Review

Specialized Pro-Resolving Mediators from Omega-3 Fatty Acids Improve Amyloid-β Phagocytosis and Regulate Inflammation in Patients with Minor Cognitive Impairment

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Abstract. In this review we discuss the immunopathology of Alzheimer’s disease (AD) and recent advances in the prevention of minor cognitive impairment (MCI) by nutritional supplementation with omega-3 fatty acids. Defective phagocytosis of amyloid-β (Aβ) and abnormal inflammatory activation of peripheral blood mononuclear cells (PBMCs) are the two key immune pathologies of MCI and AD patients. The phagocytosis of Aβ by PBMCs of MCI and AD patients is universally defective and the inflammatory gene transcription is heterogeneously deregulated in comparison to normal subjects. Recent studies have discovered a cornucopia of beneficial anti-inflammatory and pro-resolving effects of the specialized proresolving mediators (SPMs) resolvins, protectins, maresins, and their metabolic precursors. Resolvin D1 and other mediators switch macrophages from an inflammatory to a tissue protective/pro-resolving phenotype and increase phagocytosis of Aβ. In a recent study of AD and MCI patients, nutritional supplementation by omega-3 fatty acids individually increased resolvin D1, improved Aβ phagocytosis, and regulated inflammatory genes toward a physiological state, but only in MCI patients. Our studies are beginning to dissect positive factors (adherence to Mediterranean diet with omega-3 and exercise) and negative factors (high fat diet, infections, cancer, and surgeries) in each patient. The \textit{in vitro} and \textit{in vivo} effects of omega-3 fatty acids and SPMs suggest that defective phagocytosis and chronic inflammation are related to defective production and/or defective signaling by SPMs in immune cells.

Keywords: Amyloid-β, fatty acids, inflammation, mild cognitive impairment, omega-3

INTRODUCTION

Phagocytosis of amyloid-β (Aβ) and inflammation are the two faces of innate immunity underlying much of the Alzheimer’s disease (AD) immunopathology and are an excellent target for AD prevention. Curcuminoids, vitamin D3, and omega-3 fatty acids are popular preventive supplements that have been shown to enhance phagocytosis and tame inflammation [1]. Here we review the pathology of the innate immune system in AD patients and the advances in the prevention of mild cognitive impairment (MCI) by the lipidic mediators termed specialized proresolving mediators (SPMs) that are derived from omega-3 fatty acids [2].
The strategy we employed in our in vitro studies is based on investigating the clearance of Aβ1-42 by immune and endothelial cells in the brain tissues of AD patients and using omega-3 fatty acids and SPMs as agonists in macrophages. The in vivo aim of this approach is to rectify the defective clearance of Aβ1-42 by the monocyte/macrophages of AD patients using supplementation with omega-3 fatty acids and increasing SPMs [3–5]. Omega-3 fatty acids are an important component of the Mediterranean diet, which is high in fish and olive oil, low in meat, and moderate in dairy products [6]. Omega-3 fatty acids are precursors of anti-inflammatory and pro-resolving mediators that improve the immune clearance of Aβ1-42 (see below "Role of SPMs in dementia and Alzheimer’s disease").

**AMYLOID-β1-42 NEUROTOXICITY**

Neuronal damage in the AD brain is related to accumulation of Aβ1-42 and phosho-tau [7] as well as the inflammation induced by these proteins. Apolipoprotein E ε4 allele genotype is a risk factor for accumulation of Aβ1-42 in the brain related to increased amyloidogenic β- and γ-cleavage of amyloid-β protein precursor (AβPP), and loss of sirtuin T1, and reduced clearance across the blood-brain barrier. The products of β- and γ-cleavage of AβPP are the peptides sAβPPβ, Aβ1-42, Jcasp, and C31, which cause neurite retraction and cell death [8]. The mechanisms of Aβ1-42 neurotoxicity [9] involve inflammatory activation, prion-like toxicity, and production of neurofibrillary tangles. Upstream of Aβ1-42, neurotoxicity is attributed to prostaglandins, and additionally to F2-isoprostanes produced by free radical-catalyzed peroxidation of arachidonic acid [10]. In addition, possible culprits in neural damage, especially in APOE ε4 carriers, are the products of oxidative stress, 4-hydroxy-2-nonenal, which is generated by peroxidation of the ω-6 polyunsaturated fatty acid (PUFA) arachidonic acid, and 4-hydroxy-2-hexenal, which is produced by peroxidation of the ω-3 PUFAs docosahexaenoic acid (DHA) [11].

