in the Brain

A. General Evidence in Humans and Animal Models of Brain Pathologies

Inflammation in the brain is beneficial to maintain organ homeostasis in response to infection. Brain inflammation involves microglial cells, the resident macrophages of the central nervous system (CNS) (Aloisi, 2001). When activated, these cells produce pro- and anti-inflammatory cytokines. However, when the production of proinflammatory cytokines is sustained, these molecules become neurotoxic, leading to neuronal damage involved in many brain pathologies (Woodroffe and Cuzner, 1993; Woodroffe, 1995; Blais and Rivest, 2003; Laye, 2010; Solito and Sastre, 2012). Hence, limiting inflammation is of great importance, and the identification of mediators able to do that may provide new targets in brain damage prevention and treatment.

A large number of studies support the hypothesis that n-3 LC-PUFAs or their products are candidates for limiting neuroinflammation. Indeed, n-3 LC-PUFAs downregulate inflammatory gene expression, such as those of cytokine or enzymes involved in the synthesis of eicosanoids, while inducing lipid mediators involved in the resolution of inflammation (Calder, 2006; Serhan, 2014).
In animal models of acute and chronic inflammation, the effects of n-3 LC-PUFAs and their bioactive mediators have been demonstrated at the periphery (Serhan and Chiang, 2013) and in the brain (Orr and Bazinet, 2008; Rapoport, 2008; Layé, 2010; Bazinet and Layé, 2014). In humans, higher n-3 LC-PUFA consumption is associated with a lower risk of inflammation-associated neurologic disorders (reviewed in Orr and Bazinet, 2008; Layé, 2010; Bazinet and Layé, 2014). Several epidemiologic and observational studies report that subjects with higher n-3 LC-PUFA levels in blood have lower proinflammatory cytokine production (Ferrucci et al., 2006; Kiecolt-Glaser et al., 2007, 2011; Farzaneh-Far et al., 2009; Alfano et al., 2012). Moreover, supplementation of patients diagnosed with Alzheimer’s disease (AD) with a DHA-rich diet led to a reduced release of proinflammatory cytokines from blood mononuclear leukocytes (Vedin et al., 2008). In vivo, high levels of brain DHA are linked to reduced expression of proinflammatory cytokines in several rodent models of acute or chronic neuroinflammation, such as systemic administration of the bacterial endotoxin lipopolysaccharide (LPS), brain ischemia-reperfusion, spinal cord injury, or aging (see Orr et al., 2013b for review). In addition, a diet rich in EPA attenuates the production of the proinflammatory cytokine interleukin (IL)-1β and improves synaptic plasticity impairment in the hippocampus of old rats (Martin et al., 2002; Lynch et al., 2007). Aged mice exposed to a diet rich in EPA/DHA for 2 months express less proinflammatory cytokine [IL-1β, IL-6, and tumor necrosis factor (TNF)-α] compared with mice fed with a diet with a ratio of LA/ALA of 5] (Labrousse et al., 2012). Importantly, the reduction of neuroinflammation linked to diets enriched in n-3 LC-PUFA is associated with improvement of spatial memory deficits (Song et al., 2004; Labrousse et al., 2012). Moreover, increasing brain DHA by genetic or dietary means is associated with protection against LPS-induced proinflammatory cytokine production induced by LPS (Mingam et al., 2008b; Delpech et al., 2015a,b), brain ischemia-reperfusion (Lalancette-Hebert et al., 2011), or spinal cord injury (Huang et al., 2007; Lu et al., 2013). DHA’s protective activity on neuroinflammation is linked to its direct effect on microglia, as suggested by in vitro studies. For example, DHA decreases the LPS-induced nuclear factor (NF)κB activation and, as a consequence, the production of IL-1β and TNF-α (De Smedt-Peyrusse et al., 2008) and chemokines (Lu et al., 2013) by microglia. In addition, DHA enhances phagocytosis of AD-related amyloid-β (Aβ) 42 by human microglial and decreases inflammatory markers (Hjorth et al., 2013). Moreover, DHA is able to normalize the LPS-induced abnormalities in microglia (Chang et al., 2015). N-3 PUFA activity on microglia is discussed later.

Conversely, low dietary intake of n-3 PUFA has deleterious consequences in the brain, especially during the perinatal period of brain development. For instance, dietary n-3 PUFA deficiency beginning at the first day of gestation decreases DHA level, alters microglia phenotype and motility, and increases brain proinflammatory cytokine IL-6 and TNF-α expression in the offspring’s brain of mice and rats (McNamara et al., 2010; Madore et al., 2014). In mice, the early-life exposure to a n-3 PUFA-deficient diet leads to spatial memory impairment at adulthood (Moranis et al., 2012), whereas this is not the case in adult mice with a n-3 PUFA deficiency starting at weaning (Delpech et al., 2015b). However, adult mice fed a n-3 PUFA-deficient diet starting at weaning are more vulnerable to inflammatory insult as spatial memory, synaptic plasticity, microglia phenotype, and brain cytokine production is altered in response to LPS (Delpech et al., 2015b). Altogether these results pinpoint the role of dietary n-3 PUFA deficiency in regulating brain proinflammatory cytokine production and microglia profile in the absence of overt infection (sterile inflammation, development, aging) and inflammatory situation (LPS administration, stroke).

B. Overview of DHA Anti-Inflammatory / Proresolving Mechanisms

Anti-inflammatory/ resolution activities of n-3 PUFAs are potent with a variety of overlapping and/or additive mechanisms occurring either directly on the membrane, via modulation of signaling pathways or control of gene expression, or indirect through the synthesis of derivatives reviewed elsewhere (Calder, 2011; Serhan, 2017a).

1. Membrane and Signaling Effects. As described in the introduction, DHA is incorporated in membrane phospholipids. In direct link with its disordered molecular structure, DHA is believed to adopt a specific molecular orientation in the membrane likely to modify membrane domain organization and protein activity (reviewed in Shaikh, 2012). Notably, formation of DHA-enriched nanodomains in the membrane or incorporation of DHA into lipid rafts, a membrane-signaling platform rich in cholesterol and sphingomyelin, disrupts receptor-signaling interactions. In glial cells, changes in membrane fluidity due to DHA level have consequences on several proinflammatory receptor localization and associated signaling cascades. De Smedt-Peyrusse et al. (2008) reported that, in microglia, DHA impairs membrane location of the LPS receptors CD14 and Toll-like receptor (TLR)4, which in turn decreases proinflammatory activity of LPS. Rockett et al. (2011) showed that increased n-3 LC-PUFA consumption disrupts B cell lipid-raft clustering. The membrane reorganization consequence of DHA increase is in line with in vitro data showing that DHA modulates the activation of several proinflammatory transcription factors (NFκB; mitogen-activated phosphate kinase
First, EPA inhibits the activity of an inhibitory receptor (Calder, 2002). EPA is synthesized from AA. Moreover, EPA counteracts the same receptor (Bagga et al., 2003). In addition, when effective, EPA-derived eicosanoids antagonize those of AA (Calder, 2002). As an example, PGE3 is much less biologically active than the one derived from AA (De Roos et al., 2009; Arita, 2012). The subclass of oxylipins, the signaling molecule by the enzyme 5-lipoxygenase (LOX) that generates the prostaglandin (PG), prostacyclins, and leukotrienes (LT), and 3-series oxylipins (Shearer et al., 1999). In addition, eicosanoids synthesized from AA and EPA act in competition as they share the same receptor (Bagga et al., 2003). In microglia, DHA upregulates peroxisome proliferator-activated receptor (PPAR)γ nuclear translocation, a potent modulator of microglia (Ajmone-Cat et al., 2010; Corsi et al., 2015).

DHA directly modulates inflammatory gene expression via surface or intracellular receptors, as reported in inflammatory cells (Calder, 2011). Notably, DHA binds to G-coupled receptors (GPR) 120 and 40 and PPARγ, and, in turn, DHA regulates their expression (Im, 2012; Yamashima, 2012; Calder, 2013, 2015). GPR120 and PPARγ mediate DHA anti-inflammatory activity (Calder, 2011), including in the brain, as described above. Overall, these results highlight that the anti-inflammatory activity of DHA is partially attributable to its effect on cell membrane reorganization and/or through its effect on specific receptors. Although EPA is esterified in cell membranes, including those of microglial cells (De Smedt-Peyrusse et al., 2008), its effect on raft reorganization or GPR has not been extensively studied in relation to its anti-inflammatory activity (Williams et al., 2012).

