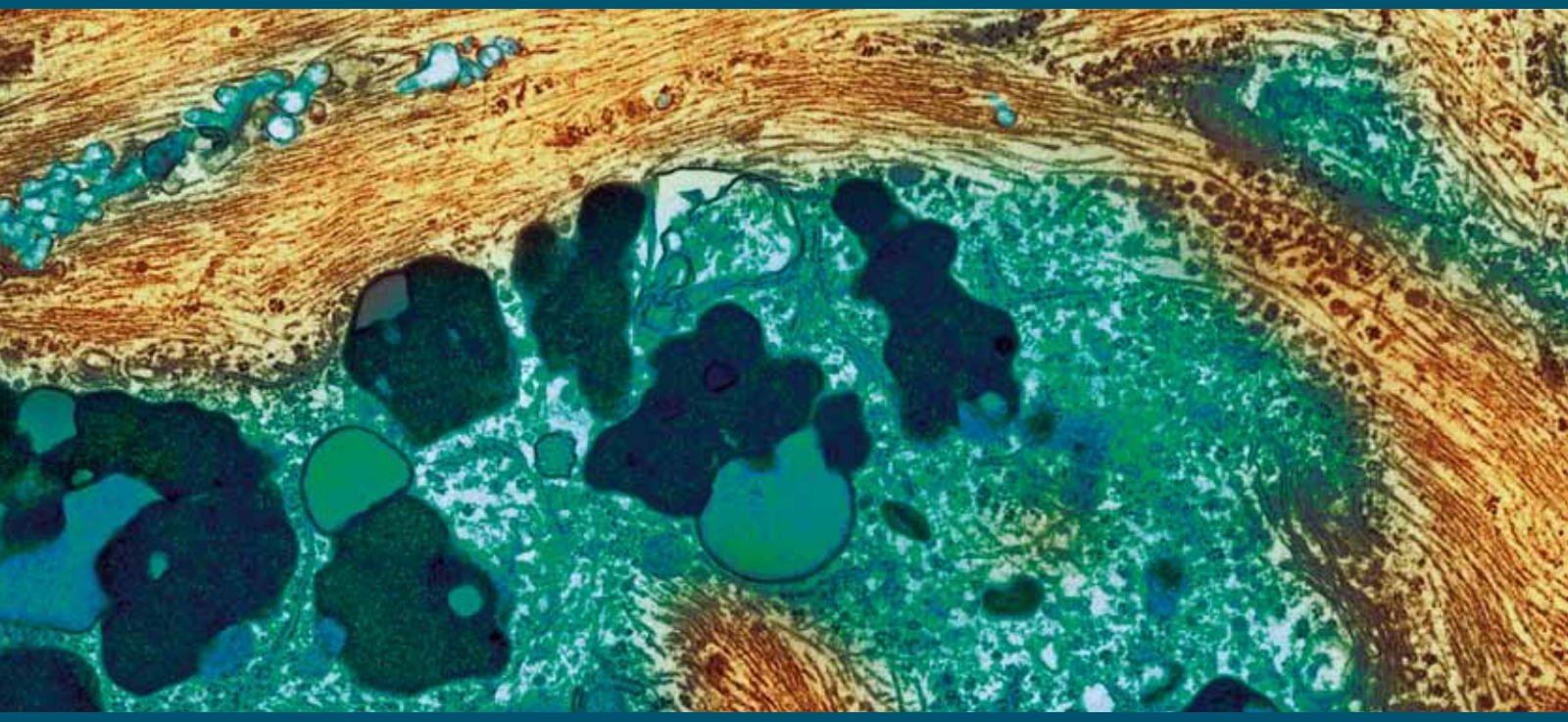
International Journal of Alzheimer's Disease

Neuroinflammation, AD, and Dementia

Guest Editors: Marcella Reale, Talma Brenner, Nigel H. Greig, Nibaldo Inestrosa, and Diana Paleacu



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Editorial Board

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Editorial

Neuroinflammation, AD, and Dementia

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This special issue of the *International Journal of Alzheimer's disease* contains a series of cuttingedge articles that deal with the broad issue of inflammatory and immunological disease mechanisms of Alzheimer disease (AD). Of necessity, these articles focus on selected topics but the mixture of contributions on signaling pathways, mediators, and articles addressing cell lines provides an overview and an insight into current areas of debate in inflammation and neurodegeneration. In particular, they highlight current interest in the interface between innate and adaptive immunity and present intriguing prospects for future therapeutic developments in neuroinflammation areas.

Immunohistochemical and molecular biological evidence accumulated over the past two decades has shown that the brain is capable of sustaining an innate immune response and that the result may be damaging to host cells. Neuroinflammation is a characteristic feature of both acute and chronic CNS disorders and is a process that results primarily from the presence of chronically activated glial cells (astrocytes and microglia) in the brain, and is a common feature of several neurodegenerative conditions. The evidence for a chronic inflammatory reaction in the brain is particularly strong in AD where it has been extensively studied. Mounting evidence indicates that microglial activation contributes to neuronal damage in neurodegenerative diseases, but beneficial aspects have also been identified. The concept that neuroinflammation is detrimental implies that glial cell activation precedes and causes neuronal degeneration, a sequence of events that appears to be at odds with experimental models of neurodegeneration in which glial cell activation occurs secondary to neuronal damage. There is abundant evidence that numerous substances involved in the promotion of inflammatory processes are present in the CNS of patients with such neurodegenerative diseases. Inflammation in the brain is silent because the brain does not possess pain fibers. It depends upon synthesis of inflammatory components by local neurons and glia, and especially resident phagocytes which, in the brain, are the microglia. Microglia, inflammatory cytokines, and the complement system appear to play significant roles.

Dementia is a decline of cognitive functions (reasoning, memory, and other mental abilities). This decline eventually impairs the ability to carry out mundane activities such as driving; household chores, and personal care such as bathing, dressing, and feeding (often denoted as *activities of daily living*), or ADL.

Proinflammatory mediators released by activated glial cells during brain inflammation have been proposed to contribute to neuropathology underlying cognitive deficits. Three major pathologies characterize the disease: senile plaques, neurofibrillary tangles, and inflammation. What distinguishes AD from other neurodegenerative diseases is the conspicuous presence of extracellular amyloid deposits in senile plaques, dystrophic neurite growth, and excessive tau phosphorylation. Senile plaques in AD brain are present in different stages of maturity, ranging from diffuse to neuritic to dense core, but they all contain amyloid beta protein (Aβ). Aβ is a peptide that forms insoluble and

pathological extracellular aggregates that attract microglial cells, as suggested by microglia clustering at Aβ deposition sites.

Duleu et al., using an improved ELISA procedure, observed in AD patient sera, circulating IgA isotype antibodies directed against tryptophan metabolites. The paper confirms that neurotoxic tryptophan metabolites are implicated in neurodegenerative diseases. The activation of the IDO pathway leads to an over expression of tryptophan metabolites, which are implicated in neuroinflammation in AD. The identification of circulating antibodies directed against IDO/THO pathway metabolites elucidates the etiology of AD.

In the paper entitled "*Microglial immunoreceptor tyrosine-based activation and inhibition motif signaling in neuroinflammation,*" Linnartz et al. reviewed the involvement of ITAM- and ITIMsignaling receptors in the central nervous system (CNS) innate immune responses and neuroinflammation as modulator of microglial phagocytosis and cytokine expression. In the mammalian CNS, essential molecules in this process are Fc receptors and DAP12-associated receptors which trigger the microglial immunoreceptor tyrosine-based activation motif (ITAM)-Syk-signaling cascade. The authors indicated how ITAM- and ITIM-signaling receptors modulate microglial phagocytosis and cytokine expression during neuroinflammatory processes. They also described how their dysfunction could lead to impaired phagocytic clearance and neurodegeneration triggered by chronic inflammation.

Hjorth et al. using human CHME3 microglia showed intracellular A$\beta_{1–42}$ colocalized with lysosome-associated membrane protein-2. This indicated that phagocytosis was increased by interferon-γ, and to a lesser degree, by Protollin, a proteasome-based adjuvant. Phagocytosis of A$\beta_{1–42}$ is associated with the expression of inflammatory markers. A$\beta_{1–42}$ and interferon-γ decreased BDNF secretion suggesting a new neuropathological role for A$\beta_{1–42}$ and inflammation accompanying AD.

In another review, Dyall described neuroprotective effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in AD. Inflammation and oxidative stress appear to be key features contributing to AD, and omega-3 polyunsaturated fats, EPA, and DHA have wellcharacterised effects on inflammation and may have neuroprotective effects in a number of neurodegenerative conditions. The role of EPA and DHA in modulating oxidative stress and inflammation and their potential as therapeutic agents were emphasized.

In the paper entitled "*Interleukin-10 promoter polymorphism in mild cognitive impairment and in its clinical evolution*," Arosio et al. strengthen the theory that the overall risk of developing AD may be governed by a multifactorial "susceptibility profile" and that polymorphisms of cytokine genes can affect neurodegeneration and its clinical progression. In their study, the authors have analysed genotype and allele frequencies of A allele of −1082 polymorphism (G/A) of interleukin-10 (IL-10) in 138 subjects with mild cognitive impairment (MCI) diagnosed respectively as amnestic (a-MCI) and multiple impaired cognitive domains (mcd-MCI). The homozygosis for the A allele of this polymorphism of IL-10 promotes an higher risk of AD and reduced IL-10 generation in peripheral cells after amyloid stimulation. Thus, the authors proposed that IL-10 may partly explain the conversion of a-MCI to AD or, at least, be a genetic marker of susceptibility.

The final paper of this special issue by Krause et al. reviews the detrimental and beneficial role of neuroinflammation in AD. The authors focus on controversies in the field of microglia activation, attempting to shed light on whether neuroinflammation is associated with brain tissue damage and functional impairment or whether damage-limiting activity occurs. The possible limitations, options and advantages of anti-inflammatory treatment are discussed and possible implications resulting from neuroinflammation for AD therapy.

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Research Article

Circulating Antibodies to IDO/THO Pathway Metabolites in Alzheimer's Disease

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In Alzheimer's disease, indoleamine 2,3-dioxygenase and tryptophan hydroxylase are known to induce an overproduction of neurotoxic compounds, such as quinolinic acid and 3-hydroxykynurenine from the former, and 5-hydroxytryptophol and 5-methoxytryptophol from the latter. Other compounds, such as kynurenic acid, serotonin, and melatonin are produced via the same pathways. An improved ELISA method identified circulating antibodies directed against these compounds, linked to proteins, as previously described for other chronic diseases. This describes how only the A isotype of circulating immunoglobulins recognized a pattern of conjugated tryptophan metabolites in the sera of Alzheimer patients. These data indirectly confirmed the involvement of tryptophan derivatives in the pathogenic processes of Alzheimer's disease. Further studies are required to evaluate the relevance of these antibody patterns in monitoring this disease.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, resulting in a gradual, irreversible loss of memory and cognitive functions [1], mainly affecting cholinergic neurons. The severity of AD depends on the dysfunction of two molecules: Amyloid protein precursor (APP) and Tau protein. The aggregation of these proteins results in senile plaque formation and neurofibrillar degeneration. Pathological mutations have been discovered on the APP gene, in the region coding for the Beta amyloid peptide (Aβ), as well as on the presenilin PS1 and PS2 genes. PS1 and PS2 proteins regulate APP catabolism. Despite this new knowledge, the etiology of AD remains largely unknown. Common pathogenic disorders reported in AD include: autoimmunity [2], excitotoxicity, and oxidative and radical processes [3], all inducing neuron death and the activation of microglia cells and astrocytes [4].