**AMYLOID-β1-42 CLEARANCE IN MODEL SYSTEMS**

Enhancement of Aβ1-42 clearance from the brain has a high priority in therapeutic research. Aβ1-42 clearance across the blood-brain barrier and choroid plexus in rodent model systems is reduced by APOE ε4 due to redirection of Aβ1-42 from the low-density lipoprotein receptor-related protein 1 to the very low-density lipoprotein receptor [12–14]. A defect of systemic Aβ1-42 elimination by liver and kidney pathologies promotes Aβ1-42 accumulation [15]. Bexarotene stimulation of the peroxisome proliferator-activated nuclear receptor γ (PPARγ) and liver X receptor, in coordination with the retinoid X receptor, in the AβPP/PS1 model increased memory and Aβ1-42 clearance and degradation [16]. Subsequent experience in other laboratories with bexarotene only partially validated these results [17]. The PPARγ agonist pioglitazone increased Aβ1-42 clearance in a mouse model and polarized microglia to pro-resolution M2 type [18]. In a mouse model, Aβ1-42 phagocytosis was enhanced by inhibition of the EP2 receptor for prostaglandin E and also by CD36 upregulation [19]. Aβ1-42 is degraded by enzymes present in the brain but reduced in APOE ε4 carriers, i.e., neprilysin and insulin-degrading enzyme [20, 21].

These strategies have not been translated into therapeutics for a number of reasons, including the following: mouse inflammatory responses show low correlation with human responses [22]; neurotoxicity of Aβ differs in mice and men [23]; and mice do not have the genetic variability in the HLA complex genes and Toll-like receptors observed in patients [24].

**IMMUNE CLEARANCE IN HUMAN BRAIN AND RECEPTORS FOR Aβ1-42 ON IMMUNE CELLS**

In patients with sporadic AD, Aβ1-42 accumulation is related to decreased clearance, but not to increased production [25]. One cogent reason for decreased Aβ1-42 clearance is the reduction of immune clearance by AD macrophages, which display reduced phagocytosis and degradation in comparison to normal macrophages [3]. Although soluble Aβ1-42 is transported across the brain endothelia, Aβ1-42 aggregates in the brain and its clearance then depends upon degradation and transport by immune cells. In animal models, Aβ1-42 phagocytosis and degradation are attributed to brain microglia recruited from the bone marrow and the blood by chemokines, such as CCL2 [26]. In the AD brain, macrophage-like cells are observed on immunohistochemical examination to traverse from the microvessels into the neuropil, as documented in HIV-1 encephalitis [27, 28] and the AD brain [3]. Macrophages are also believed to phagocytize and degrade extracellular tau [29].
Both microglia and macrophages belong to the innate immune system and have similar receptors for Aβ₁₋₄₂: (a) the scavenger receptors including class A (SCARA-1 and MARCO), class B (SCARB-1 and CD36), and other (CD68), (b) the receptor for advanced glycation end product (RAGE) called CD163; (c) the G-protein coupled receptors (GPCRs) lipoxin A₄/formyl peptide receptor 2 (ALX/FPR2) and chemokine-like receptor 1 (CMKLR1); and (d) the toll-like receptors (TLRs) [30, 31]. In addition, the studies of non-steroidal anti-inflammatory drugs targeting prostaglandins have spurred interest in the modulation of prostanoid receptors [32]. Recently an increased risk of AD was found to be related to heterozygous variants in the triggering receptor expressed on myeloid cells 2 protein (TREM2) [33]. TREM2 is expressed on alternatively activated M2 macrophages and attenuates macrophage activation [34].