2. N-3 LC-PUFA Derivatives.

a. Oxylipins. Some of the anti-inflammatory activities of n-3 PUFAs are attributed to eicosanoids (a subclass of oxylipins), the signaling molecule by the enzymatic and nonenzymatic oxidation of EPA and DHA (Fig. 3) (De Roos et al., 2009; Arita, 2012). The main EPA-derived mediators include 3-series prostaglandin (PG), 5-series leukotrienes (LT), and 3-series thromboxane (TX), and are reported to be relatively nonactive (Fig. 3). DHA is also converted into 3-series PG (Fig. 3). EPA- and DHA-derived eicosanoids are biologically less active than the one derived from AA (Calder, 2002). As an example, PGJ3 is much less effective than PGE2 to induce IL-6 and cyclooxygenase (COX)-2 expression in macrophages, despite binding to the same receptor (Bagga et al., 2003). In addition, when copresent, EPA-derived eicosanoids antagonize those synthesized from AA. Moreover, EPA counteracts AA-derived eicosanoid production, as EPA is a competitive inhibitor for the enzymes involved (Calder, 2002). First, EPA inhibits the activity of Δ5-desaturase, converting dihomo γ-linolenic acid to AA. EPA also inhibits the activity of PLA2, thus preventing the release of AA; the activity of COX-2 generating the PG, prostacyclins, and TX (Needleman et al., 1979; Obata et al., 1999); and the activity of 5-lipoxygenases (LOX) that generates the LT (Sperling et al., 1993). Overall, EPA reduces both the proportion of AA and the production of proinflammatory eicosanoids derived from AA. DHA also is able to reduce the production of PGE2 and LTB4 derived from AA in stimulated peripheral blood mononuclear cells (Kelley et al., 1999). In addition, eicosanoids synthesized from AA and EPA act in competition as they share the same G protein–coupled receptors.

Recently, SPM derived from n-3 LC-PUFAs have gained much more attention. These lipid mediators have both anti-inflammatory and proresolving properties without immune suppression (Serhan et al., 2002, 2008, 2014). They act in physiologic doses around the nanomolar level as compared with DHA, which acts at micromolar levels. Among the resolvins, resolving D1 (RvD1, 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid) and resolving E1 (RvE1, 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) are of particular interest in the resolution of inflammation because they actively turn off the inflammatory response (Fig. 3) (Fredman and Serhan, 2011). They are thought to underlie many of the beneficial effects attributed to their precursors (Calder, 2013; Serhan and Chiang, 2013; Bazinet and Laye, 2014; Headland and Norling, 2015). They act via cell surface G protein–coupled receptors: GPR32 and ALX/FPR2 for RvD1, and chemotactant receptor 23 for RvE1 (Zhang and Spite, 2012). They were mainly studied in peripheral immune cells, both in vitro and in vivo. In vitro, they act on macrophages to stimulate the clearance of apoptotic cells and inflammatory debris, inhibit the expression of the proinflammatory cytokines, and block neutrophil infiltration (Arita et al., 2005; Schwab et al., 2007; Fredman and Serhan, 2011; Zhang and Spite, 2012). RvE1 inhibits the activity of NFκB and the subsequent production of proinflammatory cytokines (Xu et al., 2013b; Rey et al., 2016). RvD1 also displays its anti-inflammatory activity through miRNA modulation (Rey et al., 2015). In vivo, RvD1 decreases proinflammatory cytokine production in acute models of kidney injury (Chen et al., 2014) or lung (Wang et al., 2011a, 2014; Zhou et al., 2013; Yaxin et al., 2014), and in a model of allergic airways (Rogério et al., 2012). RvE1 also modifies cytokine production in experimental models of colitis (Arita et al., 2005) and peritonitis (Schwab et al., 2007) and significantly modulates the inflammatory profile and activation of microglia (Harrison et al., 2015).

SPMs derived from DHA include neuroprotectin D1 (NPD1) and maresin 1 (MaR1) (Bazan, 2006; Bannenberg and Serhan, 2010; Bazan et al., 2012). NPD1 protects the brain toward leukocyte infiltration, COX-2 expression, cytokine production, and microglia activation (Hong et al., 2003; Marcheselli et al., 2003, 2010; Lukiw et al., 2005; Orr et al., 2014). MaR1 reduces cytokine production in human peripheral blood lymphocytes (Chiu et al., 2016). It protects against cerebral ischemia/reperfusion injury through the modulation of the proinflammatory response (Xian et al., 2016). It downregulates Aβ-induced inflammation in human microglial cells in culture through the stimulation of Aβ phagocytosis (Zhu et al., 2016).

b. Endocannabinoids. N-3 LC-PUFAs can also exert their effect through the modulation of the eCB system, (Meijerink et al., 2013; Bazinet and Layé, 2014; Kuda, 2017; Nadjar et al., 2017). eCBs are synthesized from PUFA, the most well-known being anandamide (AEA)
and the 2-arachidonoyl derived from AA. However, n-3 LC-PUFAs are also precursors of DHA- and EPA-derived eCBs, namely docosahexaenoyl ethanolamide (DHEA or synaptamide) and eicosapentaenoyl ethanolamide (EPEA). The formation of these compounds in mice is increased in various tissues, including the brain after consumption of a fish oil–rich diet and in the plasma of volunteers supplemented with DHA and EPA (see Meijerink et al., 2013 for review). Furthermore, the level of DHEA in the brain is higher than that of AEA even in animals fed a control diet (see Meijerink et al., 2013 for review). The immunomodulatory effect of DHEA has been demonstrated in the brain and at the periphery. Indeed, in the brain, Park et al. (2016b) showed that DHEA is a potent suppressor of LPS-induced neuroinflammation in mice, by enhancing cAMP/protein kinase A signaling and inhibiting NFκB activation. In macrophages, Meijerink et al. (2011, 2015) showed that DHEA modulates inflammation by reducing monocyte chemoattractant protein-1, nitric oxide (NO), and eicosanoid production. Moreover, Rossmeisl et al. (2012) suggested a possible role for DHEA in modulating inflammation in adipocytes. Importantly, DHEA can be oxidized to form derivatives with anti-inflammatory properties (Yang et al., 2011; Shinohara et al., 2012; Kuda, 2017).

eCBs bind mainly to the eCB receptors CB1 and CB2 (Piomelli and Sasso, 2014) that are expressed in neurons and glial cells and more specifically in microglial cells (Stella, 2009). In microglial cells, CB2 is more highly expressed than CB1 (Nunez et al., 2004). It was already shown that in inflammatory conditions eCBs act via CB2, with the role of CB1 remaining unclear (Mecha et al., 2015). The importance of CB2 in regulating microglia activity was demonstrated in vivo and in vitro. CB2 knockout (KO) mice microglia lose their phagocytic activity, and microglia motility is reduced in presence of a CB2 antagonist (Walter et al., 2003).

All of these studies highlight the central role of n-3 LC-PUFA and their derivatives in the regulation of inflammation, with emerging data in the brain, especially through their effect on microglia.

V. Microglia as a Target for n-3 PUFAs and SPMs

Microglia are a glial cell of myeloid origin whose role is to maintain brain homeostasis in a sex-, age-, and region-dependent manner (Hanisch and Kettenmann, 2007; Tay et al., 2017). Microglia are a highly plastic and multitasking cell, important from brain development to pathologic conditions, via inflammatory and noninflammatory responses (Ransohoff and Brown, 2012). Any situation leading to undesirable microglial activity at different stages of life could severely impair brain function. During development, yolk sac–derived microglia colonize the brain and spread evenly in the whole
CNS, where they shape neuronal circuits (Paolicelli et al., 2011; Schafer et al., 2012). In the adult brain, microglia sense the microenvironment with their processes in search for nonhomeostatic signals, and they also regulate neuronal architecture and function (Davalos et al., 2005; Nimmerjahn et al., 2005; Wake et al., 2009; Tremblay et al., 2010; Sipe et al., 2016). Under pathologic conditions, unusual/danger signals trigger microglial response, including release of inflammatory factors and/or redirection of its phagocytic activity to the clearance of hazardous factors (Ransohoff and Brown, 2012; Sierra et al., 2014; Tay et al., 2017). Understanding how n-3 PUFAs modulate microglial phenotypes and functions is a major challenge for future development of innovative lipid-based therapies with positive effects on physiology and behavior.