One mechanistic hypothesis focuses on the tryptophan molecule, an amino acid essential for cell growth and metabolism. In the central nervous system, tryptophan is metabolized via two pivotal biochemical pathways [5], shown in Figures 1(a) and 1(b).

In the first pathway, tryptophan is metabolized by indoleamine-2, 3-dioxygenase-1 (IDO-1), an enzyme found in many tissues. IDO-1 catalyses tryptophan to *N*-formylkynurenine, an intermediate for several biochemical compounds. Moreover, IDO-1 is an inducible enzyme, activated in AD by proinflammatory cytokines, such as interferon-gamma (IFN-γ) [6], interleukin-12 (IL-12), interleukin-18 (IL-18) [7], and the Aβ 1-42 fragment [8]. Tryptophan catabolism abnormalities have been observed in AD. The tryptophan catabolism [9] and seric kynurenine/tryptophan ratio [10] increase in AD patients. Neuroinflammation in the central nervous system (CNS) may be a major factor in this disease, due to cytotoxic tryptophan metabolite production by CNS infiltrating macrophages and glial cells [11]. Dementia in AD patients is correlated with the overproduction of quinolinic acid (Quina) [12, 13], a metabolite of tryptophan accumulated in neurons and astrocytes via proinflammatory processes [14].

In the second pathway, tryptophan hydroxylase (THO), a rate-limiting enzyme, generates serotonin (5-HT) and melatonin (Mel), among other compounds. A loss of serotoninergic neurons has also been observed [15]. Mel

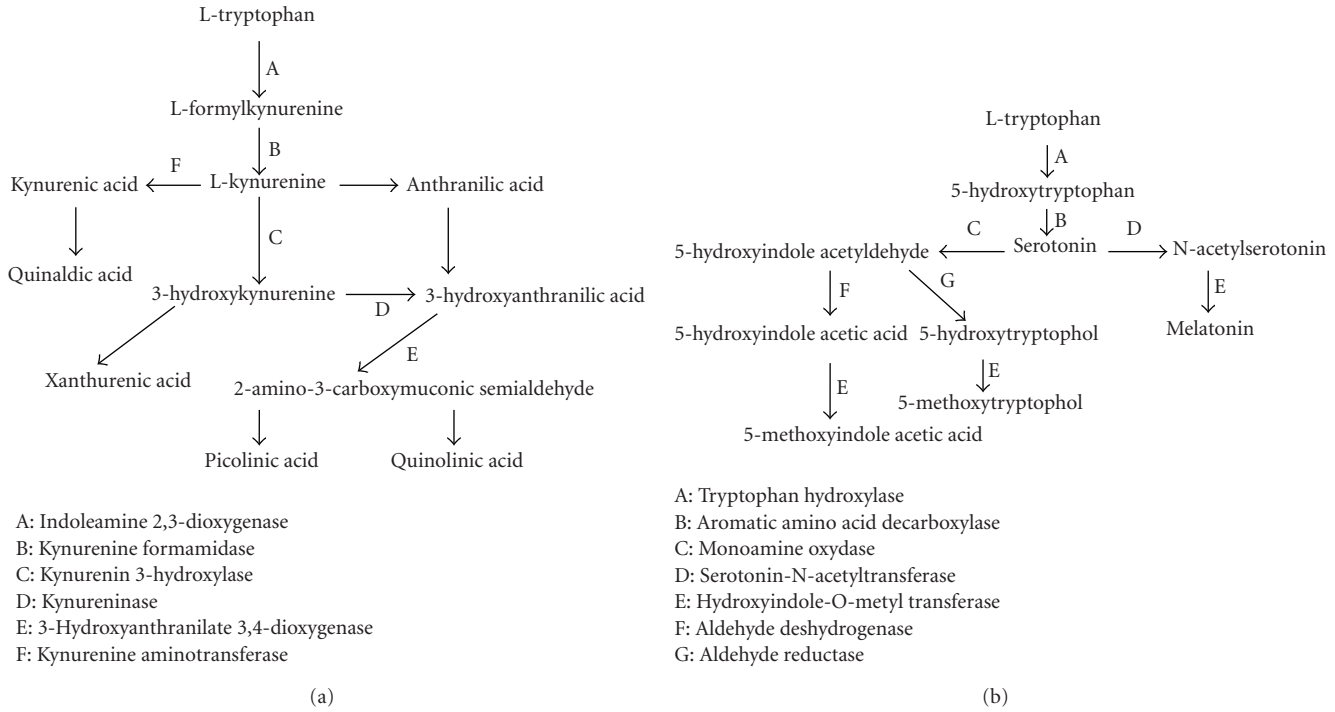

Figure 1: (a) The IDO-1 pathway. (b) the THO pathway.

is an important antioxidant, anti-inflammatory mediator [16] that interacts directly with Aβ deposition and hyperphosphorylated Tau protein. It plays a role in cholinergic neuroprotection [17]. THO activity decreases in the aging brain [18]. However, the THO pathway indirectly produces neurotoxic metabolites, such as 5-methoxytryptophol (5-MTol), 5-hydroxytryptophol (5-HTol), and the oxidative compound 5-hydroxyindole acetic acid (5-HIAA) via an enzyme cascade. All of these molecules play numerous roles in AD [19].

The aim of this study was to assay circulating antibodies directed against tryptophan derivatives conjugated to proteins in order to mimic the pathogenic mechanisms in vivo. The antibody titers in AD patient sera were thus compared to controls. The identification of specific antibodies in AD may enhances our understanding of some of the immunological processes involved.

2. Materials and Methods

2.1. Patient Sera. The study was conducted in accordance with Good Clinical Practice guidelines, with the informed consent of the patients, their caregivers, and the controls, in application of French and European law and current medical procedures. In this study, healthy control populations were matched by age and sex with the AD patients. Serum samples from 48 patients (age range: 65–85) were used. There was no subclassification among the AD states associated with dementia. Twenty serum samples were obtained from healthy controls (age range: 64–82).

AD was diagnosed according to the criteria outlined by the National Institute of Neurological and Communicative Disorders and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), in the absence of any clinical or laboratory evidence of a cause other than AD for dementia [20]. The patients had mild to moderately severe disease as defined by the Mini-Mental State Examination (MMSE) [21], with scores of 10 to 26, and screening and baseline Clinical Dementia Rating (CDR), with scores of 1 or 2 [22]. None of the patients had AD aggravated by an additional diagnosis of delusion, delirium, or depression, and none had a known or suspected history of alcoholism or drug abuse.

2.2. Conjugate Synthesis. Each tryptophan derivative was dissolved in 200 μL dimethylsulfoxide (DMSO) (Acros). Bovine serum albumin (BSA) (ID Bio) was also dissolved in 3 mL 2-morpholino-ethanesulfonic acid monohydrate (MES) buffer 10^{-1} M (pH 6.3) (Acros). Then, the tryptophan derivatives were mixed with the BSA solution and supplemented with 15 mg N-hydroxysuccinimide (Sigma) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (Acros) as coupling agents [23]. The conjugates were synthesized by linking 10 mg kynurenine (Kyn) (Sigma), or 3-hydroxykynurenine (3-OHKyn) (Sigma), kynurenic acid (Kyna) (Acros), Quina (Acros), quinaldic acid (Quinald) (Acros), 3-hydroxyanthranilic acid (3-OHAnthra) (Aldrich), anthranilic acid (Anthra) (Acros), xanthurenic acid (Xantha) (Acros), picolinic acid (Pico) (Acros), or 5-hydroxyindole acetic acid (5-HIAA) (Sigma), to 20 mg BSA. The coupling reaction took place in darkness, at 37°C, for 1 hour. The

reaction was stopped by adding 100 mg hydroxylamine (Sigma-Aldrich) per conjugate. The protein conjugates were dialyzed with 10^{-1} M NaCl solution for 72 hours and the bath solution was changed at least four times per day. The conjugated tryptophan derivative and BSA concentrations were evaluated by spectrophotometry. The coupling ratio of each conjugate was calculated from the absorbance values.

Tryptophan (Sigma), 5-hydroxytryptophan (5-HW) (Sigma), and 5HT (Sigma) were linked to BSA with glutaraldehyde (G), as previously described in [24, 25].

Mel (Sigma), 5-MTol (Sigma), and 5-HTol (Sigma) conjugates were synthesized as follows: 5 mg of each hapten were shaken in 1 mL water/ethanol (vol/vol) and mixed with 20 mg BSA (previously dissolved in 1 mL deionized water) plus 600 μL 3M acetate buffer (Sigma). One mL 2% formaldehyde solution was added to the mixture and the reaction was stabilized after 5 minutes at room temperature. The conjugates were dialyzed with a 10^{-2} M phosphate buffer solution (NaH_2PO_4, $12H_2O$) and 0.15M NaCl, (pH 7.4), 3 times per day for 72 hours.

2.3. Evaluation of the Molecular Coupling Ratio. The molecular coupling ratio of each conjugate was determined by measuring the concentration of tryptophan derivative and BSA at 310–330 nm and 280 nm, respectively, as previously described in [23], taking into account the molar extinction coefficients after coupling.

2.4. ELISA. ELISA was used to determine the titers of G, M, or A immunoglobulins (Ig). The protocol has been extensively described elsewhere [23–25]. Briefly, polystyrene 96-well plates (NUNC) were coated with 200 μL solution containing 10–50 μg/mL tryptophan-derivative conjugates in 0.05 M carbonate buffer (pH 9.6). Well plates were incubated under agitation at 4°C for 16 hours. Then, 200 μL blocking buffer A (PBS, 2.5 g/L BSA) were applied and samples were incubated at 37°C for 1 hour. Well plates were washed with PBS solution and filled with 200 μL serum diluted 1:500 in blocking buffer A for IDO derivatives and 5-HIAA or blocking buffer B (PBS, 10% glycerol and 2.5 g/L BSA) for the other conjugates. They were incubated at 37°C for 2 hours. Well plates were washed 3 times with PBS, 0.05% Tween 20, incubated with peroxidase-labeled antihuman IgG (Biorad), anti-human IgM (Pierce), or anti-human IgA (Pierce) antibodies at 37°C for 1 hour. These anti-isotype antibodies were diluted 1: 50,000, 1: 25,000, and 1: 14,000 in blocking buffer C (PBS, 0.05% Tween 20, 2.5 g/l BSA), respectively. Plates were then washed three times with PBS, 0.05% Tween 20, and incubated with the detection solution in darkness for 10 minutes. The chromogen solution consisted of 8% orthophenyldiamine (OPD, Sigma Aldrich) in a 0.1, M sodium citrate and 0.01, M phosphate buffer (pH 5.0), containing 0.01% H_2O_2 (Merck) for the peroxidase assay. The reaction was stopped using 50 μL 2-N HCl (Sigma-Aldrich). Optical densities (ODs) were measured at 492 nm using a Multiscan spectrophotometer. All assays were carried out in duplicate.

