Modulation of innate immunity of patients with Alzheimer’s disease by omega-3 fatty acids

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ABSTRACT: The innate immune system of patients with Alzheimer’s disease and mild cognitive impairment (MCI) is deregulated with highly increased or decreased transcription of inflammatory genes and consistently depressed phagocytosis of amyloid-β1-42 (Aβ) by monocytes and macrophages. Current immune therapies target single mechanisms in the adaptive immune system but not innate immunity. Here, we summarize recent advances in therapy by ω-3, ω-6, and epoxy fatty acids; specialized proresolving mediators; and vitamin D3 that have proven immune effects and emerging cognitive effects in patients with MCI. The hypothesis of this approach is that macrophages of normal participants, but not those of patients with Alzheimer’s disease and MCI, possess effective phagocytosis for Aβ and protect homeostasis of the brain and, furthermore, that defective MCI macrophages recover phagocytic function via ω-3. Recent studies of fish-derived ω-3 supplementation in patients with MCI have shown polarization of Apo3/ε3 patients’ macrophages to an intermediate M1-M2 phenotype that is optimal for Aβ phagocytosis and the stabilization of cognitive decline. Therefore, accumulating preclinical and preliminary clinical evidence indicates that ω-3 supplementation should be tested in a randomized controlled clinical trial and that the analysis should involve the apolipoprotein E genotype and intervening conditions during trial.—Fiala, M., Kooij, G., Wagner, K., Hammock, B., Pellegrini, M. Modulation of innate immunity of patients with Alzheimer’s disease by omega-3 fatty acids. FASEB J. 31, 000–000 (2017). www.fasebj.org

Alzheimer’s disease (AD) is a neurodegenerative disease that is preceded by prodromal stages of mild cognitive impairment (MCI) (1) and subjective cognitive impairment (2) that frequently are not recognized but are important for the start of preventive therapy. To date, there is no proven effective therapy to counteract the progression of MCI to AD, which has a 42.5% incidence at 5 yr (3); however, it is accepted that any therapy should address inflammatory damage and clearance of amyloid-β1-42 (Aβ) (4). Potential therapeutics include ω-3 fatty acid–derived proresolving mediators, which induce anti-inflammatory and proresolving responses and enhance macrophage phagocytosis (5), including the phagocytosis of Aβ (6). In addition, the ω-6 fatty acid arachidonic acid–derived mediator, epoxyeicosatrienoic acid (EET), displays potent anti-inflammatory and antinociceptive properties (7). Recent discoveries that have bearing on the transcriptional deregulation of innate immunity against Aβ (6) and alterations in resolution pathways in the CNS of patients with AD (8) suggest that ω-3 lipid mediators should be tested in a controlled trial.

MACROPHAGES AND MICROGLIA IN THE AD BRAIN: HARMFUL INFLAMMATION AND BENEFICIAL CLEARANCE OF Aβ

Roles in AD of microglia–vs. monocyte-derived macrophages have been discussed since the 90s. Microglia that originate from bone marrow were shown to clear Aβ (9) but also to have toxic effects on neurons (10). Inflammation in the AD brain was attributed to Aβ deposits that had been invaded by microglia and surrounded by reactive astrocytes (11). Monocytes were attracted into neuropil across the human blood-brain barrier (BBB) model by CCL2, -3, and -4 chemokines (12) and, in mouse models, by...
Subsequently, transplantation of bone marrow and bone marrow–derived microglia were tested as therapy for neurodegenerative diseases in animal models and in humans, including in patients with multiple sclerosis (14); however, further discoveries have complicated this approach, as the origin of adult microglia was shown to be prenatal from primitive myeloid progenitors in the yolk sac (15). Meanwhile, in studies relevant to AD, invasion of monocyte-derived macrophages was noticed in the human brain with ischemic plaques (16) and the AD brain (17). The investigation of brain macrophages by RNA sequencing in a mouse model has shown that CNS macrophages arise from yolk sac precursors during embryonic development and are not significantly related to circulating myeloid cells but are activated during inflammation (18, 19). However, the inflammatory model in the animal study (19) has uncertain relation to monocyte and/or macrophage infiltration of the AD brain. An extensive body of literature shows monocyte migration across the BBB in HIV-1 encephalitis (20) that histopathologically resembles the AD brain (17). In an in vitro model, monocytes are attracted by Aβ-induced chemokines to migrate across the BBB (12).