A. Modulation of Microglial Function by n-3 PUFAs

1. In Vitro Evidence. N-3 PUFAs are potent modulators of microglial functions (Nadjar et al., 2017). The first evidence came in 2007 from an in vitro study in which microglial cells (BV2 cell line) were incubated with or without EPA for 60 minutes prior to LPS application, and their inflammatory response was analyzed (Moon et al., 2007). EPA dose-dependently inhibited the expression of the two inflammatory enzymes, inducible NO synthase (iNOS) and COX-2, as well as the subsequent production of NO and PGE2 by BV2 cells. EPA also dampened the production of proinflammatory cytokines (IL-1β, IL-6, and TNF-α). This report was followed by another study in which primary cultures of rat microglia were incubated simultaneously with LPS and n-3 PUFAs (Liuuzzi et al., 2007). The authors measured the LPS-mediated induction of the matrix metalloproteinase (MMP)9 in presence or in absence of a mixture of EPA and DHA. MMP9 is an endopeptidase that degrades the extracellular matrix, and as such plays a role in inflammation by regulating processes such as cell motility, blood-brain barrier (BBB) disruption, cell infiltration, etc. Application of n-3 PUFAs concomitantly to LPS on microglial cells was sufficient to significantly reduce MMP9 expression and activity, in a dose-dependent manner (Liuuzzi et al., 2007). Since then, a total of 16 publications on various in vitro models brought converging evidence on the anti-inflammatory action of n-3 PUFAs on microglia (Nadjar et al., 2017). They also revealed new regulatory roles of n-3 PUFAs on these cells, such as modulation on microglial phenotype, migration, phagocytosis, autophagy, or lipid bodies accumulation, as well as some of the molecular mechanisms implicated (extensively reviewed in Nadjar et al., 2017). Briefly, whatever the inflammatory challenge applied on these cells (TLR3-4 or 7 agonists, Aβ, interferon-γ, hypoxia, or myelin), all studies reported a dose-dependent decrease in the production of proinflammatory factors (cytokines and/or chemokines) when treated with n-3 PUFAs (De Smedt-Peyrusse et al., 2008). N-3 PUFAs also inhibit the production and activity of the enzymes COX-2, iNOS, and the production of NO and reactive oxygen species (Moon et al., 2007; Lu et al., 2010; Antonietta Ajmone-Cat et al., 2012; Pettit et al., 2013; Chen et al., 2014; Corsi et al., 2015; Zendedel et al., 2015). This is correlated with a switch in microglial marker expression, from a proinflammatory to an anti-inflammatory phenotype (decreased CD40 and CD86, increased CD206) (Ebert et al., 2009; Chhor et al., 2013; Hjorth et al., 2013; Chen et al., 2014). The phagocytic capacity of microglia is also modulated by n-3 PUFAs in vitro. Using flow cytometry on the human microglial cell line CHME3, Hjorth et al. (2013) assessed the effects of DHA and EPA on microglial phagocytosis of the AD pathogen Aβ42. They showed that both DHA and EPA exacerbate Aβ42 engulfment by microglia in a dose- and time-dependent manner. Microglia employ a wide repertoire of mechanisms to phagocytose various types of cellular elements/debris (Sierra et al., 2013; Brown and Neher, 2014). Yet, the prophagocytic effects of n-3 PUFAs are likely to be generalizable to all stimuli, as 1 year later Chen et al. (2014) showed a significant increase in myelin engulfment by DHA- or EPA-treated microglia primary cultures. N-3 PUFAs also modulate microglia migration capacities. Using a Transwell migration assay, Ebert et al. (2009) showed that DHA dose-dependently inhibits LPS-activated microglial migration, whereas it does not affect the migratory abilities of BV2 cells in basal conditions. Very recently, by measuring the classic autophagy index LC3-I/LC3-II ratio (for the lipidded form of the microtubule-associated protein 1 light chain 3, LC3-II, and nonlipiddated LC3, LC3-I), Inoue et al. (2017) showed that application of EPA plus DHA increases autophagy in MG6 microglial cells. Autophagy is an essential process for immune cell homeostasis that leads to dampening of inflammatory processes (Levine et al., 2011). Inoue et al. (2017) highlight it as a new process by which n-3 PUFAs modulate microglial inflammatory response. Finally, lipid bodies (LBs) are functionally active organelles that are formed within immune cells, such as macrophages, in response to different inflammatory stimuli and are sites for synthesis and storage of inflammatory factors (Melo et al., 2011). Ebert et al. (2009) were the first to demonstrate that DHA significantly reduces the accumulation of LBs that is usually observed in LPS-treated microglia. These data were confirmed and extended a few years later by the group of Maysinger. Chang et al. (2015) showed in N9 cell line that DHA normalizes LPS-induced abnormalities in microglia, by promoting small LB formation and LB interaction with mitochondria, and by restoring mitochondrial function (Chang et al., 2015; Tremblay et al., 2016).
Importantly, none of these studies reported detrimental effects of PUFAs on microglia viability, except at very high doses (Moon et al., 2007; Antonietta Ajmone-Cat et al., 2012; Nadjar et al., 2017). Moreover, although EPA and DHA are both efficient almost to the same extent when applied separately, their effects are most often potentiated when combined (Zhang et al., 2010; Hjorth et al., 2013; Chen et al., 2014; Kurtys et al., 2016; Inoue et al., 2017).

Some of the studies presented above explored the molecular mechanisms by which n-3 PUFAs modulate microglial functions. Our group demonstrated that DHA significantly downregulates the cell surface expression of CD14 and TLR4, the two coreceptors that bind LPS, in LPS-stimulated BV2 cells (De Smedt-Peyrusse et al., 2008). Beyond the membrane effects of n-3 PUFAs, many studies have reported converging evidence on the modulatory role of n-3 PUFAs on signaling pathways. Indeed, the beneficial effects of n-3 PUFAs on inflammatory processes are attributable in part to their inhibitory action on inflammatory signaling pathways such as NFκB (De Smedt-Peyrusse et al., 2008; Zhang et al., 2010; Wang et al., 2015b; Inoue et al., 2017), mitogen-activated protein kinases (P38, c-Jun N-terminal kinases, or extracellular signal-regulated kinase 1/2) (Liuzzi et al., 2007; Antonietta Ajmone-Cat et al., 2012; Chang et al., 2015), or Akt (Liuzzi et al., 2007).

Finally, it was reported that DHA and EPA are natural ligands for several nuclear receptors, including peroxisome proliferator-activated receptors (PPARs) that are highly expressed in microglial cells (Xu et al., 1999; Zhang et al., 2014). These latter play an important role in the general transcriptional control of numerous cellular processes, including lipid homeostasis and inflammation (Clark, 2002). Several in vitro studies showed that DHA and EPA activate PPARγ in microglial cells as well, hence significantly decreasing the expression of inflammatory factors (Ebert et al., 2009; Antonietta Ajmone-Cat et al., 2012; Corsi et al., 2015; Wang et al., 2015; Kurtys et al., 2016).

Overall, the plethora of in vitro studies rather convincingly demonstrated the anti-inflammatory role of n-3 PUFAs. However, although they are very convenient to study molecular mechanisms, the relevance of in vitro models to study microglial function has been recently questioned (Hickman et al., 2013; Butovsky et al., 2014). By comparing gene expression in cultured microglia with in situ microglia and other myeloid cells, several groups showed that in vitro microglia do not express the same molecular signature as brain microglia and have a transcriptome signature that is closer to macrophages, putting into question the relevance of data presented above to the brain (Hickman et al., 2013; Butovsky et al., 2014). To evaluate the validity of in vitro studies, we will now review reports that assessed the effects of PUFAs on neuroinflammation in various physiologic and pathologic contexts. The data relating to their anti-inflammatory actions have been presented in the previous section. We will in this work focus on the evidence regarding microglial cells, as microglial activation was studied as a secondary outcome in most of these studies.

2. In Vivo Evidence. The first in vivo study showing a relationship between n-3 PUFAs and microglia was performed on aged rats supplemented with 125 mg/d EPA-containing chow for 4 weeks (Lynch et al., 2007). EPA supplementation was able to significantly reduce microglial activation marker expression [major histocompatibility complex (MHC)II, CD40] and microgliamediated production of IL-1β. This was paralleled by an increase in the anti-inflammatory cytokine IL-4 expression, the complete inhibition of aging-induced synaptic plasticity impairment, and a decreased vulnerability to Aβ stimulus (Lynch et al., 2007). Concomitantly, Connor et al. (2007) demonstrated, in a model of retinal degeneration, that supplementation with n-6 PUFAs increases microglial production of TNF-α in the retina, as a plausible cause for vascular growth and pathology. These effects were prevented by increasing dietary n-3 PUFAs intake (Connor et al., 2007).

Many studies have reported a close relationship between microglial function and n-3 PUFAs since then. N-3 PUFA supplementation reduces microglial activation and/or phenotype alteration in models of brain development (Kuperstein et al., 2008; Madore et al., 2014; Abiega et al., 2016), respiratory system development (Tenorio-Lopes et al., 2017), healthy aging (Grundy et al., 2014), ischemia (Zhang et al., 2010; Belaev et al., 2011; Okabe et al., 2011; Eady et al., 2012a,b, 2014; Chang et al., 2013; Zendedel et al., 2015; Jiang et al., 2016), spinal cord injury (Huang et al., 2007; Lim et al., 2013a,b; Paterniti et al., 2014; Tremoleda et al., 2016; Xu et al., 2016), Parkinson’s disease (Muntane et al., 2010; Ji et al., 2012; Tian et al., 2015; Delattre et al., 2017; Mori et al., 2017), AD (Lynch et al., 2007; Hopperton et al., 2016; Serini and Calviello, 2016; Wen et al., 2016), systemic inflammation (Delpech et al., 2015b), traumatic brain injury (Pu et al., 2013; Harrison et al., 2015; Harvey et al., 2015; Desai et al., 2016), neuropathic pain model (Xu et al., 2013b; Manzhulo et al., 2015; Huang and Tsai, 2016), aging (Labrousse et al., 2012), demyelination (Chen et al., 2014), amyotrophic lateral sclerosis (Yip et al., 2013), retinal degeneration (Ebert et al., 2009; Mirza et al., 2013), or experimental autoimmune uveoretinitis (Saraswathy et al., 2006). In all these studies, n-3 PUFAs were provided under the form of DHA or EPA supplementation, a combination of EPA plus DHA, largely as fish oil, or the precursors of LC-PUFAs. Administration was made s.c., i.v., i.p., by gavage or via dietary approaches, acutely or chronically. Microglial response was evaluated by quantifying the number of cells or by measuring expression of some phenotype markers, including Iba-1, Arg1, Ym1/2, CD16, CD32, CD40, CD36, CD68, CD86, CD206, CD11b, and MHCII.
Of all these studies, 90% found a significant decrease of microglial density and/or activation after exposure to n-3 PUFAs, as a criterion for decreased neuroinflammation, whereas 10% could not find any effect (Orr et al., 2013; Vauzour et al., 2015; Trepanier et al., 2016; Nadjar et al., 2017). Moreover, we showed that the sourcing of PUFAs (from plants or dairy products) has a differential impact on LPS-modulating microglial phenotype (Dinell et al., 2016). However, none of these studies ever addressed the intimate relationship between brain PUFA contents and microglial function.