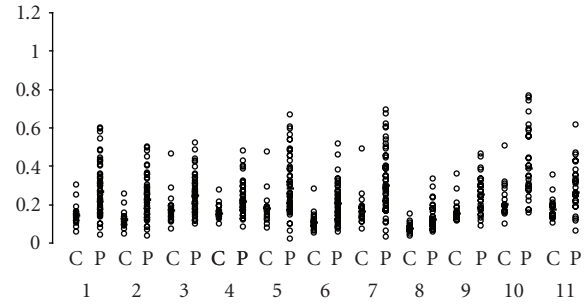

Figure 2: Scattergrams represent the OD values for AD patients (P) and controls (C) for each conjugate (statistically significant) and isotype (statistically significant). The first lane of OD values corresponds to C and the second to P. IgA for: (1) 3-OHKyn, (2) Kyna, (3) Quina, (4) 3-OHAnthra, (5) Anthra, (6) Xantha, (7) Pico, (8) 5-HT, (9) Mel, (10) 5-HIAA, and (11) 5-MTol.

2.5. Statistical Analysis. The OD of each BSA-coated well was subtracted from the OD of each well containing the tryptophan derivative. The Mann and Whitney U-test was used to compare the AD and healthy sera for each tryptophan-derivative conjugate. All statistical analyses were considered significant when $P \leq .01$. The proportion of positive sera was calculated as the number of patients with an OD above the mean control group OD value +2 standard deviations.

3. Results and Discussion

The presence of circulating antibodies directed against conjugated tryptophan metabolites indirectly revealed the overproduction of metabolites associated with hyperactivation of the IDO-1 in AD, as previously described in [23]. However, no previous study had shown the presence of circulating antibodies against THO-pathway-derived metabolites. Some statistically significant results are shown in Figure 2 and Table 1. IgA responses were observed only for the antibodies directed against the following IDO-1 pathway metabolites: 3-OHKyn, Kyna, Quina, 3-OHAnthra, Anthra, Xantha, and Pico. The role of Quina and 3OH-Kyn in neurological disorders has been previously described in numerous studies [26]. An accumulation of Quina in astrocytes and neurons is one of the events associated with depression or dementia in AD. Quina acts as an agonist of the N-methyl-d-aspartate (NMDA) receptor and plays a direct role as an excitotoxic agent [27]. Rahman et al. [28] showed that Quina was colocalized with the hyperphosphorylated Tau protein of cortical neurons in AD brains and induced Tau protein phosphorylation. Decreased concentrations of Kyna, a Quina antagonist, were found in AD patient sera: Hartai et al. [29] reported a decrease in Kyna concentrations in plasma and red-blood cells, while Kyn levels and kynurenine aminotransferase I and II activity remained unchanged.

However, our results revealed antibodies directed against conjugated Kyna in the sera. Moreover, Baran et al. [30] previously observed that a significant increase in Kyna production in the putamen and caudate nucleus of AD patients

Table 1: Significance ($P \leq .01$) of OD values (U Mann and Whitney t-test) and percentage of AD positive patients on well plates coated with tryptophan-derivative conjugates. OD values for each tryptophan-derivative conjugate were subtracted from those on BSA well plates. Percentages were calculated as follows: number of patients with OD values above mean OD value of controls +2 standard deviations.

		Conjugates coated on well plates							
Isotype		Kyn	3-0HKyn	Kyna	Quina	Quinald	3-OHAnthra	Anthra	Xantha
G	Significance	0.0836	0.5746	0.5758	0.8793	0.7513	0.0194	0.1576	0.0635
	Percentage	25.4%	10.9%	10.9%	7.2%	16.3%	9.0%	14.7%	20.0%
M	Significance	0.8275	0.8741	0.9784	0.0000	0.3450	0.2294	0.8172	0.9895
	Percentage	29%	9.0%	18.1%	7.2%	32.7%	14.5%	21.8%	21.8%
A	Significance	0.0015	**0.0001**	**0.0000**	**0.0027**	0.0119	**0.0034**	**0.0026**	**0.0005**
	Percentage	38.1%	**58.1**%	**61.8**%	**65.4**%	43.6%	**50.9**%	**54.5**%	**63.6**%
Isotype		Pico	W	5-HW	5-HT	Mel	5-HIAA	5-HTol	5-MTol
G	Significance	0.0790	0.9042	0.0000	0.0118	0.3873	0.0878	0.4244	0.6936
	Percentage	12.7%	24.1%	27.5%	20.6%	20.6%	10.3%	10.3%	6.8%
M	Significance	0.9737	0.0007	0.0000	0.7928	0.4906	0.4181	0.3696	0.6941
	Percentage	23.6%	3.4%	3.4%	10.3%	10.3%	24.1%	6.8%	3.4%
A	Significance	**0.0011**	0.0878	0.2836	**0.0001**	**0.0018**	**0.0001**	0.0010	**0.0015**
	Percentage	**65.4**%	10.3%	13.7%	**62**%	**51.7**%	**58.6**%	41.3%	**51.7**%

was associated with an elevated kynurenine metabolism. Xantha production via the IDO-1 pathway is higher in depressed patients than controls [31] and plays a role in apoptosis [32], as well as acting as a neuromodulator in the rat brain [33]. The production of 3-OH anthra, Anthra, and Pico in AD had not previously been studied in sufficient detail. The IDO-1 pathway is a key regulator of the immune response. IDO-1 induction and expression tends to limit the extracellular tryptophan pool necessary for lymphocyte proliferation [34] and pathogen invasion [35].

As in the case of the IDO-1 pathway, IgA antibodies were also found against some THO pathway metabolites, that is: the neurotransmitter 5-HT, the neuroprotector Mel, and the neurotoxic metabolites 5-HIAA, and 5-MTol. Mel and 5-MTol production is dependent on the overexpression of Hydroxyindole-O-metyl transferase, which may be indirectly responsible for the large increase in circulating antibodies directed against Mel and 5-MTol, synthesized mainly in the pineal gland [36]. Their production obeys a circadian rhythm in healthy persons, which tends to disappear in AD patients [37].

Burke et al. [38] reported that, in AD, 5-HT and 5-HIAA production was specifically localized in the raphe nucleus. A decrease in THO transport to axon terminals resulted in increased concentrations of these molecules, as well as a 4.7-fold increase in THO activity. High 5-HIAA levels have been measured in the delirious phase of AD [39].

A major finding in this work is that all the circulating antibodies detected were of the IgA isotype, associated with mucosal immunity, stimulated by exogenous factors (e.g., bacteria constituents). Along those lines, Malaguarnera et al. [40] demonstrated a correlation between the levels of circulating antibodies directed against *Helicobacter pylori* antigens and AD scores. Many authors have suggested that bacteria play a role in the etiology of AD [41]. Miklossy et al. demonstrated in vitro that exposing neuronal and glial cells to *Borrelia* spirochetes induced morphological changes related to amyloid deposition, similar to those observed in AD [42]. Moreover, IDO-1 is induced by many interleukins and gram-bacteria lipopolysaccharides [43]. IDO-1 is considered an immunomodulator, as tryptophan "starvation" prevents bacterial multiplication [44]. Moreover, the overproduction of IDO-1 pathway metabolites is linked to many cell processes associated with inflammation and apoptosis [45]. Further investigations should thus focus on IDO-1 and THO derivatives linked to endogenous proteins and/or bacteria components.

4. Conclusion

Circulating antibodies, exclusively of the IgA isotype, directed against tryptophan metabolites were found in AD patient sera, thus demonstrating that neurotoxic tryptophan metabolites are involved in this neurodegenerative disease. Activation of the IDO-1 pathway leads to overexpression of these tryptophan metabolites. The production of IgA antibodies suggests the activation of the mucosal immune system, possibly by bacterial components. The sequence of events may start when circulating bacteria components induce IDO-1 activity. The identification of circulating antibodies directed against IDO-1/THO pathway metabolites contributes to elucidating the etiology of AD.

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References

[1] S. Salloway and S. Correia, "Alzheimer disease: time to improve its diagnosis and treatment," *Cleveland Clinic Journal of Medicine*, vol. 76, no. 1, pp. 49–58, 2009.

[2] M. R. D'Andrea, "Evidence linking neuronal cell death to autoimmunity in Alzheimer's disease," *Brain Research*, vol. 982, no. 1, pp. 19–30, 2003.

[3] S. Miranda, C. Opazo, L. F. Larrondo, et al., "The role of oxidative stress in the toxicity induced by amyloid β-peptide in Alzheimer's disease," *Progress in Neurobiology*, vol. 62, no. 6, pp. 633–648, 2000.

[4] H. Akiyama, S. Barger, S. Barnum, et al., "Inflammation and Alzheimer's disease," *Neurobiology of Aging*, vol. 21, no. 3, pp. 383–421, 2000.

[5] E. Kwidzinski, J. Bunse, A. D. Kovac, et al., "IDO (indolamine 2,3-dioxygenase) expression and function in the CNS," *Advances in Experimental Medicine and Biology*, vol. 527, pp. 113–118, 2003.

[6] A. Yamada, H. Akimoto, S. Kagawa, G. J. Guillemin, and O. Takikawa, "Proinflammatory cytokine interferon-γ increases induction of indoleamine 2,3-dioxygenase in monocytic cells primed with amyloid β peptide 1-42: implications for the pathogenesis of Alzheimer's disease," *Journal of Neurochemistry*, vol. 110, no. 3, pp. 791–800, 2009.

[7] C. Liebau, A. W. A. Baltzer, S. Schmidt, et al., "Interleukin-12 and interleukin-18 induce indoleamine 2,3-dioxygenase (IDO) activity in human osteosarcoma cell lines independently from interferon-γ," *Anticancer Research*, vol. 22, no. 2, pp. 931–936, 2002.

[8] G. J. Guillemin, G. A. Smythe, L. A. Veas, O. Takikawa, and B. J. Brew, "A beta 1-42 induces production of quinolinic acid by human macrophages and microglia," *Neuroreport*, vol. 14, no. 18, pp. 2311–2315, 2003.

[9] P. Newhouse, A. Tatro, M. Naylor, K. Quealey, and P. Delgado, "*Alzheimer's* disease, serotonin systems, and tryptophan depletion," *American Journal of Geriatric Psychiatry*, vol. 10, no. 4, pp. 483–484, 2002.