Nonetheless, in mouse models, the role of monocyte-derived macrophages has remained controversial because monocyte invasion into the CNS was found to be related to radiation- or chemotherapy-induced BBB damage (21, 22). With the exception of patrolling circulating monocytes that are charged with clearing vascular Aβ (23), the role of Aβ clearance has been ascribed to microglia (24). However, there has also been strong evidence for peripheral monocytes playing a role in Aβ clearance: 1) Aβ degradation by mouse microglia was inefficient (25); 2) interference with monocyte migration by CCR2 deficiency resulted in increased levels of cerebral Aβ deposition and cognitive impairment (13); 3) CCR2 deficiency of perivascular myeloid cells impaired Aβ clearance (26); 4) PD-1 immune checkpoint blockade and regulatory T cell suppression increased recruitment of monocyte-derived macrophages in a mouse brain and cleared Aβ (27, 28); and 5) in experimental autoimmune encephalitis, the effective antigen presenting cells are CD45highCD11b/c+ transitional macrophages, not CD45lowCD11b/c+ microglia (29). Microglia are believed to clear Ab-coated Aβ via the Ab Fc domain (30, 31), but the effectiveness of Fc immune clearance is contradicted by poor results of Aβ Ab clearance (32). Conversely, there has been evidence against Aβ clearance by monocytes and/or macrophages in the mouse model, as rapid repopulation of the brain with peripheral myeloid cells, even in the presence of anti-Aβ Ab, failed to clear Aβ plaques (33, 34).

The genetic mouse model that overexpresses the amyloid-precursor protein with pathogenic mutations does not express the pathognomonic immune defect of Aβ phagocytosis by macrophages of patients with AD (35). In addition, the mouse response to 1α,25-dihydroxy vitamin D3 (1,25D3) is different compared with the human response (36), and, in general, inflammatory responses differ between mice and humans (37). Finally, immune activation of the hNOS2 gene is crucial in an animal model to mimic the pathologic phenotype of AD (38). The examination of brain tissues and immune cells from human patients with AD demonstrates that the immunopathology and clearance of Aβ depend upon monocytes and macrophages (17). In the AD brain tissue assay, normal monocytes, but not AD monocytes, clear Aβ from neurons (35). Monocytes that had phagocytized Aβ appear fat and do not transmigrate across the BBB, whereas lean monocytes do. AD macrophages are defective in Aβ phagocytosis and degradation and are prone to apoptosis from Aβ (39); thus, in the healthy brain, monocytes may immigrate into the neuropil, clear Aβ by phagocytosis, and emigrate, whereas in the AD brain, AD monocytes may not. This hypothesis is supported by a study of the AD brain that shows apopotic macrophages that contain fibrillary Aβ on the abluminal side of microvessels that circumferentially surround the congoophilic vessel wall and also colocalize with neurons (39) and plaques (17) (Fig. 1). In some patients, increased expression of CD33 is associated with the rs3865444C risk allele for AD and decreased Aβ clearance (40, 41). These considerations suggest that peripheral blood monocytes are diagnostic and therapeutic tools.

Early epidemiologic studies have pointed to the role of inflammation because the prevalence of AD was reduced in patients who were treated with nonsteroidal anti-inflammatory drugs (42) and the brain showed the presence of membrane attack complex and T cells (43). The roles of the initiating factor of the classic complement cascade, C1q and C3 component, have been revisited in mouse models in which they were found to be associated with early synapse loss (44). In a recent study, macrophages of most patients with AD displayed inflammatory markers, including CD14, TLR 2, TLR4, IL6, and CCR2, as well as MHC-II/Aβ complexes (45). Exposure of microglia to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) decreased inflammatory M1 markers and increased Aβ phagocytosis and anti-inflammatory M2 markers (46). Translation of these results to patients with MCI is not straightforward because the inflammatory activation of peripheral blood mononuclear cells is heterogeneous: down-regulated in a subgroup of noninflammatory patients and up-regulated in a subgroup of inflammatory patients (47, 48). The macrophage phenotype of patients with MCI is also heterogeneous and either highly proinflammatory M1 or very low inflammatory M2 (49). Thus, ω-3 therapy should lead to an intermediate proresolution M1-M2 phenotype, as shown in a pilot study (49).