Notably, our group demonstrated that low dietary consumption of n-3 PUFA precursors over the perinatal period not only impairs microglia phenotype but also its morphologic dynamic (assessed by two-photon microscopy of microglial processes motility) in the postnatal developing brain (Madore et al., 2014). On the same model of developmental n-3 PUFA deficiency, we also showed that the phagocytic activity of microglia was enhanced in the offspring, as a consequence of an increased density in apoptotic cells (Abiega et al., 2016). It is important to highlight that these data are not in accordance with in vitro studies that show higher phagocytic activity in n-3 PUFA-treated cells (Hjorth et al., 2013; Chen et al., 2014), emphasizing even more the need for thorough in vivo work. To improve our knowledge of how PUFA specifically modulate microglia in vivo, new technological tools are required. The recent development of new tools to study these cells, such as CX3CR1-Cre mice, will surely provide informative results in the coming years (Wieghofer and Prinz, 2016).

B. Modulation of Microglial Function by SPMs

1. Lipid Derivatives Target Microglia. Hundreds of biologically active metabolites of DHA and EPA have been described in the literature (see details in previous section) (Dyall, 2015; Kuda, 2017) (Fig. 3). Briefly, DHA derivatives can be divided into oxygenated metabolites (SPMs, epoxides, electrophilic oxo-derivatives (EFOX), and neuroprostanes) and conjugates of DHA (ethanolamides, acylglycerols, docosahexaenoyl amides of amino acids or neurotransmitters, and branched DHA esters of hydroxy fatty acids). EPA can also be metabolized via oxygenation, hydroxylation, or peroxidation processes that lead to the production of eicosanoids. Some of them have been studied in relation to microglial functions, and data are summarized hereafter.

The EPA-derived RvE1 inhibits LPS-induced microglial activation following peripheral nerve injury (Xu et al., 2013b). DHA-derived lipid mediators (resolvins of the D-series, MaR, and neuroprostecins) also modify microglial functions. RvD1 promotes anti-inflammatory phenotype in BV2 cells, enhancing Arg1 and Ym1 expression, IL-4 synthesis, and subsequent NFκB and PPARγ activation and decreasing CD11b expression (Li et al., 2014; Zhu et al., 2015), via the regulation of miRNA expression (Rey et al., 2016). In vivo, RvE1 and the aspirin-triggered (AT) 17R-epimer of RvD1 (AT-RvD1) significantly modify microglial morphology in a model of traumatic brain injury, decreasing the proportion of rod/activated microglia at the expense of ramified microglia (Harrison et al., 2015). The DHA derivative MaR1 also modulates microglial response to Aβ42 application in vitro, downregulating Aβ42-mediated phenotype alterations (CD40 and CD11b expression) in CHME3 cells (Zhu et al., 2016). MaR1 also promotes Aβ42 phagocytosis by microglial cells in culture (Zhu et al., 2016). Finally, they provide the demonstration that microglia can produce SPMs [e.g., PD1, lipoxin A4 (LxA4), and RV-D1] (Zhu et al., 2015). RvD2 is also a modulator of microglial cells in vitro, as shown by Tian et al. (2015). In this study, they incubated microglia with LPS and increasing doses of RvD2 for 24 hours. Using Western blot, they showed that RvD2 inhibits LPS-mediated activation of TLR4 and its downstream signaling pathway NFκB (Tian et al., 2015). Finally, NPD1 signaling induces an increase of microglial ramification size typical of nonactivated phenotype and coincident with attenuation of retina structural alterations (Sheets et al., 2013), whereas the AT-NPD1 significantly reduces the number of ED1-positive cells (microglia/macrophages) in a model of cerebral ischemia (Bazan et al., 2012).

Even though this review focuses on the anti-inflammatory effects of n-3 PUFAs in the brain and the complexity of the lipid-dependent inflammatory response, one should mention in this work that some AA-derived lipid mediators also display anti-inflammatory activity via the modulation of microglial function (Fig. 3). LxA4 for instance inhibits interferon-γ–mediated inflammatory response (TNF-α release and P38 mitogen-activated phosphate kinase activation) in primary cultures of microglia (Martini et al., 2016). It also dampens microglial proliferation, TNF-α upregulation, and the expression of microglial markers such as P2Y12, in a model of spinal cord injury (Martini et al., 2016). In a transgenic model of AD (3xTg-AD mice), the AT-LxA4 significantly decreases the number of CD11b, Iba-1, and CD45-positive cells (Medeiros et al., 2013; Dunn et al., 2015). In vitro experiments on LPS-treated BV2 cells revealed that AT-LxA4 inhibits NFκB signaling pathway and NADPH oxidase activity, hence reducing proinflammatory cytokines and reactive oxygen species production, respectively, as potential mechanisms to explain its anti-inflammatory action (Wang et al., 2011; Wu et al., 2012). Another class of DHA-derived oxygenated metabolites has been linked to inflammation. DHA can be converted into EFOX by a COX-2–catalyzed mechanism (Groeger et al., 2010) (Fig. 3). Whereas EFOX have been shown to
modulate macrophage inflammatory activity, no data are yet available on microglia. However, EFOX are natural ligands for PPARy and modulate the Nr2 and NFkB inflammatory pathways (Groeger et al., 2010), all these molecules being highly expressed in microglial cells (Zhang et al., 2014). Hence, EFOX also look like good candidates to explain modulatory activity of DHA on microglial cells, claiming for more studies on these lipids.

Besides oxidation, DHA can be conjugated with alcohols and amines to form esters and amides, respectively (Kuda, 2017). Among all DHA conjugates, the amine conjugate N-docosahexanoyl dopamine has been shown to modulate microglial function in vitro (BV2 cells) (Wang et al., 2017). N-docosahexanoyl dopamine dose-dependently (1 or 2 µM) inhibits LPS-induced IL-6 and CCL20 production in BV2 cells, whereas neither DHA nor dopamine alone (at the same, low concentrations, 1 or 2 µM) is able to produce the same effects. This was paralleled by a decreased production of PGE2, whereas COX-2 gene expression remained stable (Wang et al., 2017).

2. Lipid Derivatives and Their Receptors. Only few receptors for DHA and EPA derivatives have been determined to date. These include ALX/FPR2 and GPR32, both receptors for Lxa4 and RvD1; ChemR23, receptor for RvE1; and LTB4R (or BLT1), receptor for LTB4 and RvE1 (Serhan et al., 2011). Most of these receptors have been found on microglial cells in vitro (Rey et al., 2016; Zhu et al., 2016). Data mining in the “Barres brain RNA-seq” database, which provides information on the transcriptome of glial cells (including microglia) and neurons sorted from adult cortex, provides the information that microglia in vivo also express the genes cmklr1 (for ChemR23), ltb4r1 (for LTB4R or BLT1), and fpr2 (for ALX/FPR2) at very high levels. Even more interestingly, they express these genes at much higher concentration than any other cell type (Zhang et al., 2014). Beyond receptors, some of these lipid mediators target PPARs, also highly expressed by microglia (Forman et al., 1997; Zhang et al., 2014). Hence, based on this evidence, it is highly likely that microglia are a target for EPA and DHA derivatives within the brain.

Overall, all of these reports highlight the modulatory activity of DHA and EPA derivatives on microglial functions. However, this promising field is still in its infancy and requires more studies to unravel the molecular mechanisms involved and validate the activity of these lipids in vivo.