[10] B. Widner, F. Leblhuber, J. Walli, G. P. Tilz, U. Demel, and D. Fuchs, "Tryptophan degradation and immune activation in Alzheimer's disease," *Journal of Neural Transmission*, vol. 107, no. 3, pp. 343–353, 2000.

[11] E. J. Roy, O. Takikawa, D. M. Kranz, A. R. Brown, and D. L. Thomas, "Neuronal localization of indoleamine 2,3-dioxygenase in mice," *Neuroscience Letters*, vol. 387, no. 2, pp. 95–99, 2005.

[12] G. J. Guillemin, K. R. Williams, D. G. Smith, G. A. Smythe, J. Croitoru-Lamoury, and B. J. Brew, "Quinolinic acid in the pathogenesis of Alzheimer's disease," *Advances in Experimental Medicine and Biology*, vol. 527, pp. 167–176, 2003.

[13] G. J. Guillemin and B. J. Brew, "Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease," *Redox Report*, vol. 7, no. 4, pp. 199–206, 2002.

[14] B. E. Leonard, "Inflammation, depression and dementia: are they connected?" *Neurochemical Research*, vol. 32, no. 10, pp. 1749–1756, 2007.

[15] A. Toledano-Gasca, "Hypotheses concerning the aetiology of Alzheimer's disease," *Pharmacopsychiatry*, vol. 21, no. 1, pp. 17–25, 1988.

[16] E. Matsubara, T. Bryant-Thomas, J. P. Quinto, et al., "Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease," *Journal of Neurochemistry*, vol. 85, no. 5, pp. 1101–1108, 2003.

[17] J.-Z. Wang and Z.-F. Wang, "Role of melatonin in Alzheimer-like neurodegeneration," *Acta Pharmacologica Sinica*, vol. 27, no. 1, pp. 41–49, 2006.

[18] A. M. Hussain and A. K. Mitra, "Effect of aging on tryptophan hydroxylase in rat brain: implications on serotonin level," *Drug Metabolism and Disposition*, vol. 28, no. 9, pp. 1038–1042, 2000.

[19] H. J. Stuerenburg, S. Ganzer, and T. Müller-Thomsen, "5-hydroxyindoleacetic acid and homovanillic acid concentrations in cerebrospinal fluid in patients with Alzheimer's disease, depression and mild cognitive impairment," *Neuroendocrinology Letters*, vol. 25, no. 6, pp. 435–437, 2004.

[20] G. McKhann, D. Drachman, and M. Folstein, "Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease," *Neurology*, vol. 34, no. 7, pp. 939–944, 1984.

[21] M. F. Folstein, S. E. Folstein, and P. R. McHugh, ""Mini mental state". A practical method for grading the cognitive state of patients for the clinician," *Journal of Psychiatric Research*, vol. 12, no. 3, pp. 189–198, 1975.

[22] C. P. Hughes, L. Berg, and W. L. Danziger, "A new clinical scale for the staging of dementia," *British Journal of Psychiatry*, vol. 140, no. 6, pp. 566–572, 1982.

[23] S. Duleu, A. Mangas, F. Poulletier de Gannes, M.-C. Tranchant, and M. Geffard, "Circulating antibodies to conjugated tryptophan derivatives of the IDO pathway in amyotrophic lateral sclerosis, Alzheimer's, Parkinson's and multiple sclerosis patients," *Immuno-Analyses & Biologie Spécialisée*, vol. 23, no. 1, pp. 27–34, 2008.

[24] S. Duleu, C. Van Der Velden, F. Poulletier de Gannes, M.-C. Tranchant, and M. Geffard, "Circulating antibodies to NO- and ONOO-modified antigens in amyotrophic lateral sclerosis, Alzheimer's disease and multiple sclerosis," *Immuno-Analyses & Biologie Spécialisée*, vol. 22, no. 5, pp. 273–281, 2007.

[25] M. Geffard, J. Dulluc, and A.-M. Rock, "Antisera against the indolealkylamines: tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-methoxytryptophan, and 5-methoxytryptamine tested by an enzyme-linked immunosorbent assay method," *Journal of Neurochemistry*, vol. 44, no. 4, pp. 1221–1228, 1985.

[26] K. K. Ting, B. Brew, and G. Guillemin, "The involvement of astrocytes and kynurenine pathway in Alzheimer's disease," *Neurotoxicity Research*, vol. 12, no. 4, pp. 247–262, 2007.

[27] N. Braidy, R. Grant, S. Adams, B. J. Brew, and G. J. Guillemin, "Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons," *Neurotoxicity Research*, vol. 16, no. 1, pp. 77–86, 2009.

[28] A. Rahman, K. Ting, K. M. Cullen, N. Braidy, B. J. Brew, and G. J. Guillemin, "The excitotoxin quinolinic acid induces tau phosphorylation in human neurons," *PLoS One*, vol. 4, no. 7, article e6344, 2009.

[29] Z. Hartai, A. Juhász, A. Rimanóczy, et al., "Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease," *Neurochemistry International*, vol. 50, no. 2, pp. 308–313, 2007.

[30] H. Baran, K. Jellinger, and L. Deecke, "Kynurenine metabolism in Alzheimer's disease," *Journal of Neural Transmission*, vol. 106, no. 2, pp. 165–181, 1999.

[31] M. J. Hoes and N. Sijben, "The clinical significance of disordered renal excretion of xanthurenic acid in depressive patients," *Psychopharmacology*, vol. 75, no. 4, pp. 346–349, 1981.

[32] H. Z. Malina, C. Richter, M. Mehl, and O. M. Hess, "Pathological apoptosis by xanthurenic acid, a tryptophan metabolite: activation of cell caspases but not cytoskeleton breakdown," *BMC Physiology*, vol. 1, article 7, 8 pages, 2001.

[33] S. Gobaille, V. Kemmel, D. Brumaru, C. Dugave, D. Aunis, and M. Maitre, "Xanthurenic acid distribution, transport, accumulation and release in the rat brain," *Journal of Neurochemistry*, vol. 105, no. 3, pp. 982–993, 2008.

[34] A. L. Mellor, B. Baban, P. Chandler, et al., "Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion," *Journal of Immunology*, vol. 171, no. 4, pp. 1652–1655, 2003.

[35] C. R. MacKenzie, K. Heseler, A. Müller, and W. Däubener, "Role of indoleamine 2,3-dioxygenase in antimicrobial defence and immuno-regulation: tryptophan depletion versus production of toxic kynurenines," *Current Drug Metabolism*, vol. 8, no. 3, pp. 237–244, 2007.

[36] J. B. Zawilska, M. Bereziska, J. Rosiak, et al., "Daily variation in the concentration of melatonin and 5-methoxytryptophol in the goose pineal gland, retina, and plasma," *General and Comparative Endocrinology*, vol. 134, no. 3, pp. 296–302, 2003.

[37] D. J. Skene, B. Vivient-Roels, D. L. Sparks, et al., "Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease," *Brain Research*, vol. 528, no. 1, pp. 170–174, 1990.

[38] W. J. Burke, D. H. Park, H. D. Chung, G. L. Marshall, J. H. Haring, and T. H. Joh, "Evidence for decreased transport of tryptophan hydroxylase in Alzheimer's disease," *Brain Research*, vol. 537, no. 1-2, pp. 83–87, 1990.

[39] T. J. M. van der Cammen, H. Tiemeier, M. J. Engelhart, and D. Fekkes, "Abnormal neurotransmitter metabolite levels in Alzheimer patients with a delirium," *International Journal of Geriatric Psychiatry*, vol. 21, no. 9, pp. 838–843, 2006.

[40] M. Malaguarnera, R. Bella, G. Alagona, R. Ferri, A. Carnemolla, and G. Pennisi, "Helicobacter pylori and Alzheimer's disease: a possible link," *European Journal of Internal Medicine*, vol. 15, no. 6, pp. 381–386, 2004.

[41] S. R. Robinson, C. Dobson, and J. Lyons, "Challenges and directions for the pathogen hypothesis of Alzheimer's disease," *Neurobiology of Aging*, vol. 25, no. 5, pp. 629–637, 2004.

[42] J. Miklossy, A. Kis, A. Radenovic, et al., "Beta-amyloid deposition and Alzheimer's type changes induced by Borrelia spirochetes," *Neurobiology of Aging*, vol. 27, no. 2, pp. 228–236, 2006.

[43] I. D. Jung, C.-M. Lee, Y.-I. Jeong, et al., "Differential regulation of indoleamine 2,3-dioxygenase by lipopolysaccharide and interferon gamma in murine bone marrow derived dendritic cells," *FEBS Letters*, vol. 581, no. 7, pp. 1449–1456, 2007.

[44] C. Oberdörfer, O. Adams, C. R. MacKenzie, C. J. A. De Groot, and W. Däubener, "Role of IDO activation in anti-microbial defense in human native astrocytes," *Advances in Experimental Medicine and Biology*, vol. 527, pp. 15–26, 2003.

[45] M. Staniszewska and R. H. Nagaraj, "Detection of kynurenine modifications in proteins using a monoclonal antibody," *Journal of Immunological Methods*, vol. 324, no. 1-2, pp. 63–73, 2007.

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Review Article

Microglial Immunoreceptor Tyrosine-Based Activation and Inhibition Motif Signaling in Neuroinflammation

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Elimination of extracellular aggregates and apoptotic neural membranes without inflammation is crucial for brain tissue homeostasis. In the mammalian central nervous system, essential molecules in this process are the Fc receptors and the DAP12-associated receptors which both trigger the microglial immunoreceptor tyrosine-based activation motif- (ITAM-) Syk-signaling cascade. Microglial triggering receptor expressed on myeloid cells-2 (TREM2), signal regulatory protein-β1, and complement receptor-3 (CD11b/CD18) signal via the adaptor protein DAP12 and activate phagocytic activity of microglia. Microglial ITAM-signaling receptors are counter-regulated by immunoreceptor tyrosine-based inhibition motif- (ITIM-) signaling molecules such as sialic acid-binding immunoglobulin superfamily lectins (Siglecs). Siglecs can suppress the proinflammatory and phagocytic activity of microglia via ITIM signaling. Moreover, microglial neurotoxicity is alleviated via interaction of Siglec-11 with sialic acids on the neuronal glycocalyx. Thus, ITAM- and ITIM-signaling receptors modulate microglial phagocytosis and cytokine expression during neuroinflammatory processes. Their dysfunction could lead to impaired phagocytic clearance and neurodegeneration triggered by chronic inflammation.