**IMMUNE THERAPIES BY RECEPTOR ANTAGONISTS, ω-3 AND ω-3–DERIVED SPECIALIZED PRORESOLVING MEDIATORS, AND EPOXY FATTY ACIDS**

Immune approaches in animal models, such as chemokine receptor CCR2 antagonists, purinergic receptor P2Y6 antagonists, or a CX3CR1 agonist (31), target single genes in CNS myeloid cells and do not consider the heterogeneity of inflammation or immune exhaustion with deregulation of many genes and pathways in amyotrophic lateral sclerosis (50, 51) and AD (49). Remarkably, lipid mediators...
from ω-3 regulate the activation of a multitude of genes and pathways that are related to inflammation and resolution in vitro and in vivo (52) and provide a different approach to AD therapy by multitargeting. Inflammation is normally terminated by an active process called resolution, which limits the entry of immune cells, increases the clearance of apoptotic cells by macrophage phagocytosis, and enables tissue restoration (53). Incomplete resolution leads to unabated inflammation, which is an underlying cause of many chronic diseases (54–56).

During the resolution of acute inflammation, host-protective mediators are biosynthesized from polyunsaturated fatty acids (PUFA) via up-regulation of the key biosynthetic enzyme 15-lipoxygenase type 1 in leukocytes (57). This promotes the conversion of ω-6 arachidonic acid to lipoxin A₄ (LXA₄) and of ω-3 DHA to d-series resolvins and protectins/neuroprotectins (57). DHA is also converted in macrophages via 14-lipoxygenation to maresins (macrophage mediators in resolving inflammation). The first identified member of this family was maresin 1 (MaR1; 7R,14S-dihydroxy-docosa-4Z,8E,10E,12Z,16Z,19Z-hexaenoic acid). Specific members of these families of mediators from the DHA metabolome are named D-series resolvins [resolvin D1 (RvD1) to -6], protectins (including protectin D1-neuroprotectin D1), and maresins (MaR1 and -2) and E-series resolvins (RvE1 and -2) (58). These mediators limit neutrophil recruitment and potently stimulate macrophage phagocytosis of apoptotic cells in a stereospecific manner. They also regulate both inflammation and chemotherapy-induced pain by inhibiting transient receptor potential vanilloid 1 currents in neurons (59).
novel family of macrophage-derived proresolving mediators, called Maresins Conjugates in Tissue Regeneration, carry potent organ-protective, tissue-regenerative, and pro-
resolving actions (60). In addition, protectin and resolvin sulfido-conjugates were recently identified as novel resolution mediators that control regeneration (60, 61).

Specialized proresolving mediators (SPMs) bind to their receptors on leukocytes and regulate anti-inflammatory and pro-
resolving actions. LXA4 inhibits leukocyte trafficking in vivo by activating the LXA4 receptor denoted ALX/FPR2. RvD1 binds to the ALX/FPR2 receptor and another GPCR called GPR32, but RvD1 does not activate nuclear receptors retinoid X receptor (RXR)-α and peroxisome proliferator-activated receptor (PPAR)-α, δ, and γ (62). RvD1 inhibits neutrophil migration and promotes their phagocytosis by macrophages (62). Aspirin-triggered RvD1 (AT-RvD1) inhibits the release of IL-6 and protects the brain after surgical injury from neuroinflammation, synaptic dysfunction, and cognitive decline (63). RvD2 binds to GPCR GPR18 and mediates bacterial phagocytosis and organ protection (64). RvE1 binds to the receptor on macrophages called ChemR23 (65), also named human chemokine-like receptor 1. 135,145-Epoxide-maresin intermediate stimulates M1 to
M2 phenotype switch (66). PD1 inhibits inflammatory cy-
tokines, COX-2, and NF-κB in the CNS (67).