C. A Link between n-3 PUFAs and Microglial Extracellular Vesicles?

Extracellular vesicles (EVs) transport represents a fundamental mechanism of communication in the CNS. EVs are released from almost all cell brain types, including microglia, into the microenvironment and are involved in cell-to-cell communication (Potolicchio et al., 2005; Subra et al., 2010; Turola et al., 2012). EVs include exosomes, which are small vesicles (40–100 nm in diameter) derived from the endosomal multivesicular bodies that fuse with the plasma membrane via exocytosis. They serve as shuttles for intercellular delivery of cargo, including specific lipids (Turola et al., 2012). Microglia-derived exosomes were discovered in 2005 in N9 microglial cell lines (Bianco et al., 2005; Potolicchio et al., 2005). Several studies have further shown that microglia-derived exosomes can deliver a proinflammatory signal on neighboring cells (Bianco et al., 2005; Verderio et al., 2012; Prada et al., 2013). A recent study elegantly showed that eCBs such as AEA can also be secreted through extracellular membrane vesicles produced by microglial cells and hence inhibit presynaptic transmission in target GABAergic neurons in a CB1-dependent manner (Gabrielli et al., 2015).

Interestingly, lipidomic and proteomic analyses of exosomes from various cell types have shown that these EVs are not randomly filled with cellular content but rather display a highly specific pattern in terms of lipid and protein expression (Subra et al., 2010; Connolly et al., 2015; Haraszti et al., 2016). Specifically, exosomes contain free fatty acids, including AA and DHA (Haraszti et al., 2016), but also AA derivatives such as prostaglandins (PGE2, PGJ2) (Subra et al., 2010). Exosomes also express specific proteins, including enzymes, such as PLA2 or FABP (Subra et al., 2010). Computer-based analyses of proteomic data also revealed that they specifically contain proteins involved in functions such as immune response, cell adhesion, or integrin signaling (Haraszti et al., 2016). Overall, exosomes are considered as signalosome carriers to neighboring cells (Subra et al., 2010).

Based on the currently available evidence, studies are needed to decipher the lipid and protein composition of microglial exosomes under various dietary situations. One might speculate that, depending on the amount of n-3 PUFAs, exosome composition might change, leading to differential effects in neighboring cells.

D. Sex, Age, and Regional Differences in the Relationship between Microglia and n-3 PUFAs

As already exposed in previous sections, n-3 PUFA distribution varies according to sex, age, and structure, and so do microglial functions. Hence, microglia/n-3 PUFA interactions could vary according to these factors, which would have to be considered to understand whether and how n-3 PUFAs modulate CNS homeostasis and neuroinflammation.

1. Evidence for Sexual Dimorphism of Microglia.

The colonization of the brain by yolk sac–originating microglia takes place very early during development, even before formation of neurons, astrocytes, or oligodendrocytes. Once in the CNS, microglia then proliferate and spread evenly in all structures all along their development (Tay et al., 2017). Although entry of microglia in the CNS is likely to be sex-independent, postnatal
microglial colonization and proliferation are sex-dependent (McCarthy et al., 2015; Nelson and Lenz, 2017b). Indeed, male and female rats do not show sex differences in microglial number before the testosterone surge at embryonic day E17 (Schwarz et al., 2012). At the time of testicular androgen secretion onset, sex differences in microglia begin to emerge. At P0, females have a transient increase in the number of ameboid microglia and microglia with stout processes relative to males, in the CA3 region of the hippocampus, para-ventricular nucleus of the hypothalamus, and amygdala (Schwarz et al., 2012). By P4, the situation is reversed with males showing greater numbers of ameboid microglia and microglia with stout or thick processes relative to females, in the amygdala, hippocampus, and cortex (Schwarz et al., 2012). These sex differences remain until adolescence period with females displaying more microglia with thick processes than males (Schwarz et al., 2012). Slighter sex differences have been reported in the cerebellum, with males having less ramified microglia than females, but no overall differences in ameboid microglia or total microglia during the first 3 weeks of life (Perez-Pouchoulen et al., 2015). At adulthood, the number of microglia is significantly higher in females than in males (as quantified at 3, 14, or 24 months old), at least in the dentate gyrus of the hippocampus (Mouton et al., 2002).

Microglia are also essential for CNS masculinization by standing at the interface of both endocrine and nervous systems during development (Lenz et al., 2013). In the preoptic area, an essential structure for brain masculinization, male neonates have 30% more microglia and twofold more ameboid microglia than females (Lenz et al., 2013). At the time of testicular surge, estradiol aromatized from testosterone promotes microglia-mediated PGE2 synthesis, a critical step in gene expression profile (Crain et al., 2009, 2013; Crain and Watters, 2015). Microglia freshly isolated from males and females differentially express P2X and P2Y purinergic receptors (Crain et al., 2009). The functional significance of this differential expression is still unknown, yet purinergic receptors are involved in functional modal switch of microglia, i.e., transition from one phenotype/activity to another phenotype/activity (Koizumi et al., 2013). Female microglia also display higher expression of pro- and anti-inflammatory cytokines (IL-1β, TNF-α, IL-6, and IL-10) relative to males (Crain et al., 2013). This sexual dimorphism is structure-dependent as microglia from cerebral cortex of females expresses more iNOS mRNA than those of males, whereas no such difference is observed in the brainstem (Crain and Watters, 2015). Finally, microglia also display sexual dimorphisms in function. In female neonates, a higher number of microglia exhibit phagocytic phenotype as compared with males (Nelson and Lenz, 2017a,b). However, the molecular and cellular mechanisms of sexual dimorphism of microglia and consequences on brain function are still to be unraveled.

The dynamics of microglia across the developmental period suggest that gonadal hormones regulate sex differences in microglia. Microglia specifically express the anti-inflammatory estrogen receptor (ERα), as ERβ was never found on these cells (Vegeto et al., 2003; Sierra et al., 2008; Crain et al., 2013; Crain and Watters, 2015). However, ERα is expressed to the same extent in males and females at all ages, suggesting that sexual dimorphism of microglia is ER-independent (Crain et al., 2013).

2. Evidence for Regionalization of Microglia. Microglia are essential in monitoring the environment and sense variations, and react accordingly to maintain CNS homeostasis (Kettenmann et al., 2011). Hence, the local environment, such as BBB permeability or neuronal activity, is key in driving microglial phenotype. Lawson et al. (1990) described regional variations in microglial morphology and density as the first evidence for heterogeneity of these cells. More recently, using genome-wide transcriptional profiling of adult microglia from various brain regions, Grabert et al. (2016) thoroughly addressed the question of regional heterogeneity of microglia and found that the mouse microglial transcriptome is regionally heterogeneous. Moreover, regional microglial heterogeneity in immunophenotype suggests differences in immune vigilance (Grabert et al., 2016). Overall, these data reveal microglia as highly diverse cells under steady-state conditions. In addition, only recently has it become clear that microglial sensitivity to stimuli may be region-specific (Lucin and Wyss-Coray, 2009; Ransohoff and Perry, 2009; Olah et al., 2011; Doorn et al., 2015), as a potential substrate for differential and evolving pattern of neuropathologies.

3. Evidence for Age Dependence of Microglia. Many groups have examined age-dependent modulation of microglial morphology, density, phenotype, and function (Tay et al., 2017). This literature is too vast to be described in an exhaustive way in this work. In substance, microglial activity is continuously evolving, in a structure and age-dependent manner, from
guidance of axons or phagocytosis of neuronal elements during development (Paolicelli et al., 2011; Schafer et al., 2012; Squarzoni et al., 2014), to fine remodeling of neuronal circuits at adolescence and adulthood (Tremblay et al., 2010; Parkhurst et al., 2013) or control of neuroinflammation in normal and pathologic ageing (Kettenmann et al., 2011; Tay et al., 2017). This list is far from being exhaustive but shows that microglia are versatile cells, constantly adapting to the environment to maintain homeostasis.

In conclusion, age-, sex-, and region-specific variances in microglial function may allow differential responses to the same stimulus at different ages, perhaps contributing to altered CNS vulnerabilities and/or disease courses. Combined to age, sex, and region dependence of n-3 PUFA brain composition, microglia–lipid interactions are likely to be extremely diverse. Hence, more studies taking into account this complexity are needed to fully understand how n-3 PUFAs modulate microglial activity in time and space and in a sex-specific way.

VI. Pharmacological Considerations on the Use of LC-PUFAs or SPMs as Effective Anti-Inflammatory Drugs in the Brain—Clinical Use

A. LC-PUFA Dietary Interventions to Limit Neuroinflammation in Humans

Considering the role of LC PUFA status, in particular DHA and/or EPA, to promote SPMs in the brain, the increase of these fatty acids in the brain using dietary approaches represents a potential strategy to control inadequate neuroinflammatory processes.