1. Microglia and Alzheimer's Disease

Microglial cells originate from myeloid cells of the hematopoietic lineage and are the resident immune cells of the central nervous system (CNS). They are involved in the active immune defense by their ability to phagocytose invading bacteria and to release reactive oxygen species acting as microbicides. In the healthy brain, microglia are relative evenly distributed and predominantly found in a so-called "resting" state, displaying a small cell body with many highly branched processes, which are highly motile and continuously monitor the brain parenchyma [1–3]. Microglia are involved in tissue maintenance, execution of innate immunity, and participation in adaptive immune responses [1–3]. They are regarded as active sensors, searching for and reading biochemical signals of pathogenic changes in the brain environment [2]. In response to injury, ischemia and inflammatory stimuli microglia change from an immunologically silent state to an activated state that is reflected in different morphological appearance—amoeboid, rodlike, or phagocytic. They can migrate to the site of disturbance, secrete a wide range of soluble factors including cytokines as well as neurotrophic factors, and phagocytose cellular debris. Thereby, microglia contribute to tissue homeostasis and regeneration [2–5]. The effects of activated microglia can be highly diverse. On the one hand, they are neurotoxic by producing pro-inflammatory mediators including cytokines and reactive oxygen species such as interleukin-1β, tumor necrosis factor-α and nitric oxide, which are potent inducers of neuronal damage and cell death [3]. On the other hand, they can also initiate anti-inflammatory and immunosuppressive signaling that results in repair, resolution of inflammation and turning back to tissue homeostasis [3, 6, 7]. Furthermore, microglia act as regulators of neuronal survival and development through cytokines and chemokines such as interleukin-6 and CCL5 (RANTES). Activated, interleukin-6 producing microglia have been shown to decrease *in vitro* the neurogenesis of neural stem cells and increase the number of apoptotic cells in differentiating cultures [8]. Moreover, upon stimulation,

RANTES is produced by microglia [9]. Due to the observation that similar amounts of RANTES are produced by fetal and adult microglia, Hu et al. suggest this chemokine to be acquired early in brain development [9].

Increasing evidence indicates that microglia are involved in almost all types of brain pathology. In the aging brain and most chronic neurodegenerative diseases including Parkinson's disease and Alzheimer's disease (AD), microglial cells become activated and provoke ambivalent effects. They can either be deleterious by enhancing neurodegeneration through secreting cytokines and neurotoxins [10], or might be beneficial by principally migrating to the amyloid-β (Aβ) plaques and phagocytosing Aβ deposits. Recently, it was shown by *in vivo* multiphoton microscopy in different animal models of AD that Aβ plaques could appear within 24 hours and microglial cells are activated and recruited to the newly formed plaques within one day [11]. Additionally, within one week after the onset of plaque formation dysmorphic neurites were present [11]. Interestingly, microglial cells seem to contribute to AD progression. Although maintaining their ability to produce pro-inflammatory cytokines, microglia of aging APP/PS1-transgenic mice, a mouse model of Aβ plaque formation associated with AD, become dysfunctional and display a reduced Aβ clearance capability [12], suggesting that Aβ plaques might partially result from impaired microglial removal. However, in APP-transgenic mice that barely exhibit resident microglia, formation and maintenance of Aβ plaques have been lately demonstrated unchanged [13]. Nevertheless, several lines of *in vitro* evidence suggest the involvement of innate immune signaling during recognition of Aβ. Different receptors expressed on microglia such as CD14 and toll-like receptors (TLR) 2 and 4 are known to contribute to the clearance of Aβ plaques in AD [14–16]. CD14 is involved in the uptake of the bacterial component lipopolysaccharide (LPS) [15]. To transduce activation signals, CD14 interacts with TLR2 and TLR4 containing dimeric complexes [16, 17]. Additionally, CD14 has been shown to specifically mediate Aβ phagocytosis *in vitro*. Cells expressing CD14 internalized significantly higher amounts of Aβ compared to CD14-deficient cells while the uptake of microbeads was unaffected [15]. Moreover, CD14 acts together with TLR2 and TLR4 to bind Aβ and subsequently activate intracellular signaling leading to phagocytosis *in vitro*. Cells deficient for either CD14, TLR2 or TLR4 could not initiate the cascades inducing phagocytosis [16]. Recently, CD36, another coreceptor of TLRs, has been described *in vitro* to facilitate the assembly of a heteromeric complex of CD36, TLR4, and TLR6 upon binding of Aβ [14]. However, the exact receptors which might scavenge Aβ and/or induce microglial phagocytic responses and signaling pathways that impair microglial phagocytosis *in vivo* are still unclear.

2. Microglial ITAM-ITIM Signaling

Latest publications indicate that immunoreceptor tyrosine-based activation motif (ITAM) signaling plays an important role in the phagocytic process. ITAM-containing signaling adaptor proteins are associated with receptor subunits. After the binding of ligand and receptor, the tyrosine residues of the ITAMs become phosphorylated by members of the Src kinase family (Figure 1, left side). These phosphotyrosine residues are docking sites for Src homology 2 (SH2) domains of Syk protein kinases which upon activation mediate cellular activation via a number of downstream cascades [18–20]. The processes involved in phagocytosis of apoptotic material are well conserved from worms to mammals [21]. Draper is a phagocytic receptor of the fruit fly *Drosophila* with an ITAM in the intracellular domain. Recently, it has been described that upon phosphorylation of ITAM tyrosine residues, Draper can bind the nonreceptor tyrosine kinase Shark which is similar to the mammalian Syk. Moreover, not only the activity of Shark but also the ITAM-phosphorylation of Draper is required for Draper-mediated signaling events such as the attraction of glial membranes to damaged axons and the glial phagocytic activity [21]. Interestingly, the Draper-ITAM signaling pathway of *Drosophila* and the DAP12-ITAM signaling of mammalian immunoreceptors have a lot in common. The mammalian DAP12 molecule is a transmembrane adaptor protein that contains two ITAMs. It is expressed by microglia and associates with cell membrane receptors such as triggering receptor expressed on myeloid cells 2 (TREM2) [18] or signal regulatory protein-β1 (SIRPβ1) [22]. Stimulation of SIRPβ1

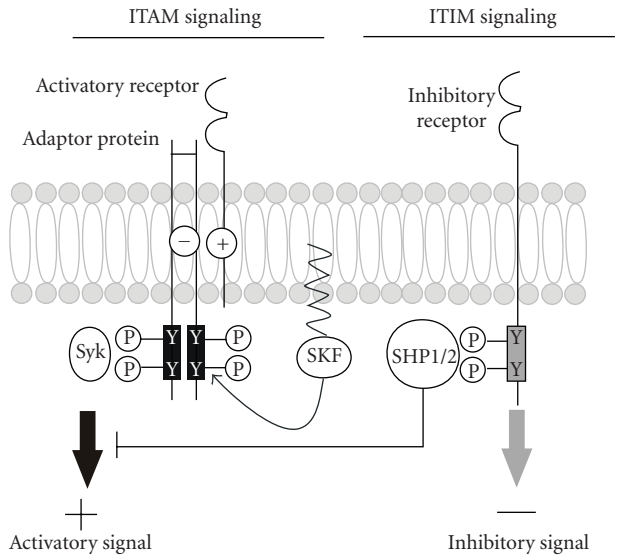

Figure 1: ITAM-/ITIM-signaling cascade. *Left side:* Upon ligand binding, activatory receptors like TREM2, SIRPβ1, FcγRI or FcγRIIIA associate with ITAM containing adaptor proteins such as DAP12 or the common γ chains through interactions between charged amino acids (−/+) within the transmembrane regions of each protein. Subsequently, members of Src kinase family (SKF) phosphorylate tyrosine residues of ITAMs. Phosphotyrosine residues are docking sites for Syk protein kinases that upon activation mediate cellular activation via a number of downstream cascades. *Right side:* Upon ligand binding, inhibitory receptors like most Siglecs recruit SHP1 and SHP2 which can in turn terminate intracellular signals emanating from ITAM receptors.

or TREM2 occurs by yet unknown endogenous ligands. For TREM2 it has been suggested that it binds to lipo-oligosaccharides of Gram-positive and -negative bacteria. The observation that the binding can be disrupted by anionic carbohydrates led to the suggestion that a charge-dependent ligand recognition takes place [23]. Upon stimulation of SIRPβ1 or TREM2, a phosphorylation of DAP12-ITAM is induced and the phagocytic activity of microglial cells is increased *in vitro* [22, 24, 25]. TREM2-DAP12 signaling via ITAM also promotes phagocytosis of bacteria. It has been demonstrated *in vitro* that TREM2 mediates binding of bacteria and promotes their internalization dependent on Src kinase mediated tyrosine phosphorylation [26]. ITAMs are counter-regulated by immunoreceptor tyrosine-based inhibition motifs (ITIMs; Figure 1, right side). Upon ligand binding, inhibitory receptors with ITIMs prevent the activation signals that originate from receptors associated with ITAMs through the recruitment of SH2 domain containing tyrosine phosphatases (SHP1 and SHP2) which in turn can modulate the function of various signaling pathways [27, 28]. Most CD33-related sialic acid-binding immunoglobulin superfamily lectins (Siglecs), a subgroup of the immunoglobulin superfamily that recognizes sialic acid residues of glycoproteins and glycolipids, have one or more ITIMs in the cytoplasmic domain [27, 29]. Binding of Siglecs to highly sialylated proteins and lipids such as clusterin, apolipoprotein E and gangliosides that are abundantly present in AD plaques could in turn mediate an inhibitory signaling cascade. Thereby, microglial phagocytosis is possibly suppressed and the AD plaques might be left untouched [28].

3. Microglial Fc Receptors

An important group of receptors on the surface of phagocytes, which signal via ITAM-Syk signaling and mediate phagocytosis function, include the Fc receptors (FcR). FcR interact with the Fc part of immunoglobulin (Ig) G bound to antigen presented on microbial pathogens or autoantigens [20, 30, 31]. Except for the human FcγRIIA (CD32a), which itself possesses an ITAM located in the cytoplasmic region, activating FcRs like FcγRI (CD64) and FcγRIIIA (CD16a) have to interact with adaptor molecules, the common γ chain of FcR that contain the required ITAMs. The common γ chain of FcR is a homolog of the adaptor protein DAP12 and functionally close related to it. Subsequently, tyrosine residues of the ITAM are phosphorylated by members of the Src kinase family resulting in the establishment of docking sites for Syk kinases (Figure 1, left side). Activated Syk kinases in turn initiate a variety of downstream signals mediated through calcium, protein kinase C, phospholipase A2, phosphatidyl-inositol 3-kinase, extracellular signal-regulated kinase and GTPases of the Rho family leading to phagocytosis of IgG coated and opsonized particles and antigens [31–33]. Accordingly, microglial cells express the activating FcRs CD16, CD32 and CD64 and phagocytose antigens via the corresponding IgG subtypes [34, 35]. Moreover, in brain areas displaying neurodegeneration such as multiple sclerosis lesions, the expression of those FcRs on microglia is increased [34], suggesting a role of FcRs in protecting the surrounding tissue from IgG-opsonized antigens [35]. Furthermore, there is an ongoing discussion whether FcRs play a role in AD by contributing to microglial Aβ clearance [36]. In APP-transgenic mice it has been demonstrated that antibodies directed against Aβ can enter the CNS [36]. One study described that immunization of APP-transgenic mice with Aβ1-42, which induced Aβ1-42 specific antibodies, reduced Aβ deposition regardless of whether the mice were genetically deficient of the FcR domain FcRγ. The authors suggest that FcR-mediated mechanisms are irrelevant for the effectiveness of Aβ immunotherapy *in vivo* [37]. However, another study clearly demonstrated by using an *ex vivo* assay, in which primary microglial cells were cultured with unfixed cryostat sections of AD brains, that Aβ antibodies could evoke FcR-mediated microglial phagocytosis of Aβ plaques and subsequent Aβ degradation [36].