These SPMs are active in animal models of different infectious, metabolic, ischemic, arthritic, and oncogenic pathologies, such as RvD2 in microbial sepsis (68), RvE1 in periodontal disease (69), PDI in ocular herpes simplex (70), LXA4 in liver and renal fibrosis related to obesity (71), LXA4 in cerebral ischemia (72), LXA4 in brain amyloidosis (73), RvD1 in arthritis (74), and resolvins in cancer (75). Emerging studies in human participants show positive therapeutic effects of ω-3 supplementation on clot re-
sorption by macrophages of patients with coronary artery disease (76) and on phagocytosis of Aβ mentioned above (48).

ω-3 PUFAs are also a source for the formation of epoxy fatty acids (EpFAs), epoxydocosapentaenoic acids from DHA, and EETs from EPA (77) (Table 1). EpFAs are antinociceptive in several models of pain, and, like resolvins, they dampen prostaglandin- and cytokine-driven inflammation (7, 78, 79). Of interest, ω-6 PUFA arachidonic acid is also a precursor to anti-inflammatory lipid mediators that are formed in different branches of the arachidonic acid cascade. The biologic activities of LXA4 formed by lipoxygenase and those of EETs generated by cytochrome

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SPMS IN THE AD BRAIN
In the brain, DHA is a precursor to the neuroprotective and anti-inflammatory mediator, PD1 (93), which is decreased in the AD brain (94) and reduces Aβ-induced neurotoxicity (95). Moreover, it has recently been shown that LXA4 was decreased in the AD brain and CSF and that LXA4 and RvD1 levels in the CSF positively correlate with minimal state examination (MMSE) scores (8), thereby suggesting a connection with cognitive function in AD. In line with these findings, Zhu and colleagues (55) recently reported lower levels of MaR1, PD1, and RvD5 in the entorhinal cortex of patients with AD, thereby providing more evidence of a disturbed resolution pathway in AD. Addition of these SPMs exerted neuroprotective activity and boosted Aβ uptake by microglia, which illustrated the feasibility of use of SPMs in a clinical setting. Indeed, supplementation with AT-RvD1 prevented memory loss in an animal model of surgery-induced cognitive decline (63), and AT-LXA4 improved cognition in a transgenic mouse model of AD (96).
MACROPHAGE PHENOTYPES IN PATIENTS WITH AD AND MCI AND POLARIZATION BY \( \omega-3 \) AND SPMS

Blood-borne monocyte-derived macrophages have critical importance in various neurodegenerative diseases, as these cells cross the BBB and produce specific immune pathologies in HIV-1 encephalitis (20), AD (17), and amyotrophic lateral sclerosis (97). Macrophages are bifunctional in A\( \beta \) phagocytosis: in healthy individuals, they are effective in phagocytosis and the degradation of A\( \beta \), whereas in patients with MCI and AD, macrophages are defective in both functions (98).

Regulation of the macrophage phenotype is at the core of therapy for a variety of disorders, including atherosclerosis, allergy, infection, tissue repair, obesity, emphysema, cancer, and neurodegenerative diseases (99). The extremes of a macrophage phenotype continuum in model systems are described as M1 proinflammatory and M2 proresolving and wound-healing macrophages, but in vivo intermediate and unique macrophage phenotypes are frequently found (100, 101). M1 macrophages produce cytokotoxic and inflammatory cytokines (TNF-\( \alpha \) and IL-12), chemokines (CXCL9, -10, -11), and NO2. M2 macrophages up-regulate the mannose receptor (Mrc1) and chitinase 3-like3 (Chi3L3). Macrophage polarization is activated in the following ways: 1) to M1 phenotype by IFN-\( \alpha \)/\( \beta \) or IFN-\( \gamma \) signaling via IFN regulatory factor-5 (IRF5) and signal transducer and activator of transcription 1 (STAT1), or alternatively by LPS via TLR4 and NF-\( \kappa \)B signaling; 2) to M2 phenotype by IL-4 and IL-13 signaling via STAT6, PPAR-\( \gamma \), and PPAR-\( \delta \) (102); IL-4 induces Jmjd3-IRF4 axis to inhibit IRF5-mediated M1 polarization (103); 3) by epigenetic changes, including those via a histone demethylase (104); and 4) by miR-155 (105). In addition, T cells influence macrophage polarization: Th1 stimulate M1 type and regulatory T cells stimulate M2 type phenotypes (106).