Several studies highlighted that increasing DHA and/or EPA by oral administration leads to increasing SPMs. In healthy humans, basal plasma levels of DHA-derived SPMs are low (Markworth et al., 2016). Fish oil supplementation containing a mixture of EPA and DHA is reported to increase DHA metabolites (Schuchardt et al., 2014; Keelan et al., 2015; Mas et al., 2016; Zulyniak et al., 2016). D-series SPMs are also reported as increased by fish oil dietary supplementation (RvD1; Keenan et al., 2012; Mas et al., 2016), whereas other studies report no change (Zulyniak et al., 2013; Skarke et al., 2015). Changes in SPMs appear to be specific, as a 14-day oral intake of EPA (2.7 mg/d) leads to increased blood level of 18-HEPE, with no effect on RvE2 and 3 (Endo et al., 2014). A 7-day oral supplementation with purified n-3 DPA increases DHA metabolites (EpDPE, DiHDoPE, and MaR) as compared with oral olive oil (Markworth et al., 2016). Conversely, oral supplementation with fish oil or EPA, but not with n-3 EPA, decreases AA-derived LOX products (Fischer et al., 2014; Markworth et al., 2016). Although the rapid increase in SPMs linked to oral intake of LC-PUFAs is promising, whether this is also the case in the brain has not been evaluated in humans yet. Recent works report that SPMs are detectable in postmortem brain tissue and cerebrospinal fluid (CSF) samples, with a dramatic decrease in LxA4, RvD1, and MaR in the CSF and brain of AD patients as compared with control age-matched subjects (Wang et al., 2015).

One puzzling question is whether increases in dietary DHA intake are sufficient to increase brain DHA and target neuroinflammation in humans. As previously reviewed, the plasma unesterified pool of DHA is the principal source for brain DHA (Chen et al., 2015). Positron-emission tomography studies using C11-DHA showed that DHA half-life in the plasma of healthy humans is about 2 hours, and the brain incorporation corresponds to 3.8 mg/d with a half-life of 2.5 years (Umhau et al., 2009). It is important to understand that this predicts a drop of 5% in the brain when DHA decreases in the plasma for 49 days. Dietary supplementation aims at maintaining high level of blood DHA to consequently increase its levels in the brain. However, the efficiency of DHA metabolism has to be taken into account to better predict brain DHA increase (Yassine et al., 2017). For example, lipoprotein APOE largely influences DHA half-life, suggesting that fish oil supplementation of APOE4 carriers (at risk of AD) is less efficient to increase DHA in the brain (Chouinard-Watkins and Plourde, 2014). Parenteral administration of DHA (Omegaven, LipoPlus, SMOFLipid containing 10%–15% fish oil) is also useful to rapidly increase plasma DHA but has been poorly researched in the context of neuroinflammatory diseases, but more commonly in peripheral inflammatory diseases (Hall et al., 2016; Klek, 2016).

1. LC-PUFA Dietary Intervention and Neuroinflammation in AD Patients

Clinical trials using DHA in patients with moderate to severe AD are representative of current strategies using nutritional approaches to protect and/or treat neuroinflammation. The aim is not to perform an exhaustive review of trials (14 trials of “Alzheimer and DHA” currently declared on clinicaltrials.gov) or mechanistic evidence of DHA in pathologic pathways of AD, as elegantly reviewed elsewhere (Devassy et al., 2016), but rather to highlight recent data questioning the use of DHA to protect or to treat AD-related neuroinflammation.

Several epidemiologic studies report that patients suffering from AD display relatively low blood DHA (Conquer et al., 2000; Tully et al., 2003; Wang et al., 2008), and conversely, that poor DHA consumption is associated with increased risk for AD (Quinn et al., 2010). In addition to the protective effect of DHA on neuronal death and function, together with Aβ pathogenesis, neuroinflammatory processes occurring in AD are also targeted by DHA (Cole and Frautschy, 2010; Calon, 2011; Joffre et al., 2014). Neuroinflammation and microglia cell proliferation in AD patient brain surrounding amyloid deposit are prominent (recently reviewed in Perry et al., 2010). This is consistent with the observation that in the 3xTg-AD, an AD animal
In the model, microglia number is increased before the apparition of Aβ deposition (Rodriguez et al., 2016). Microglia are involved in Aβ phagocytosis, therefore containing Aβ-induced neuronal damage, except in APOE carrier (Mulder et al., 2014). Of note, APOE, which plays a critical role in cholesterol and phospholipid transport to neurons, has recently been highlighted as being important in microglia phagocytic activity of Aβ (Yeh et al., 2016). Triggering receptor expressed on myeloid cells 2 (TREM2), a transmembrane protein exclusively expressed in microglia and an AD risk gene (Lucin et al., 2013), binds APOE, especially after lipidation of this apolipoprotein and facilitates Aβ phagocytosis when coupled to lipoprotein (Walter, 2016). In addition, microglia inflammatory response, as exacerbated by aging or inflammatory insult, decreases microglia phagocytic activity and may promote Aβ aggregation and neuronal death (Heneka et al., 2015). Interestingly, microglial activation leading to increased Aβ phagocytosis and anti-inflammatory profile, is achieved through the use of agonists of several receptors (Yamanaka et al., 2012), in particular PPARs and retinoid X receptors, which are DHA receptors, as previously discussed. In this context, and in the frame of this review, a high brain DHA status is of interest to protect from neuroinflammation and for maintenance of optimal phagocytic activity of microglia. Recent work highlighted that dietary supplementation with n-3 PUFAs and antioxidants dramatically increases Aβ phagocytosis by monocytes (Fiala et al., 2015). As discussed before, increasing DHA levels via dietary means or genetic approaches potently reduces brain proinflammatory cytokine production in animal models of acute and chronic inflammation (Orr et al., 2013; Delpech et al., 2015a,b; Taha et al., 2017), aging (Labrousse et al., 2012; Moranis et al., 2012), or AD (Casali et al., 2015; Hopperton et al., 2016). Concomitantly, DHA potently promotes microglia phagocytic activity (Chouinard-Watkins and Plourde, 2014), including toward Aβ in vitro (Hjorth et al., 2013). Experimental evidence of n-3 PUFA-protective effects on neuroinflammation in animal models of AD is corroborated by several clinical studies. The OmegaD study, a prospective clinical trial using DHA and EPA (1.7 g DHA and 0.6 g EPA/d), as primary intervention, shows that this supplementation reduces cognitive decline in very mild AD cases (Freund-Levi et al., 2014). At these doses, a 6-month dietary supplementation increases circulating DHA level in healthy subjects. Importantly, despite no significant increase in DHA in peripheral blood mononuclear cells (PBMC) of mild cognitive impairment patients, the dietary intervention restores the decrease of LxA4 and RvD1 production by these cells upon Aβ ex vivo treatment (Wang et al., 2015). However, DHA levels are not increased in the cerebral spinal fluid of supplemented subjects, although accompanied by decreased expression of proinflammatory genes and an increase in anti-inflammatory factors, including the soluble form of the type II IL-1 receptor, a decoy target for IL-1 (Vedin et al., 2008, 2012; Freund-Levi et al., 2014). Despite these encouraging clinical data showing that primary intervention aiming at increasing brain DHA prevents from neuroinflammatory processes (Devassy et al., 2016), overall the results of LC n-3 PUFAs dietary supplementation on cognitive deficits, the main outcome of AD disease, are poor (Jiao et al., 2014). Some authors suggest that these interventions might not be beneficial when the disease is at advanced stages, with effectiveness at earlier stages of the disease, when resolution of inflammation is disturbed. Importantly, a large clinical trial with a multidomain intervention (multidomain Alzheimer prevention trial [MAPT]) highlighted that a 3-year supplementation with DHA plus EPA (800 and 225 mg, respectively) of nondemented elderly does not protect from cognitive decline, except in subgroup starting with a low n-3 PUFA index (Andrieu et al., 2017). The effectiveness of DHA in stimulating a phagocytic (noninflammatory) phenotype of microglia is consistent with this hypothesis (Hjorth et al., 2013). It is important to note that APOE4 carriers, which represent 40% of AD patients, and patients with low Aβ levels in the CSF have a lower DHA brain transport, as revealed by identical plasma DHA and reduced DHA in the CSF after DHA supplementation (Yassine et al., 2017). Other studies report a lower DHA increased in APOE4 carriers as compared with noncarriers (Plourde and Cunnane, 2007; Chouinard-Watkins et al., 2013). All together, these data suggest that in humans, APOE4 carriers have an impairment in fatty acid homeostasis, even after DHA supplementation, further highlighting the necessity to pay attention to this allele when supplementing with PUFA (Chouinard-Watkins and Plourde, 2014; Hennebelle et al., 2014). However, a very recent study highlights that unesterified DHA uptake is increased in the brain of APOE4 carriers (Yassine et al., 2017). Noteworthy, no attention has been paid to APOE4 carriers in studying the efficiency of DHA supplementation on neuroinflammatory processes or microglia phagocytic activity of Aβ. This deserves attention, as it could help design personalized nutrition to specific subtypes of patients at risks of neuroinflammation and/or microglia activity impairment, such as Trem2, the complement receptor 1, or cluster of differentiation 33, which are involved in microglial phagocytic activity and are regulated by DHA (Griciuc et al., 2013).