4. Microglial DAP12 Associated Receptors

Several DAP12 associated receptors are known including activating natural killer cell receptors, like KIR2DS and NKG2D, and myeloid receptors, such as signal regulatory protein-β1 (SIRPβ1), TREM1, -2, -3 [38], complement receptors [39], and certain Siglecs, such as Siglec-16 [40]. This review will focus on some of them.

4.1. TREM2. The glycoprotein TREM2 is expressed on microglia [41], and consists of one extracellular Ig-like domain, a transmembrane region with a charged lysine residue and a short cytoplasmic tail [18]. As TREM2 lacks an intracellular signaling tail, it is completely dependent on the presence of the adaptor protein DAP12 [18, 42]. As mentioned before, the mammalian adaptor molecule DAP12 is another protein besides the common γ chain of FcR that activates an ITAM-Src kinase signaling pathway. Via signaling through the adaptor protein DAP12, TREM2, a phagocytic receptor with still unknown endogenous ligand, leads to activation of microglial cells. Activated microglia in turn can clear cellular apoptotic material, thereby contributing to tissue repair [18, 24, 43]. Therefore, a non-functional TREM2 might be involved in brain damage by causing accumulation of toxic products. Interestingly, loss-of-function mutations of DAP12 or TREM2 both lead to a chronic neurodegenerative disease called Nasu-Hakola or polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), an autosomal recessive inherited disease [18]. While this disease is characterized by early onset presenile dementia followed by delayed bone symptoms in patients carrying TREM2 mutations [44], patients with mutations in DAP12 display an early onset combination of presenile dementia and systemic bone cysts [45, 46]. Moreover, it has been demonstrated that TREM2 is down-regulated by inflammatory signals [47]. All these data indicate that TREM2 might be functionally crucial for the prevention of neurodegenerative processes.

4.2. SIRPβ1. Recently, other new microglial receptors like SIRPβ1 with a phagocytic ITAM signaling capacity have been identified [25]. Like TREM2, SIRPβ1 is expressed on microglial cells. In APP-transgenic mice and a mouse model for experimental autoimmune encephalomyelitis, the expression levels of both proteins are increased [25]. Furthermore, TREM2 and SIRPβ1 are plaque-associated and increase the phagocytic activity of microglia [24, 25, 42, 43]. Upon neurodegenerative signals, TREM2 expression is induced leading to increased phagocytosis and decreased pro-inflammatory responses of microglial cells [42]. However, SIRPβ1 does not only specifically clear Aβ but also neural debris and microsphere beads [25]. A strong increase of microglial SIRPβ1 gene transcript has been revealed in the cerebral hemispheres and cerebellum of an animal model of AD, while the gene transcript of DAP12 has only been increased slightly. Thus, up-regulation of SIPRβ1 does not simply reflect a higher number of microglia. Moreover, it is not directly triggered by the amyloid plaques but by other disease-associated processes including interferons (IFNs) like IFNβ and IFNγ. In cultured microglia, IFNs have been shown to influence the gene transcription of SIRPβ1 [25]. So far, concrete *in vivo* evidence for a direct pathophysiological relevance of SIRPβ1 is missing. While SIRPβ1 could not only be detected on microglial cells associated with plaques, but also in those not directly associated with plaques, it is regarded as a potent regulator of Aβ42 fibril clearance *in vitro* [25].

4.3. Complement Receptor 3 (CD11b/CD18). Complement receptor 3 (CD11b/CD18). Another potential microglial DAP12-associated receptor is the complement receptor 3 (CR3), a major heterodimeric receptor consisting of the integrins CD11b and CD18, which is involved in the complement system. Complement 1q (C1q), the first component of the classical pathway, mediates complement 3 (C3) deposition on apoptotic cells. The phagocytic receptor CR3 plays an important role in the subsequent clearance of C3-opsonized structures [48]. Moreover, sequence similarities to C1q-binding peptides in CD18 suggest direct binding of CR3 (CD11b/CD18) to C1q [49]. As for immunoreceptors, signal transduction by CD18 could follow the ITAM-DAP12 signaling cascade although a direct binding of integrins with ITAM-containing proteins has not been demonstrated so far. But, it has been shown that CD18-mediated Syk activation requires the ITAM-associated molecules DAP12 and FcRγ [39]. Furthermore, both DAP12 and CD11b are required for targeted contact of microglia, like for the contact with hippocampal neurons during development that induces cell death [50]. Switching on the complement system plays an important role in initiating inflammatory reactions in the CNS as observed in AD [51] by upregulation of phagocytosis induced via activation and migration of immune cells [52]. Indeed, during formation of amyloid in APP-transgenic mice increased mRNA and protein levels of components of the complement system have been detected. Among those there have been C1q and C3, at which the classical and alternative pathway merge [53]. Moreover, complement activation has been described to occur in amyloid plaques in AD brains [54, 55] and complement products like the membrane attack complex (C5b-9) have been reported to be associated with amyloid plaques [56]. Additionally, C3 seems to be involved in the process of plaque clearance. APP-transgenic mice either deficient in C3 or expressing a C3 complement inhibitor display accelerated Aβ plaque deposition and prominent neurodegeneration [51, 57] as well as a changed activation state of the microglial cells simultaneously [51]. However, while evidences for the direct induction of a phagocytic ITAM signaling by activation of the complement signaling cascade are missing so far, these data suggest an involvement of complement components in microglia for an effective Aβ clearance.

5. Microglial Siglecs

Siglecs are members of a subgroup of the Ig superfamily that recognize specific sugar residues on the periphery of cell surface glycans, the sialic acids. Because of their sequence similarity and evolutionary conservation, Siglecs can be separated into two subsets [58]. While CD33-related Siglecs including CD33, Siglec-5 to -11, Siglec-14, and Siglec-16 evolve very rapidly by means of gene duplication or conversion and exon shuffling or loss and show a similarity of ~50–99% in their protein sequences, other members of the Siglec family such as sialoadhesin, CD22, myelin-associated glycoprotein (MAG) and Siglec-15 are more conserved and quite distantly related [27, 40, 59]. Humans display ten CD33-related Siglecs and one Siglec-like protein; mice however express only five CD33-related Siglecs, which seem to have largely lost their CD33-related Siglec genes [27, 40, 59–62]. Siglecs are type 1 transmembrane proteins showing an amino-terminal Ig-like variable (V-set Ig-like) domain that binds sialic acid and variable numbers of Ig-like constant region type 2 (C2-set Ig-like) domains [27, 58, 62, 63]. Mostly, Siglecs function as inhibitory receptors via one or more ITIMs in their cytoplasmic domain [27, 29]. Receptors with ITIMs can counteract signals emanating from ITAM receptors via the recruitment of tyrosine phosphatases such as SHP1 and SHP2 which can lead to the termination of intracellular signals (Figure 1, right side) [27, 59]. Most CD33-related Siglecs, such as the human Siglec-11, are predominantly expressed on mature cells of the immune system such as monocytes and macrophages. Therefore, CD33-related Siglecs are suggested to be important regulators of the innate immunity [27, 59, 62–64]. Siglec-11 has been shown to interact with SHP1 and SHP2 upon tyrosine phosphorylation [29]. Interestingly, SHP1 seems to be involved in antiinflammatory signaling of microglia. Microglia deficient for SHP1 have been demonstrated to produce higher amounts of neurotoxic substances upon LPS stimulation [65]. It has been shown that via interaction of microglial Siglecs with the neuronal glycocalyx microglial neurotoxicity is alleviated. Furthermore, it has been demonstrated that Siglec-11 expressing microglial cells show a reduced phagocytic capacity of apoptotic material in microglia-neuron coculture experiments [66], indicating that ITIM-signaling could be the opponent of the phagocytosis-associated ITAM-Syk signaling pathway [21].

In addition to inhibiting cellular activation, CD33-related Siglecs participate in the induction of apoptosis and the release of pro-inflammatory cytokines [27, 67–69]. However, few Siglecs have been demonstrated to associate with the ITAM-containing adaptor protein DAP12, including the recently discovered human Siglec-16. It contains a positively charged lysine residue in its transmembrane domain but lacks ITIM in its short cytoplasmic tail [40]. Cao et al. [40] have shown that Siglec-16 is expressed on macrophages and on rare microglial-like cell populations in the normal human brain. Phylogenetic analysis of the transmembrane and cytoplasmic tail domain of human and mammalian CD33-related Siglecs revealed that Siglec-16 and the before mentioned Siglec-11 are found in humans, but no direct othologues exist in rodents [40]. This indicates that these two proteins expressed on human myeloid cells could especially be involved in diseases that are uniquely occurring with their whole peculiarities only in humans such as AD. So far, it is not known whether DAP12 associated Siglecs also have a sialic acid binding specificity as observed for the ITIM bearing Siglecs. This should be investigated in the future to find out whether such Siglecs could counter-regulate each other. However, the involvement of different Siglecs in all processes including apoptosis and inflammation indicates a modulatory role of Siglecs in neuroinflammatory and neurodegenerative diseases.

6. Conclusion

The biological functions of ITAM-/ITIM-signaling in microglia are not fully understood. Several publications indicate the involvement of ITAM- and ITIM-signaling receptors in CNS innate immune responses and neuroinflammation. It is now becoming evident that those receptors also play a major role in modulating microglial phagocytosis and cytokine expression. Thus, dysfunctional ITAM-/ITIM-signaling receptors lead to chronic neurodegenerative diseases like Nasu-Hakola disease characterized by presenile dementia. These new insights might have important implications for the pathogenesis and treatment of the neuroinflammatory component of neurodegenerative diseases.

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References

[1] K. Biber, H. Neumann, K. Inoue, and H. W. G. M. Boddeke, "Neuronal 'On' and 'Off' signals control microglia," *Trends in Neurosciences*, vol. 30, no. 11, pp. 596–602, 2007.

[2] U.-K. Hanisch and H. Kettenmann, "Microglia: active sensor and versatile effector cells in the normal and pathologic brain," *Nature Neuroscience*, vol. 10, no. 11, pp. 1387–1394, 2007.

[3] R. M. Ransohoff and V. H. Perry, "Microglial physiology: unique stimuli, specialized responses," *Annual Review of Immunology*, vol. 27, pp. 119–145, 2009.

[4] F. Aloisi, "Immune function of microglia," *GLIA*, vol. 36, no. 2, pp. 165–179, 2001.

[5] M. J. Carson, "Microglia as liaisons between the immune and central nervous systems: functional implications for multiple sclerosis," *GLIA*, vol. 40, no. 2, pp. 218–231, 2002.