Defects in AD macrophage phagocytosis seem to be related to defective protein glycosylation as a result of down-regulation of the enzyme, \( \beta \)-1,4-mannosyl-glycoprotein4-\( \beta \)-N-acetylgalactosaminyltransferase (GlcNAc). This enzyme is up-regulated during A\( \beta \) phagocytosis in normal, but not AD, monocytes (107). Low expression of GlcNAc is associated with poor prognosis (108). As the inhibition of N-glycosylation decreases anti-inflammatory and proresolution M2 macrophage polarization (109), reduced transcription of GlcNAc may be an important defect in patients with AD. \( \beta \)-Site amyloid precursor protein cleaving enzyme-1 is modified by bisecting GlcNAc in the brain of patients with AD, which possibly leads to greater \( \beta \)-site amyloid precursor protein cleaving enzyme-1 degradation and, thus, increased A\( \beta \) production, as observed in a mouse model (110).

Macrophage polarization toward the proresolving M2 type by SPM has a favorable effect in many disorders. \( \omega-3 \) protect against obesity-associated metabolic disorders and cardiovascular disease via modulation of eicosanoid production and stimulation of anti-inflammatory mediators. RvD1 inhibits macrophage accumulation in adipose tissues and increases the proportion of alternatively activated macrophages (111, 112). Resolvins, maresins, and 13S,14S-epoxy-maresin shift macrophage phenotype to a less inflammatory type (62, 113). SPMs polarize inflammatory M1 macrophage type in a mouse-air-pouch model to a proresolution M2 type (114). M2 macrophages have elevated levels of proresolving lipid mediators, including maresin, and decreased prostaglandins and leukotrienes in relation to M1 macrophages (115). In addition, M2 macrophages specifically produce the recently identified protectin conjugate in tissue regeneration 1, which seems to be a potent novel inducer of macrophage phagocytosis (116). SPMs also modulate the metabolism of macrophages (117).

RvD1 interacts with GPCR GPR32 (62), whereas fatty acids employ PPAR-\( \alpha \) (118–120). \( \omega-3 \) DHA induces M2 macrophage polarization and efferocytosis via PPAR-\( \gamma \) (121). In mouse models of AD and in vitro microglia and astrocytes, DHA has anti-inflammatory and proresolving effects via RXR and PPAR-\( \gamma \) (122).

STIMULATION OF INNATE IMMUNITY BY NATURAL SUBSTANCES

In 2016, the monumental action against AD includes the development of nontoxic therapies with Abs and natural substances. This approach has gained traction after clinical failures and amyloid-related imaging abnormalities of A\( \beta \) Abs (123), which also increased cortical hyperactivity in a mouse model (124). The next generations of the vaccine and Abs in development are designed to clear A\( \beta \) by actively or passively induced A\( \beta \) Abs, not by improving the function of macrophages and/or microglia, and may have various adverse effects on chronic administration (125). Recent A\( \beta \) Ab approaches include gantenerumab (126) and aducanumab (127). Aducanumab reduced brain A\( \beta \) and putatively slowed clinical decline but had A\( \beta \)-related imaging abnormalities.

Natural substances have been used for centuries to boost the immune system with manifest results, such as that observed with curcuminoids (128) and vitamin D\( \gamma \) (129). Despite extensive anecdotal experience, clinical investigation of natural substances in MCI and AD is difficult because of the chronic nature of these diseases that requires large prospective studies. A large compendium of nutritional supplements for dementia (130) includes a cornucopia of micronutrients, minerals, vitamins (1,25D\( \gamma \) and vitamin B complex), \( \omega-3 \) fatty acids DHA and EPA, flavonoids (e.g., resveratrol), polyphenols (e.g., curcuminoids), and alkaloids (caffeine, ashwagandha), as well as nutraceuticals, such as herbs, diets, and probiotics. Currently, the following substances have a potential for prevention of MCI progression via increased A\( \beta \) clearance on the basis of clinical or experimental experience (131): \( \omega-3 \) fatty acids (48), vitamin D\( \gamma \) (132), vitamin B complex (133), and curcuminoids (35, 134–136).