GPCRs are the most interesting from the omega-3 therapeutic viewpoint as the ALX/FPR2 antagonist blocked Aβ₁₋₄₂-induced IL-1β in monocytes [35] and ALX/FPR2 antibody blocked enhancement of Aβ₁₋₄₂ phagocytosis by resolvin D1 [36]. CMKLR1 is a receptor for another lipid mediator resolvin E1 [37]. Thus GPCR signaling may be crucial for the balance between inflammation and phagocytosis. The roles of Aβ receptors are summarized in an excellent review of animal studies [38]: a) CD14 functions in the recognition and binding of Aβ for its internalization and clearance from the brain parenchyma [31, 39]; b) RAGE has a role in the induction of inflammatory cascade; c) TREM has a role in clearance of Aβ; and d) complement and Fc receptors, ALX/FPR2, CD36, and TLRs are important in both inflammation and phagocytosis. The multiplicity and heterogeneity of these receptors reduces therapeutic potential of the receptor blockade. The use of endogenous SPMs discussed below may provide a physiological approach to optimize the balance between effective phagocytosis and moderate inflammation.

IMMUNE PATHOLOGIES IN AD PATIENTS

Peripheral blood mononuclear cells (PBMCs) of AD patients display specific immune pathologies. Defective phagocytosis of Aβ₁₋₄₂ by immune cells of AD patients was first noted with AD macrophages [40]. In subsequent studies, almost all AD patients have been found defective in Aβ₁₋₄₂ phagocytosis in macrophages and monocytes, and deregulated with respect to inflammatory activation of PBMCs. The defect in phagocytosis was found to be specific for genuine Aβ₁₋₄₂ as scrambled Aβ₁₋₄₂ was not phagocytized, whereas E. coli was phagocytized by both normal and AD macrophages [41]. In a “tissue assay” with sections of the AD brain incubated with macrophages, strikingly different results were observed with normal macrophages phagocytizing and clearing Aβ₁₋₄₂ compared to AD macrophages aggregating and becoming apoptotic after up loading Aβ₁₋₄₂ in the AD brain tissue [3]. Fibrillar Aβ₁₋₄₂ was cleared by normal macrophages but caused apoptosis of AD macrophages. These defects may explain the apoptosis of macrophages around brain microvessels and release of Aβ₁₋₄₂ in the wall of congophilic brain vessels [42].

ENHANCEMENT OF IMMUNE CLEARANCE OF Aβ₁₋₄₂ IN THE AD BRAIN

In the AD brain, Aβ plaques are cleared by macrophages, which appear distinct from ramified microglia [28]. According to a confocal microscopic study of the AD brain in a “tissue assay” (monocytes are co incubated with the AD brain tissue slices), monocyte/macrophages intrude into neurons and upload Aβ [42]. Thus monocyte/macrophages, depending on their fitness, may upload and clear Aβ₁₋₄₂ from both the plaques and neurons.

Epidemiological studies of dementia in various populations have produced strong rationale for AD prevention using natural substances based on local customs [16, 17, 19]. Fortuitously, these substances have been shown to increase Aβ₁₋₄₂ phagocytosis [1]. The nutrients believed to be related to better cognition according to population studies include omega-3 fatty acids, vitamin D3, curcumin, and other natural substances [1]. Using these substances in vitro, Aβ₁₋₄₂ phagocytosis by monocytes and macrophages was modulated by vitamin D3, curcumin, and, in particular, by the omega-3 fatty acids DHA and eicosapentaenoic acid [36]. Administration of omega-3 fatty acids to patients with dementia in a randomized, double-blind, placebo-controlled clinical trial significantly slowed the decline of Mini-Mental State Examination (MMSE) scores in a subgroup of MCI patients with MMSE >27 points [43]. A recent study of supplementation by omega-3 fatty acids, antioxidants, and resveratrol in the drink Smartfish (Smartfish Inc., Oslo, Norway) showed that MCI patients on this supplement maintained their cognition with initial MMSE 25.9 and final MMSE 25.7 after 4–17 months [5].
OMEGA-3 FATTY ACID
ANTI-INFLAMMATORY AND
PRO-RESOLVING MECHANISMS