2. LC-PUFA Dietary Intervention and Neuroinflammation in Patients with Mood Disorders. In the last 20 years, epidemiologic studies have linked dietary PUFAs in the pathophysiology of mood disorders (recently reviewed in Bazinet and Layé, 2014; McNamara, 2015). In particular, dietary intake of food rich in n-3 LC-PUFAs (fish, seafood) is associated with reduced prevalence of major depression, postpartum depression, or bipolar disorder. In addition, patients suffering from
mood disorders display reduced levels of EPA in erythrocyte and DHA in specific brain regions (Edwards et al., 1998). These observations are consistent with experimental studies linking low dietary intake of n-3 PUFAs, decreased brain DHA, emotional behavior disorders, and increased stress response (Harauma and Moriguchi, 2011; Lafourcade et al., 2011; Larrieu et al., 2012, 2014, 2016). Recent data from our group highlighted that neuropathological mechanisms linking brain DHA decrease and emotional/cognitive behavior disturbances involve eCB-dependent synaptic plasticity (Lafourcade et al., 2011; Bosch-Bouju et al., 2016; Thomazeau et al., 2016), glucocorticoid (Larrieu et al., 2014, 2016), and neuroinflammatory processes (Mingam et al., 2008; Labrousse et al., 2012; Moranis et al., 2012; Delpech et al., 2015a,b). In the scope of this review, neuroinflammation is implicated in the pathophysiology of mood disorders (Dantzer et al., 2008; Capuron and Miller, 2011), becoming a target of interest in the treatment of major depression, postpartum depression, and bipolar disorder. However, it has to be noted that inflammation contributes to symptoms only in a subset of patients diagnosed with mood disorders, with noticeable increase of inflammatory markers (mainly C-reactive protein) in about one third of depressed patients (Raison and Miller, 2011). In addition to genetic factors, environmental factors (diet, adversity, etc.) are considered as important risk factors for inflammation and depression, with a specific attention given to n-3 PUFA metabolism and dietary content (Kiecolt-Glaser, 2010; Laye, 2010; Bazinet and Laye, 2014; Kiecolt-Glaser et al., 2015). Observational studies linked n-6/n-3 PUFA ratio to IL-6 and TNF-α circulating levels together with depressive symptoms in older adults and students undertaking an examination (Maes et al., 2000; Kiecolt-Glaser et al., 2007). Population-based study revealed a lower level of DHA only in depressed elderly with a C-reactive protein concentration <1.5 mg/l (Tiemeier et al., 2003). Interestingly, low blood DHA levels predict depression incidence in depressive episode triggered by interferon-α administration in a subset of hepatitis patients (Su et al., 2010, 2014; Lotrich et al., 2013).

Dietary LC-PUFAs, DHA, and/or EPA have been used in 49 trials (mood disorders and omega3) referenced on clinical.trials.gov., with mitigated and heterogeneous results as revealed by several meta-analysis (Lin et al., 2010; Bloch and Hannestad, 2012; Mocking et al., 2016). Based on meta-analyses, EPA has been suggested as a predictor of mood disorder treatment efficiency with supplements containing less than 60% of total EPA plus DHA as EPA (between 200 and 2200 mg/d) or DHA alone being noneffective (Martins, 2009; Sublette et al., 2011; Mocking et al., 2016). However, few studies have studied the impact of dietary supplementation with LC-PUFAs on inflammation and depression. Medical students with no diagnosis of depression supplemented with 2085 mg EPA plus 348 mg DHA/d for 12 weeks have less LPS-induced IL-6 release in cultured PBMC and anxiety symptoms compared with control (Kiecolt-Glaser et al., 2011). Based on the observation that LC-PUFAs have anti-inflammatory activities and that they are efficient only in a subset of depressed patients, Rapaport et al. (2016) analyzed whether high EPA supplementation was more efficient in those with inflammation. Using several markers of inflammation as markers of inflammation, they found that patients with high IL-1ra and C-reactive protein and low adiponectin blood levels have a greater improvement in mood symptoms in response to a EPA-enriched dietary supplement (1060 mg EPA plus 260 mg DHA/d, 8 weeks), whereas it was not the case when supplemented with a DHA-enriched dietary supplement (180 mg EPA plus 900 mg DHA/d). Additional studies with a higher number of patients are warranted to confirm this interesting first study. The mechanisms underlying the higher efficiency of high EPA rather than DHA dietary supplementation on inflammation and depressive symptoms are still poorly understood. It can be speculated that EPA preferentially targets directly or indirectly (through specific EPA-derived SPMs) immune cells and correct immune system dysfunctions, including in the brain. In this regard, a recent study indicates that patients diagnosed with major depression had a significant decrease in cytosolic PLA2 gene expression in PBMC when treated with EPA (3.5 g/d), but not with DHA (1.75 g/d) for 12 weeks, whereas both treatments improved depressive symptoms (Su et al., 2017). To our knowledge, the effect of EPA-derived SPMs has not been tested in depression accompanied by inflammation.

All together, these examples illustrate that in humans, targeting DHA and/or EPA represents a potent strategy to regulate microglia activity and to reduce chronic inflammation. Whether DHA or EPA administration via a parenteral route controls neuroinflammatory pathways efficiently has to be further studied in humans, and could represent a way to accelerate DHA access to the brain and local SPM production.

B. The Use of SPMs or n-3 PUFA-Derived Fatty Amides to Target Brain Neuroinflammation in Humans

Some data reveal that DHA and its SPMs are anti-inflammatory in non-neural tissues (Serhan, 2017a), with indirect and direct evidence existing for their anti-inflammatory effects in the brain, including on microglia (Marcheselli et al., 2003; Lukiw et al., 2005; Orr et al., 2013a; Rey et al., 2016). As mentioned above, SPMs are found in brain cells at pM, whereas DHA is at nM, suggesting that the activity of SPMs requires less quantity than DHA. Some, but not all DHA and SPM receptors, have been identified, including very recently (see previous sections). Despite that some receptors are identified, affinity and function of these receptors are poorly known; hence, pharmacological use of SPMs is
still in emergence (Chiurchiu et al., 2016). Recent studies revealed that DHA status has also to be considered in the effectiveness of SPM activity in inflammation (Chiurchiu et al., 2016). The challenge now will be to develop clinical applicable forms of SPMs to target the brain and especially microglia. Experimental studies mainly use intrathecal route of administration, which is poorly applicable to humans. The study of the route of administration, oral or i.v., is a necessary proof of concept of the utility of SPMs to treat neuroinflammation and to reduce associated symptoms. Noteworthily, age and sex, which are crucial in DHA metabolism, neuroinflammatory regulation, and drug activity, will also have to be taken into account.

Experimental data revealed that DHEA and EPEA, the main fatty acyl amides derived from DHA and EPA, respectively, display anti-inflammatory activities in microglia in vitro and in vivo (Meijerink et al., 2011, 2013; Park et al., 2016). However, no studies have been conducted in humans, probably due to the lack of knowledge of the mechanisms underlying their activity, but also to the difficulty to manipulate eCBs in humans (Fowler, 2015). Increasing n-3 PUFAs in the diet leads to the decreased formation of n-6 PUFA-derived eCBs 2AG and AEA, whereas DHEA and/or EPEA increase (Watanabe et al., 2003; Banni and DiMarzo, 2010; Bosch-Bouju et al., 2016). Such a correlation is also reported in humans (Joosten et al., 2010; Jones et al., 2014; Pu et al., 2016). Recent data highlight that in humans, genetic variants of the AEA hydrolytic enzyme fatty acid amide hydrolase, but not of N-acetyl phosphatidylethanolamine-specific phospholipase D, may also contribute to DHEA circulating level (Pu et al., 2016). DHEA is a substrate of fatty acid amide hydrolase, which degrades it into DHA (Kim et al., 2011). Whether plasma DHEA, which is a lipophilic factor that crosses the BBB, brain-derived DHEA, or both contribute to the anti-inflammatory activity of DHA remains to be investigated (Meijerink et al., 2013). Also, receptors mediating the anti-inflammatory effects of DHEA are poorly known. In macrophages, none of the classic eCB receptors (CB1, CB2, and PPAR) mediate the effect of DHEA on LPS-induced cytokine release (Meijerink et al., 2015). No studies have ever evaluated whether GPR110, a DHEA receptor recently identified (Bosch-Bouju et al., 2016), is involved in DHEA anti-inflammatory activity.

C. Pharmacological Strategy to Promote Endogenous SPM Production in the Brain: Combination of PUFAs and Anti-Inflammatory Drugs

Increases in COX-1 and COX-2 expression are a hallmark of brain inflammation and occurs in ischemic or traumatic brain injuries and neurodegenerative diseases such as AD (reviewed in McGeer and McGeer, 2007). Consequent rise in PGE and TX in endothelial, neuronal, and glial cells is believed to be causative of neurodegeneration (Farooqui et al., 2006; Phillis et al., 2006). Not only AA is converted to cyclic endoperoxides by the action of COX, but also DHA and EPA, as described above. However, these latter are less prone to be metabolized through the COX pathway, and eicosanoid generated are less active than the ones generated from AA. Importantly, DHA and EPA reduce COX-2 activity, therefore reducing the production of inflammatory eicosanoids and TX from AA. The use of nonsteroidal anti-inflammatory drugs (NSAIDs), which block COX activity, is considered as attractive to counteract neurodegeneration associated to neuroinflammation, especially microglia. Of note, as COX-2 is also highly expressed in healthy neurons (Yamagata et al., 1993), NSAIDs have adverse effects on neuronal activity in noninflammatory conditions, whereas reduction of neuronal COX-2 activity in inflammatory condition improves pain. COX-3 is a more recently identified variant of COX-1 (Chandrasekharan et al., 2002), which is preferentially expressed in the brain and involved in pain and fever. In addition, to be sensitive to NSAIDs, COX-3 is inhibited by paracetamol (acetaminophen), whereas COX-1 and 2 are not (Botti, 2003). To the best of our knowledge, no specific effect of DHA has been reported on COX-3 activity. A new class of COX-2 inhibitors (tricyclics such as coxibs and nontricyclics) emerged, allowing the specific targeting of COX-2 (Zarghi and Kakhki, 2014).