MECHANISMS OF SPMS, 1,25D\( \gamma \), AND OTHER MEDIATORS THAT ENHANCE A\( \beta \) PHAGOCYTOSIS

\( \omega-3 \) and 1,25D\( \gamma \) are the best-understood natural immune therapeutics for human use in patients with
MCI, but their effects in patients are heterogeneous. The effects of \( \omega-3 \) and \( 1,25D_3 \) are different from each other and do not completely normalize the AD transcriptome (6). Both mediators up-regulated IL-1 in noninflammatory patients and down-regulated IL-1 and IL-6 in inflammatory patients. In general, \( 1,25D_3 \) had more pronounced effects on normalization of chemokine and cytokine transcription than did RvD1. \( 1,25D_3 \) down-regulated IL-1R1, CCL23, -2, and CD40 in both groups of patients. RvD1 profoundly down-regulated NF-kB, IL-1\( \beta \), CCL2, -4, and -24 in inflammatory patients, but up-regulated IL-1\( \alpha \) and IL-1\( \beta \) in noninflammatory patients. \( 1,25D_3 \) forms heterodimers with RXR and stimulates A\( \beta \) phagocytosis via non-genomic signaling by increasing intracellular calcium and potentiating MEK1/2 signaling, which leads to the opening of the CIC3 channel, as well as via nuclear signaling (47).

Other chemicals that enhance phagocytosis include bexarotene and resveratrol. Bexarotene is an RXR agonist that activates the PPAR-\( \gamma \) and RXR heterodimer. Bexarotene potentiates phagocytosis of A\( \beta \) in an animal model (137, 138), but this has been only incompletely reproduced in other laboratories (139). Resveratrol altered certain CSF biomarkers (A\( \beta \), -40) without consistent cognitive changes in a controlled clinical trial (140). Resveratrol has a broad spectrum of activities against A\( \beta \) and \( \tau \) neuropathologies, including attenuation of inflammatory mechanisms and increasing intracellular A\( \beta \) clearance, in part by activating autophagy and proteolysis via modulation of the AMPK/sirtuin1/PPAR-\( \gamma \) coactivator1-\( \alpha \) signaling network (141). PPAR-\( \gamma \) coactivator1-\( \alpha \) is a transcriptional coactivator that regulates the genes that are involved in energy metabolism and mitochondrial biogenesis and function and induces polarization of macrophages toward M2 via STAT6/PPAR-\( \gamma \) (142).

**DESIGN OF AN IDEAL CLINICAL TRIAL OF \( \omega-3 \) SUPPLEMENTATION**

It is clear that MCI and AD involve the deregulation of many pathways and thus requires combination therapy, as suggested by the glimmers of success with this approach (150). Effective therapy should be based on \( \omega-3 \) of the highest quality, protected against oxidation [\( \omega-3 \) are oxidized in 50% of commercial supplements (151)]. It is not clear whether there are advantages of fish-derived vs. algal \( \omega-3 \) and whether DHA, EPA, or both are needed. Whey protein is necessary for emulsification and nutrient delivery (152). Additional natural supplements with central and peripheral immune effects, such as resveratrol (153), vitamin D_3 (154), vitamin B complex (folic acid, vitamins B_6 and B_12) (155), and curcumin (35), should be evaluated for inclusion in the supplement. The design should be randomized, placebo-controlled, and double-blind, with a sample size of adequate power. Participants should be observed in 2-mo intervals and comorbidities, such as fractures, infections, and cancer, which may interfere with the immune response, must be analyzed. As it is impossible to know the genotype in advance of trial enrollment, results must be analyzed according to the APOEe3/e4 vs. e3/e4 genotype. Use of over-the-counter supplements should be discontinued. According to the results of our recent trial of \( \omega-3 \) (49), we estimate that in APOEe3/e4 patients with MCI, the effect of Smartfish supplementation on MMSE could be 60–70% greater than that of a placebo. This is more likely to be achieved if patients follow