The roles of prostaglandin E₂ in inflammation, thromboxanes in platelet aggregation and constriction of vascular smooth muscle, and leukotrienes in asthma were gleaned in the laboratory of Samuelson [44] in the 1960s and 1970s [44]. The anti-inflammatory and pro-resolving lipoxins derived from arachidonic acids were discovered in 1980s at Karolinska Institute, and the first resolvins in Serhan's laboratory in 2000 [45]. The concept of active resolution of inflammation became apparent in inflammatory exudates when the lipid mediator class switched from pro-inflammatory prostaglandins and leukotrienes to anti-inflammatory and pro-resolving lipoxins [45, 46]. Therefore, this class switch process leads to a shift in the exudate lipid mediator profile from one that is pro-inflammatory during the initial pro-inflammatory response to an anti-inflammatory and pro-resolving with elevated SPMs [47].

The molecular basis of the omega-3 actions in the central nervous system was discovered in Serhan's [48] and Bazan's laboratories [49]. These researchers observed the production of 10R,17S-docosatriene called neuroprotectin D1 (NPD1; 10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid) in oxidative stress-challenged human retinal pigment epithelial cells [50] and the rat brain undergoing ischemia-reperfusion [49]. Interestingly, IL-1β enhances the production of NPD1 which in turn inhibits IL-1β-stimulated production of COX-2 and caspase-3 activation [51], suggesting that during inflammation the SPMs production is tightly regulated to achieve optimal phagocytosis and neuroprotection.

STIMULATION OF INFLAMMATION BY
PROSTAGLANDINS AND LEUKOTRIENES
AND TERMINATION OF ACUTE
INFLAMMATION BY SPECIALIZED
PRO-RESOLVING MEDIATORS

Inflammation is induced by the pro-inflammatory leukotrienes LTB₄ (potent leukocyte chemotactant), LTC₄, LTD₄, LTE₄ (vascular leakage, smooth muscle contraction), and the prostaglandins E₂ and D₂ (PGE₂ and PGD₂), and cytokines that stimulate vascular leakage and promote neutrophil diapedesis in the early phase of inflammation. LTB₄ stimulates inflammation by binding to the G-protein coupled receptors (GPCRs) BLT1 and BLT2 that are present in the membranes of the relevant cell types and triggers the expression of chemokines and cytokines (IL-6 and IL-8). After the early phase, PGE₂ and PGD₂ switch production of lipid mediators to SPMs by the upregulation of the key biosynthetic enzyme 15-lipoxygenase (15-LO) type 1 in leukocytes [52]. This promotes the conversion of (a) arachidonic acid to lipoxin A₄ (LX A₄) and LX B₄, and (b) the omega-3 PUFA DHA to the D-series resolvins and protectins/neuroprotectins [52]. The mediators are produced in humans by 15-LO and 5-lipoxygenase that convert the PUFA substrate via subsequent lipoxygenation reactions [52]. For recent detailed reviews on the biosynthetic pathways, interested readers are directed to the reviews [53, 54]. Specific members of these new families of mediators from the DHA metabolome are named D-series resolvins (Resolvin D1 to Resolvin D6), protectins (including protectin D1-neuroprotectin D1), and maresins (MaR1 and MaR2) [53].

Resolution of inflammation involves limiting of neutrophil recruitment, promotion of nonphlogistic recruitment, and activation of monocytes, and nonphlogistic phagocytosis and lymphatic clearance of apoptotic neutrophils by macrophages [55]. Alternatively, chronic inflammation can result from excessive and/or unresolved inflammatory responses. Resolution of inflammation is mediated by SPMs, each with a specialized function. LXA₄ inhibits leukocyte trafficking in vivo by activating the lipoxin A₄ receptor (denoted ALX/FPR2). Resolvin D1 directly activates CMKLR1/ERV1 on mononuclear cells and dendritic cells and acts as a competitive antagonist at the leukotriene B₄ receptor (denoted BLT). Resolvin D1 binds to ALX/FPR2 and another G protein coupled receptor called GPR 32, and inhibits neutrophil migration and promotes their phagocytosis by macrophages [56]. Aspirin-triggered resolin D1 (AT-RvD1) inhibits the release of IL-6 and protects the brain after surgical injury from neuroinflammation, synaptic dysfunction, and cognitive decline [57]. DHA is also converted by macrophages via 14-lipoxygenation to the Maresins (macrophage mediators in resolving inflammation). The first member of this family identified was Maresin 1 (MaR1; 7R,14S-dihydroxy-docosa-4Z,8E,10E,12Z,16Z,19Z-hexaenoic acid). These mediators potently stimulate macrophage phagocytosis of apoptotic cells in a stereospecific manner. They also regulate both inflammation and chemotherapy-induced pain by inhibiting TRPV1 currents in neurons and limits neutrophil recruitment [58]. Recently, a novel family of macrophage-derived pro-resolving