[6] A. E. Cardona, E. P. Pioro, M. E. Sasse et al., "Control of microglial neurotoxicity by the fractalkine receptor," *Nature Neuroscience*, vol. 9, no. 7, pp. 917–924, 2006.

[7] H. Neumann, M. R. Kotter, and R. J. M. Franklin, "Debris clearance by microglia: an essential link between degeneration and regeneration," *Brain*, vol. 132, no. 2, pp. 288–295, 2009.

[8] M. L. Monje, H. Toda, and T. D. Palmer, "Inflammatory blockade restores adult hippocampal neurogenesis," *Science*, vol. 302, no. 5651, pp. 1760–1765, 2003.

[9] S. Hu, C. C. Chao, L. C. Ehrlich et al., "Inhibition of microglial cell RANTES production by IL-10 and TGF-β," *Journal of Leukocyte Biology*, vol. 65, no. 6, pp. 815–821, 1999.

[10] R. E. Mrak and W. S. T. Griffin, "Glia and their cytokines in progression of neurodegeneration," *Neurobiology of Aging*, vol. 26, no. 3, pp. 349–354, 2005.

[11] M. Meyer-Luehmann, T. L. Spires-Jones, C. Prada et al., "Rapid appearance and local toxicity of amyloid-β plaques in a mouse model of Alzheimer's disease," *Nature*, vol. 451, no. 7179, pp. 720–724, 2008.

[12] S. E. Hickman, E. K. Allison, and J. El Khoury, "Microglial dysfunction and defective β-amyloid clearance pathways in aging alzheimer's disease mice," *Journal of Neuroscience*, vol. 28, no. 33, pp. 8354–8360, 2008.

[13] S. A. Grathwohl, R. E. Kälin, T. Bolmont et al., "Formation and maintenance of Alzheimer's disease β-amyloid plaques in the absence of microglia," *Nature Neuroscience*, vol. 12, no. 11, pp. 1361–1363, 2009.

[14] C. R. Stewart, L. M. Stuart, K. Wilkinson et al., "CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer," *Nature Immunology*, vol. 11, no. 2, pp. 155–161, 2010.

[15] Y. Liu, S. Walter, M. Stagi et al., "LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide," *Brain*, vol. 128, no. 8, pp. 1778–1789, 2005.

[16] E. G. Reed-Geaghan, J. C. Savage, A. G. Hise, and G. E. Landreth, "CD14 and toll-like receptors 2 and 4 are required for fibrillar Aβ-stimulated microglial activation," *Journal of Neuroscience*, vol. 29, no. 38, pp. 11982–11992, 2009.

[17] T. Kielian, "Toll-like receptors in central nervous system glial inflammation and homeostasis," *Journal of Neuroscience Research*, vol. 83, no. 5, pp. 711–730, 2006.

[18] M. Colonna, "Trems in the immune system and beyond," *Nature Reviews Immunology*, vol. 3, no. 6, pp. 445–453, 2003.

[19] J. A. Hamerman, M. Ni, J. R. Killebrew, C.-L. Chu, and C. A. Lowell, "The expanding roles of ITAM adapters FcRγ and DAP12 in myeloid cells," *Immunological Reviews*, vol. 232, no. 1, pp. 42–58, 2009.

[20] F. Nimmerjahn and J. V. Ravetch, "Fc-receptors as regulators of immunity," *Advances in Immunology*, vol. 96, pp. 179–204, 2007.

[21] J. S. Ziegenfuss, R. Biswas, M. A. Avery et al., "Draper-dependent glial phagocytic activity is mediated by Src and Syk family kinase signalling," *Nature*, vol. 453, no. 7197, pp. 935–939, 2008.

[22] E. Tomasello, C. Cant, H.-J. Bhring et al., "Association of signal-regulatory proteins β with KARAP/DAP-12," *European*

Journal of Immunology, vol. 30, no. 8, pp. 2147–2156, 2000.

[23] M. R. Daws, P. M. Sullam, E. C. Niemi, T. T. Chen, N. K. Tchao, and W. E. Seaman, "Pattern recognition by TREM-2: binding of anionic ligands," *Journal of Immunology*, vol. 171, no. 2, pp. 594–599, 2003.

[24] K. Takahashi, C. D. P. Rochford, and H. Neumann, "Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2," *Journal of Experimental Medicine*, vol. 201, no. 4, pp. 647–657, 2005.

[25] S. Gaikwad, S. Larionov, Y. Wang et al., "Signal regulatory protein-β1: a microglial modulator of phagocytosis in Alzheimer's disease," *American Journal of Pathology*, vol. 175, no. 6, pp. 2528–2539, 2009.

[26] E.-N. N'Diaye, C. S. Branda, S. S. Branda et al., "TREM-2 (triggering receptor expressed on myeloid cells 2) is a phagocytic receptor for bacteria," *Journal of Cell Biology*, vol. 184, no. 2, pp. 215–223, 2009.

[27] P. R. Crocker, J. C. Paulson, and A. Varki, "Siglecs and their roles in the immune system," *Nature Reviews Immunology*, vol. 7, no. 4, pp. 255–266, 2007.

[28] A. Salminen and K. Kaarniranta, "Siglec receptors and hiding plaques in Alzheimer's disease," *Journal of Molecular Medicine*, vol. 87, no. 7, pp. 697–701, 2009.

[29] T. Angata, S. C. Kerr, D. R. Greaves, N. M. Varki, P. R. Crocker, and A. Varki, "Cloning and characterization of human Siglec-11: a recently evolved signaling molecule that can interact with SHP-1 and SHP-2 and is expressed by tissue macrophages, including brain microglia," *Journal of Biological Chemistry*, vol. 277, no. 27, pp. 24466–24474, 2002.

[30] J. V. Ravetch and S. Bolland, "IgG Fc receptors," *Annual Review of Immunology*, vol. 19, pp. 275–290, 2001.

[31] F. Nimmerjahn and J. V. Ravetch, "Fcγ receptors: old friends and new family members," *Immunity*, vol. 24, no. 1, pp. 19–28, 2006.

[32] L. Stephens, C. Ellson, and P. Hawkins, "Roles of PI3Ks in leukocyte chemotaxis and phagocytosis," *Current Opinion in Cell Biology*, vol. 14, no. 2, pp. 203–213, 2002.

[33] E. García-García and C. Rosales, "Signal transduction during Fc receptor-mediated phagocytosis," *Journal of Leukocyte Biology*, vol. 72, no. 6, pp. 1092–1108, 2002.

[34] E. Ulvestad, K. Williams, C. Vedeler et al., "Reactive microglia in multiple sclerosis lesions have an increased expression of receptors for the Fc part of IgG," *Journal of the Neurological Sciences*, vol. 121, no. 2, pp. 125–131, 1994.

[35] N. S. Peress, J. Siegelman, H. B. Fleit, M. W. Fanger, and E. Perillo, "Monoclonal antibodies identify three IgG Fc receptors in normal human central nervous system," *Clinical Immunology and Immunopathology*, vol. 53, no. 2 I, pp. 268–280, 1989.

[36] F. Bard, C. Cannon, R. Barbour et al., "Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease," *Nature Medicine*, vol. 6, no. 8, pp. 916–919, 2000.

[37] P. Das, V. Howard, N. Loosbrock, D. Dickson, M. P. Murphy, and T. E. Golde, "Amyloid-β immunization effectively reduces amyloid deposition in FcR$\gamma^{-/-}$ knock-out mice," *Journal of Neuroscience*, vol. 23, no. 24, pp. 8532–8538, 2003.

[38] M. Colonna, "DAP12 signaling: from immune cells to bone modeling and brain myelination," *Journal of Clinical Investigation*, vol. 111, no. 3, pp. 313–314, 2003.

[39] A. Mócsai, C. L. Abram, Z. Jakus, Y. Hu, L. L. Lanier, and C. A. Lowell, "Integrin signaling in neutrophils and macrophages uses adaptors containing immunoreceptor tyrosine-based activation motifs," *Nature Immunology*, vol. 7, no. 12, pp. 1326–1333, 2006.

[40] H. Cao, U. Lakner, B. de Bono, J. A. Traherne, J. Trowsdale, and A. D. Barrow, "SIGLEC16 encodes a DAP12-associated receptor expressed in macrophages that evolved from its inhibitory counterpart SIGLEC11 and has functional and non-functional alleles in humans," *European Journal of Immunology*, vol. 38, no. 8, pp. 2303–2315, 2008.

[41] C. D. Schmid, L. N. Sautkulis, P. E. Danielson et al., "Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult murine microglia," *Journal of Neurochemistry*, vol. 83, no. 6, pp. 1309–1320, 2002.

[42] S. Frank, G. J. Burbach, M. Bonin et al., "TREM2 is upregulated in amyloid plaque-associated microglia in aged APP23 transgenic mice," *GLIA*, vol. 56, no. 13, pp. 1438–1447, 2008.

[43] K. Takahashi, M. Prinz, M. Stagi, O. Chechneva, and H. Neumann, "TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis," *PLoS Medicine*, vol. 4, no. 4, pp. 675–689, 2007.

[44] E. Chouery, V. Delague, A. Bergougnoux, S. Koussa, J. L. Serre, and A. Megarbane, "Mutations in TREM2 lead to pure early-onset dementia without bone cysts," *Human Mutation*, vol. 29, no. 9, pp. E194–E204, 2008.

[45] J. Paloneva, M. Kestilä, J. Wu et al., "Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts," *Nature Genetics*, vol. 25, no. 3, pp. 357–361, 2000.

[46] L. L. Lanier and A. B. H. Bakker, "The ITAM-bearing transmembrane adaptor DAP12 in lymphoid and myeloid cell function," *Immunology Today*, vol. 21, no. 12, pp. 611–614, 2000.

[47] A. Bouchon, C. Hernández-Munain, M. Cella, and M. Colonna, "A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells," *Journal of Experimental Medicine*, vol. 194, no. 8, pp. 1111–1122, 2001.

[48] J. Lu, X. Wu, and B. K. Teh, "The regulatory roles of C1q," *Immunobiology*, vol. 212, no. 4-5, pp. 245–252, 2007.

[49] V. Lauvrak, O. H. Brekke, ∅. Ihle, and B. H. Lindqvist, "Identification and characterisation of C1q-binding phage displayed peptides," *Biological Chemistry*, vol. 378, no. 12, pp. 1509–1519, 1997.

[50] S. Wakselman, C. Béchade, A. Roumier, D. Bernard, A. Triller, and A. Bessis, "Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor," *Journal of Neuroscience*, vol. 28, no. 32, pp. 8138–8143, 2008.

[51] M. Maier, Y. Peng, L. Jiang, T. J. Seabrook, M. C. Carroll, and C. A. Lemere, "Complement C3 deficiency leads to accelerated amyloid β plaque deposition and neurodegeneration and modulation of the microglia/macrophage phenotype in amyloid precursor protein transgenic mice," *Journal of Neuroscience*, vol. 28, no. 25, pp. 6333–6341, 2008.