**OBSERVATIONAL STUDIES AND CLINICAL TRIALS OF \( \omega-3 \): COGNITIVE, IMMUNE, AND MOLECULAR EFFECTS**

Retrospective epidemiologic studies in various populations have shown positive cognitive results in relation to optimal DHA plasma levels or a high consumption of fish in 14 of 17 studies (143). In a controlled study of \( \omega-3 \) supplementation, patients with MCI with MMSE score \( >27 \) reduced cognitive decline compared with placebo (144). \( \omega-3 \) supplementation in a double-blind study improved memory functions in healthy older adults (145). Recently, 3 trials (144–147) were analyzed according to the Cochrane Handbook for Systematic Reviews of Interventions and found no evidence for the efficacy of \( \omega-3 \) supplements in the treatment of mild to moderate AD (148). Seafood consumption (\( \geq 1 \) meal/wk) was significantly correlated with less AD pathology, but this effect was observed only among apolipoprotein E (APOE) e4 carriers (149). The choice of fish-derived DHA vs. algal DHA has not been considered an issue, but the 2 largest clinical trials of DHA supplementation had different results: fish-derived DHA showed the slowing of decline in patients with early MCI, whereas algal DHA was not different from placebo.

A recent observational study of supplementation by the \( \omega-3 \) drink, Smartfish (Smartfish, Oslo, Norway), analyzed the immune and molecular effects of \( \omega-3 \) supplementation in patients with MCI. This drink is a well-characterized, stabilized emulsion of \( \omega-3 \) lipids, antioxidants, vitamin D_3, whey protein, and resveratrol. This small study showed significant enhancement of A\( \beta \) phagocytosis after only 1 mo in parallel with cognitive stabilization (48). Immune improvement was marred in some patients by lack of daily compliance, infections, diabetes mellitus, cancer, and surgeries. RvD1 increased in macrophages in 80% of patients with MCI. \( \omega-3 \) regulated the inflammatory activation of peripheral blood mononuclear cells—either too high or too low at baseline—into a middle green zone (48). This study has been recently extended to 14.7 mo (average follow-up) (49). The results have shown that macrophage M1 or -2 type at baseline was modulated in \( \omega-3 \)-supplemented APOEe3/e4 patients to an intermediate type M1-M2 with improved A\( \beta \) phagocytosis, whereas macrophages of APOEe3/e4 patients were modulated in an irregular fashion. Of importance, MMSE score significantly increased in APOEe3/e4 patients (by 2.2 points/yr) vs. no increase in APOEe3/e4 patients (0 points/yr) (49).
recommendations for physical and mental exercise, adequate sleep, and eating a diet low in saturated fat and high in fish (156). Assuming 60% effect, we calculated the sample size in a study with a 2-yr follow-up (with 80% power, \( \alpha = 0.05 \)) as 30 patients on \( \omega-3 \) preparation and 30 controls on a placebo in an ideal situation with identical proportion of APOE e3/e3 vs. e3/e4 genotype subjects; however, the APOE genotype distribution cannot be estimated in advance in small samples. Therefore, the number of the participants in the trial should be at least 2 or 3 times higher. Trials of mAbs against Aβ required 2000 patients (157, 158) and still failed; however, even a small-size (100–200 participants) trial of \( \omega-3 \) preparation will definitively produce a wealth of data on immune responses that may explain why certain patients respond cognitively better than others.

CONCLUSIONS

A retrospective (143) and a prospective study (144) demonstrated cognitive benefits with \( \omega-3 \) supplementation. Our observational study with the \( \omega-3 \) drink suggests that the beneficial effects are associated with macrophage polarization to M1-M2 type, particularly in APOE e3/e3 patients (49). Conversely, 2 large prospective studies seem to disclaim the benefits of \( \omega-3 \) because oral supplementation with algal DHA (but without antioxidants and other components) had no effect on the cognitive function in patients who were at risk of macular degeneration (159) and in those with AD (146). The benefits of \( \omega-3 \)-need to be confirmed in a randomized controlled trial that will analyze not only the overall cognitive results but also baseline cognitive and health status, APOE type, compliance (i.e., a reliable caregiver), intercurrent inflammatory and immunosuppressive conditions, and correlation of cognitive and immune results during the trial.

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AUTHOR CONTRIBUTIONS

M. Fiala and M. Pellegrini reviewed \( \omega-3 \) studies in macrophages and human patients; G. Kooij reviewed specialized pro-resolving mediators; and K. Wagner and B. Hummock reviewed epoxy fatty acids.

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