296
M. Fiula et al. / Pro-Resolving Mediators Improve Amyloid-β Immunity
mediators coined Maresins Conjugates in Tissue Regeneration (MCTR) was identified. These novel resolution agonists carry potent organ-protective, tissue-regenerative, and pro-resolving actions [59]. The production of proresolving mediators is evolutionarily conserved being identified in a number of species from planaria, to mice, baboons and humans [60, 61]. Whereas in planaria RvE1, MaR1, MCTR1 and MCTR2 accelerate tissue regeneration, in mice and humans, this process is regulated by select macrophage subtypes [62].

ROLE OF MACROPHAGES IN RESOLUTION OF INFLAMMATION

Macrophages are key players in various host protective processes ranging from protection against pathogen infections to clearance of cellular debris and tissue repair and regeneration, with different macrophage subsets being associated with the propagation or resolution of inflammation [63, 64]. Classically M1 macrophages are regarded as pro-inflammatory, whereas macrophages displaying the M2 phenotype are anti-inflammatory/pro-resolving. Recent evidence demonstrates that macrophage phenotypes in vivo display intermediate characteristics to these extreme descriptions giving rise to more physiologically relevant phenotypes, such as those described by Stables and colleagues [65] and Schif-Zuick and colleagues [66]. A number of studies have now investigated the actions of pro-resolving mediators on regulating the macrophage phenotype switch. Resolvin D1 promotes the adipose macrophage phenotype switch toward an M2-like phenotype in a mouse model of diet induced obesity [67], and in obese-diabetic mice in hepatic macrophages of Type I (non-inflammatory) and Type II (inflammatory), but even in the inflammatory group, the upregulated cytokines and chemokines and the responses to anti-inflammatory therapy by omega-3 differed between patients (see next paragraph). Another example of the heterogeneity is found in the study of β-secretase inhibitors, which failed to achieve overall significance but observed an effect in an MCI subgroup [70]. Thus anti-inflammatory and other therapeutic approaches in MCI and AD patients need to be personalized.

ROLE OF SPMS IN DEMENTIA AND ALZHEIMER'S DISEASE

SPMs are investigated in AD on the basis of their anti-apoptotic effects, down regulation of inflammation in neural cells, shift to non-amyloidogenic processing of AβPP, and activation of PPAR signaling. A protective role of the neuroprotectin D1 (NPD1) in the AD brain has been suspected due to its decreased level and a decreased expression of phospholipase A2 and 15-LOX type 1 in the hippocampal cornu ammonis region 1 [71]. Furthermore, in a mouse model of surgery-induced cognitive decline, aspirin-triggered resolvin D1 prevented surgery-induced neuronal dysfunction by modulating astrocyte activity and synaptic plasticity in the hippocampus, thus ameliorating cognitive loss [57]. Surgery and anesthesia have also been implicated in the progression of AD pathology, suggesting a possible role for inflammation and oxidative stress damage in modulating Aβ accumulation and tau phosphorylation [72, 73]. However, not all anesthetic agents have been shown to exacerbate AD pathology [74]); some may exert even neuroprotective actions, including jumpstarting endogenous resolution and providing anti-inflammatory actions [75]).