Combination of NSAIDs and DHA or EPA has been proposed as an efficient strategy to prevent or treat neuroinflammatory pathways associated to neurodegenerative diseases (Pomponi et al., 2011), with no results in humans yet. In vitro, microglia treatment with DHA or aspirin or both reveals that the combination of aspirin and DHA promotes the antioxidative endogenous enzyme glutathione production, with no potentiation on proinflammatory cytokine production (Pettit et al., 2013). Attention has been paid to aspirin, a NSAID, which acetylates COX-1 and COX-2. Whereas COX-1 acetylation leads to a loss of its activity, the acetylated form of COX-2 leads to the synthesis of AT-LxA4, primarily identified to limit neutrophil activation and recruitment to inflamed tissues (Serhan et al., 2002) with anti-inflammatory and proresolving activities (Ye et al., 2010; Martins et al., 2009). Importantly, low-dose aspirin treatment has a gender effect on AT-LxA4 production in healthy subjects (Chiang et al., 2006). In AD animal models, chronic administration of AT-LxA4 improves memory deficits through an Aβ-independent mechanism (Medeiros et al., 2013; Dunn et al., 2015). In addition, this compound promotes the alternative phenotype of microglia, with a higher phagocytic activity toward Aβ (Ye et al., 2013) and reduces microglia activation (Wang et al., 2011) and neuropathic pain (Martini et al., 2016). A randomized trial with low-dose aspirin in AD patients did not observe a beneficial effect upon 2 years on cognitive outcomes, rather it increases risk of bleeding (Bentham et al., 2008).
Since 2010, studies using aspirin in AD patients led to the conclusion that the beneficial effect of this drug on AD symptoms is unclear and that bleeding risks are high (Thoonsen et al., 2010). As high-dose DHA is recognized to affect blood fluidity, one could wonder whether it is safe to combine aspirin and DHA as a chronic treatment of neuroinflammation.

D. The Use of COX or Lipoxygenase Inhibitors to Target Neuroinflammation: a Paradox?

Clinical trials with NSAIDs on AD are overall disappointing with mitigated results. COX-2 generates both pro- and anti-inflammatory compounds with a balance depending on the ratio AA/DHA-EPA that is largely linked to nutritional status (Bazan et al., 2011; Mouchlis and Dennis, 2016; Serhan, 2017b); one could wonder about the beneficial effect of inhibiting this enzyme in the context of neuroinflammation. In humans, using a lipidomic approach, Ibuprofen (a non-selective inhibitor of COX) was shown to block exercise-induced increase in prostanoids and leukotrienes, but also anti-inflammatory/proresolving lipid mediators, possibly explaining the mitigated beneficial effect of COX inhibitors (Markworth et al., 2016). Also, combination of DHA and NSAIDs should be considered cautiously as it could augment the production of leukotrienes that are proinflammatory, as recently suggested by an elegant in vitro study (Norris and Dennis, 2012). The analysis of DHA status, together with dietary patterns and genetic overview of alleles such as fatty acid desaturase 2, which influences fatty acid metabolism (Martinelli et al., 2008; Schuchardt et al., 2016), should help to phenotype AD patients and treat them with NSAIDs in combination or not with DHA. Such a strategy could be used in other diseases with an inflammatory component, such as depression, which overall show mitigated effect of DHA/EPA supplementation on symptoms, except when patients display a proinflammatory profile (Rapaport et al., 2016).

LOX are iron-containing dioxygenases, which oxygenate AA, DHA, and EPA into several derivatives with proinflammatory (leukotrienes, HETE) and anti-inflammatory (resolvins, protectin D1) activities. Importantly, as AA and DHA compete for their metabolism into LOX-mediated derivatives, the quantity of these precursors is critical. A specific system, called suicidal inactivation, allows for the stopping of LOX activity after a certain number of cycles of oxygenation through specific oxidation of LOX amino acids (Kuhn and O’Donnell, 2006). LOX also participate to membrane lipid peroxidation with structural modifications as observed in the aged brain (Praticò et al., 2004). All together, these data suggest that LOX may be involved in pathologic mechanisms of brain diseases, such as AD through their pro-oxidative and proinflammatory signaling, but may also affect processes directly related to neurotoxic cascades dependent on Aβ and Tau. There are five existing LOX isoforms (5, 8, 12, and 15-LOX and 3-LOX) with 5-LOX particularly expressed in the inflamed brain. When catalyzing AA, 5-LOX leads to the synthesis of the instable form LTA4 that is rapidly metabolized into LTB4 or LTC4 as observed in brain injury, multiple sclerosis, Parkinson’s disease, or AD (Czapski et al., 2016). LOX-derived LTA4 synthesis is either occurring in a cell-specific manner or through endothelial cells or platelets that do not express LOX (Kuhn et al., 2015). In this last case, LTA4 produced by surrounding neutrophils is used to generate LTC4 via transcellular mechanism (Papayianni et al., 1996). Transcellular mechanisms have been described in neurons and glial cells to produce LTC, LTD, and LTE4 as neutrophils provide LTA4 (Farias et al., 2007). This suggests that brain entry of neutrophils is a critical step for local leukotriene production, as it is the case in traumatic brain injury (Jickling et al., 2015). In line with this, depletion in neutrophils in an animal model of AD improves memory (Zenaro et al., 2015). However, more work has to be done to understand the mechanisms underlying 5-LOX activity in AD as its expression modulates both Aβ and Tau processing (recently reviewed in Joshi and Praticò, 2015).

Zileuton and meclofenamate sodium are 5-LOX inhibitors. Long-term zileuton treatment potently reduces amyloidogenesis and improves memory of Tg2576 and 3xTg mice, used as animal models of AD (Chu and Pratico, 2011; Chu et al., 2013), which is in accordance with data obtained in 3xTg-KO 5-LOX mice (Gianopoulos et al., 2014). In vitro, inhibitors of 5-LOX are less potent than COX inhibitors to protect neurons against toxicity triggered by activated monocyctic or microglial cells (Klegeris and McGeer, 2002, 2003). Dual COX/5-LOX inhibitors (licofelone and flavocoxid) show anti-inflammatory activities in several brain disease models such as stroke (Minutoli et al., 2015), experimental allergic encephalitis (Kong et al., 2016), muscular dystrophy, AD (Bitto et al., 2017), or sepsis (Bitto et al., 2012), with a direct effect on microglia (Kong et al., 2016). The recent discovery that 5-LOX not only metabolized AA, but also DPA, whose metabolites display anti-inflammatory activities, highlights the need to evaluate whether inhibiting this enzyme in the context of high n-3 PUFA status is beneficial under neuroinflammation. In addition, human microglia stimulate a 5-LOX resolution pathway when activated by classic pathogen-associated molecular patterns such as LPS, although it is not the case not when activated by Aβ (Zhu et al., 2016), suggesting that the nature of the stimulus is crucial in the mobilization of 5-LOX-dependent resolution pathways in microglia.

In humans, 12/15-LOX expression is constitutive in several immune cells (macrophage, immature red blood cells, eosinophil, airway epithelial cells, T lymphocytes) (Nadel et al., 1991). Animal studies using genetic deletion of these enzymes reveal the importance of 12/15-LOX
products in the regulation of inflammation (reviewed in Kuhn and O’Donnell, 2006). Classic anti-inflammatory cytokines IL-4 and IL-13 activate 12/15-LOX expression in monocytes, which do not express these enzymes in noninflammatory conditions (Brinckmann and Kühn, 1997). 15-LOX–induced lipoxin LxA4 promotes a shift from acute inflammation to resolution (Levy et al., 2001). However, different isoforms of 15-LOX identified in humans play opposing roles in prostate cancer progression through LA and AA metabolic products, respectively, 13-hydroxyoctadecatrienoic acid (promotion of cancer, 15-LOX1) and 15-HETE (inhibition of cancer, 15-LOX2) (Hu et al., 2013). The 17-series DHA metabolites, through 13-hydroxyoctadecadienoic acid (promotion of cancer, suppression through LA and AA metabolic products, respectively, reduced phagocytic receptor expression triggering apoptosis/phagocytosis uncoupling. PLoS Biol 14:e1002466.


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