Omega-3 fatty acids are popular supplements taken by many people for prevention of health problems including dementia. Studies in healthy volunteers showed a significant increase in RvE1, 18R/S-HEPE, 17R/S-HDHA, and 14R/S-HDHA, but not other SPMs, after short-term omega-3 supplementation [76, 77]. In another study, plasma concentrations of all SPMs were increased after a single administration of omega-3
capsule and aspirin [60] We have evaluated immuno-supportive and pro- and anti-inflammatory effects of nutritional supplementation in MCI patients by the Smartfish drink (Smartfish, Oslo, Norway) containing omega-3, natural anti-oxidants, resveratrol and vitamin D3. After a 4- to 17-month supplementation, the phagocytosis of Aβ by monocytes was significantly increased and the pro-resolving mediator RvD1 increased in the macrophages of 80% of the MCI patients [5].

The brain damage in AD has been ascribed to inflammatory cytokines, in particular IL-6, TNF-α, IL-1β, and TGF-β [78]. However, the transcriptome of AD patients’ PBMCS is either “inflammatory” (with increased TLRs, IL-1, IL1R1, and chemokines) or “non-inflammatory” (with opposite findings); however, both groups have increased IL1RN, ITGB2, and NFκB [36]. Omega-3 nutritional supplementation up regulated transcription of cytokines in “non-inflammatory” patients (but not to the “inflammatory” level) and down regulated transcription in “inflammatory” patients [5, 36]. Omega-3 attenuated cognitive decline in MCI patients with MMSE >27 in a placebo-controlled study [43]. In our study, which was not placebo-controlled, the mean MMSE score was 25.9 at baseline and 25.7 after supplementation [79]. However, there are caveats in the effects of omega-3 supplementation. Previous studies [80] showed that omega-3 supplementation has less protective effect against dementia in APOE e4 carriers. In our study of 14 MCI patients, three had the APOE e4 genotype. In these patients, the improvement of Aβ1-42 phagocytosis on supplementation and the MMSE score were not sustained in two patients. The Aβ1-42 phagocytosis was generally adversely affected by intercurrent infections, surgeries, cancer, and lack of adherence to the supplementation (even for a short time). Some MCI patients did not produce RvD1 in macrophages despite immune and cognitive improvements, indicating a defect in the biosynthetic pathway for this potent pro-resolving mediator.

**BLOOD BIOMARKER TEST OF Aβ1-42 PHAGOCYTOSIS**

The benefits of omega-3 fatty acids are difficult to analyze in small samples by psychological tests due to the heterogeneity of their cognitive effects in individual patients. However, Aβ1-42 phagocytosis and inflammatory activation are the key pathologies in AD and MCI patients, which can be accurately measured by flow cytometry and PCR. The flow cytometric test of fluorescent Aβ1-42 phagocytosis by monocytes had the following results: (a) Aβ1-42 uptake of <450 mean fluorescence intensity (MFI) units in AD and most MCI patients; (b) Aβ1-42 uptake of >450 MFI units in cognitively-normal subjects; (c) Aβ1-42 uptake >1000 MFI Units in cognitively-highly active University professors; (d) Aβ1-42 uptake in the AD range in some stressed caregivers and apparently cognitively normal persons not mentally and physically active [4]. Our recent results show that the test score deteriorates over time in AD and MCI patients and, contrariwise, the test is improving or is stabilized in some MCI patients who are nutritionally supplemented with the omega-3 drink Smartfish [79].

**FUTURE DIRECTIONS**

Omega-3–derived specialized pro-resolving mediators have strong effects on acute inflammation in vitro and in vivo and may attenuate chronic inflammation in patients with neurodegenerative diseases. Yet, large studies of omega-3 supplementation have not produced a consensus about their beneficial cognitive effects in MCI and AD patients. These difficulties can be partially overcome by nutritional supplementation of individual patients while monitoring adherence to daily therapy, Aβ1-42 phagocytosis, inflammation, and lipid mediators. Nutritional supplementation is compromised by lack of daily adherence, intercurrent infections, surgeries, cancer, endocrine and metabolic factors, and lack of a healthy and active lifestyle.

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[21] M. Fiala et al. / Pro-Resolving Mediators Improve Amyloid-β Immunity 299