[52] K. M. Lucin and T. Wyss-Coray, "Immune activation in brain aging and neurodegeneration: too much or too little?" *Neuron*, vol. 64, no. 1, pp. 110–122, 2009.

[53] J. Reichwald, S. Danner, K.-H. Wiederhold, and M. Staufenbiel, "Expression of complement system components during aging and amyloid deposition in APP transgenic mice," *Journal of Neuroinflammation*, vol. 6, Article ID 35, 2009.

[54] P. Eikelenboom, C. E. Hack, J. M. Rozemuller, and F. C. Stam, "Complement activation in amyloid plaques in Alzheimer's

dementia," *Virchows Archiv Abteilung B Cell Pathology*, vol. 56, no. 4, pp. 259–262, 1989.

[55] P. L. McGeer, H. Akiyama, S. Itagaki, and E. G. McGeer, "Activation of the classical complement pathway in brain tissue of Alzheimer patients," *Neuroscience Letters*, vol. 107, no. 1–3, pp. 341–346, 1989.

[56] S. Webster, L.-F. Lue, L. Brachova et al., "Molecular and cellular characterization of the membrane attack complex, C5b-9, in Alzheimer's disease," *Neurobiology of Aging*, vol. 18, no. 4, pp. 415–421, 1997.

[57] T. Wyss-Coray, F. Yan, A. H.-T. Lin et al., "Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 16, pp. 10837–10842, 2002.

[58] A. Varki and T. Angata, "Siglecs—the major subfamily of I-type lectins," *Glycobiology*, vol. 16, no. 1, 2006.

[59] H. Cao, B. De Bono, K. Belov, E. S. Wong, J. Trowsdale, and A. D. Barrow, "Comparative genomics indicates the mammalian CD33rSiglec locus evolved by an ancient large-scale inverse duplication and suggests all Siglecs share a common ancestral region," *Immunogenetics*, vol. 61, no. 5, pp. 401–417, 2009.

[60] T. Angata, T. Hayakawa, M. Yamanaka, A. Varki, and M. Nakamura, "Discovery of Siglec-14, a novel sialic acid receptor undergoing concerted evolution with Siglec-5 in primates," *FASEB Journal*, vol. 20, no. 12, pp. 1964–1973, 2006.

[61] T. Angata, Y. Tabuchi, K. Nakamura, and M. Nakamura, "Siglec-15: an immune system Siglec conserved throughout vertebrate evolution," *Glycobiology*, vol. 17, no. 8, pp. 838–846, 2007.

[62] A. Varki, "Natural ligands for CD33-related Siglecs?" *Glycobiology*, vol. 19, no. 8, pp. 810–812, 2009.

[63] P. R. Crocker, "Siglecs in innate immunity," *Current Opinion in Pharmacology*, vol. 5, no. 4, pp. 431–437, 2005.

[64] A. F. Carlin, S. Uchiyama, Y.-C. Chang, A. L. Lewis, V. Nizet, and A. Varki, "Molecular mimicry of host sialylated glycans allows a bacterial pathogen to engage neutrophil Siglec-9 and dampen the innate immune response," *Blood*, vol. 113, no. 14, pp. 3333–3336, 2009.

[65] J. Zhao, D. M. Brooks, and D. I. Lurie, "Lipopolysaccharide-activated SHP-1-deficient motheaten microglia release increased nitric oxide, TNF-α, and IL-1β," *GLIA*, vol. 53, no. 3, pp. 304–312, 2006.

[66] Y. Wang and H. Neumann, "Alleviation of neurotoxicity by microglial human Siglec-11," *Journal of Neuroscience*, vol. 30, no. 9, pp. 3482–3488, 2010.

[67] E. Nutku, H. Aizawa, S. A. Hudson, and B. S. Bochner, "Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis," *Blood*, vol. 101, no. 12, pp. 5014–5020, 2003.

[68] S. Von Gunten, S. Yousefi, M. Seitz et al., "Siglec-9 transduces apoptotic and nonapoptotic death signals into neutrophils depending on the proinflammatory cytokine environment," *Blood*, vol. 106, no. 4, pp. 1423–1431, 2005.

[69] F. Lajaunias, J.-M. Dayer, and C. Chizzolini, "Constitutive repressor activity of CD33 on human monocytes requires sialic acid recognition and phosphoinositide 3-kinase-mediated intracellular signaling," *European Journal of Immunology*, vol. 35, no. 1, pp. 243–251, 2005.

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Research Article

Effects of Immunomodulatory Substances on Phagocytosis of A$\beta_{1–42}$ by Human Microglia

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Glial activation and increased inflammation characterize neuropathology in Alzheimer's disease (AD). The aim was to develop a model for studying phagocytosis of β-amyloid (Aβ) peptide by human microglia and to test effects thereupon by immunomodulatory substances. Human CHME3 microglia showed intracellular A$\beta_{1–42}$ colocalized with lysosome-associated membrane protein-2, indicating phagocytosis. This was increased by interferon-γ, and to a lesser degree with Protollin, a proteosome-based adjuvant. Secretion of brain-derived neurotrophic factor (BDNF) was decreased by A$\beta_{1–42}$ and by interferon-γ and interleukin-1β. These cytokines, but not A$\beta_{1–42}$, stimulated interleukin-6 release. Microglia which phagocytosed A$\beta_{1–42}$ exhibited a higher degree of expression of interleukin-1 receptor type I and inducible nitric oxide synthase. In conclusion, we show that human microglia are able to phagocytose A$\beta_{1–42}$ and that this is associated with expression of inflammatory markers. A$\beta_{1–42}$ and interferon-γ decreased BDNF secretion suggesting a new neuropathological role for A$\beta_{1–42}$ and the inflammation accompanying AD.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia. The major pathological hallmarks of AD besides neuronal loss are amyloid plaques and neurofibrillary tangles (NFTs). Both amyloid plaques and NFTs have been implicated in neuronal impairment and death in a large number of studies. Although a great deal of controversy exists about the relative importance of amyloid plaques *contra* NFTs in AD [1], there is an overwhelming body of evidence showing that amyloid plaques and the peptides they are composed of are culprits in the neurodegenerative processes in AD [2]. The main component of amyloid plaques is the β-amyloid (Aβ) peptide, that is secreted by neurons and other cells through cleavage of the larger, membrane-bound, amyloid precursor protein (APP). APP can be processed by two major pathways: the amyloidogenic pathway that yields Aβ, and the nonamyloidogenic yielding fragments believed to be nonpathogenic. The Aβ peptides are prone to self-aggregation and deposition into insoluble plaques. This is especially true for the 42 amino acid form (A$\beta_{1–42}$), which is the predominant form in the dense core plaques [3]. Aβ-species also exist in the forms of soluble monomers and oligomers which similarly to the amyloid plaques are more abundant in the AD brain than in the non-AD brain [4]. Somehow, the balance between production and clearance/degradation of Aβ is disturbed in AD.

Damage to brain tissue induces an inflammation, a response which is present in many, if not all neurodegenerative conditions. In the central nervous system (CNS), glial cells, that is, microglia and astrocytes, represent the main source of inflammatory reactions. In normal conditions, glia have supportive functions, including maintenance of ionic homeostasis, clearance of neurotransmitters, and in the case of astrocytes, providing nutrients to the energy-demanding work of the neurons [5]. Glial, particularly microglial, cell responses can also serve in the elimination of debris from damaged cells and to remove pathogens. Removal of pathogens is executed by the process of phagocytosis, a

capability that glial cells share with peripheral immunocompetent cells including monocytes and macrophages. In the brain, phagocytosis is believed to be performed mainly by microglia, but astrocytes also have this capability [6].

Under stress, glial cells proliferate and become activated, which leads to production of neurotoxic molecules such as free radical species and proinflammatory cytokines [7, 8]. In contrast to the potentially tissue-damaging responses, glia can also produce factors that promote neuroprotection and neuronal plasticity [9]. These protective and supportive functions may be downregulated during stress. Therefore, inflammation in the brain may worsen the outcome of an already existing trauma or pathological condition.

In the AD brain, activated microglia and astrocytes are present in the areas of neurodegeneration and amyloid plaques [10] and there is an increased production of proinflammatory cytokines such as interleukin- (IL)-1 [11] and IL-6 [12]. In addition, elevated levels of IL-1 and IL-6 have been found in serum and cerebrospinal fluid (CSF) from AD patients [13, 14]. In vitro studies have demonstrated that Aβ peptides can indeed activate glial cells to produce inflammatory factors [15–18], which can contribute to the neurodegenerative process [19]. Evidence from studies on rat cortical microglia showed that the smaller aggregational forms of Aβsuch as oligomers are more potent in stimulating glial secretion of proinflammatory cytokines [18]. IL-1 increases the production of APP in human glia [20], and the APP gene contains a binding site for the prime inflammatory transcription factor nuclear factor κB (NFκB) [21]. Furthermore, inflammation may shift processing of APP towards the amyloidogenic pathway [22]. A consequence of the interactions between inflammation and the APP/Aβ-peptide may be a vicious circle [23], in which inflammation increases Aβ levels through increased production and reduced clearance, which in turn results in neuronal cell death and a perpetuated and increased glial activation and release of proinflammatory factors, as well as neuronal cell death, and so forth.

Removal of a disease-causing pathogen is probably the most effective way of treating a disease. To stimulate cellular uptake, phagocytosis of Aβ is a promising strategy. In studies on animal models of AD, active and passive immunizations have been shown to be effective in removing plaques and to improve cognitive performance [24]. Human clinical trials with active immunization have been started but were aborted due to serious side-effects in a few cases [25]. However, several clinical trials with modified protocols, including passive immunizations, are currently being carried out. Phagocytosis is an activity that is performed primarily by cells of the immune system, notably cells of the monocyte lineage. In the light of the studies on active and passive immunization we are presented with a strategy for treatment of AD, to activate the immune system into phagocytosis of Aβ. Although inflammation generally is believed to stimulate phagocytic activities, there is also evidence indicating that inflammation may inhibit phagocytosis [26], whereas anti-inflammatory cytokines such as transforming growth factor-β (TGF-β) can stimulate phagocytosis [27]. Thus, it is of importance to search for compounds that can promote phagocytosis without starting the damaging processes of inflammation. In short, directed and differential activation of immune cells residing in, or destined for, the CNS constitutes a potential therapeutic target.

In the present study, the aim was to characterise the responses of human microglia to the exposure of Aβ. In order to investigate the possibilities to increase glial uptake of Aβ we have analysed the effects of different immunomodulatory substances. The effects of the adjuvant Protollin, as well as those of the archetypical proinflammatory cytokines, IL-1β and interferon-γ (IFNγ), were analysed in an in vitro model of human microglial cells with regard to A$\beta_{1–42}$ uptake, microglial phenotype, and the secretion of IL-6 and brain-derived neurotrophic factor (BDNF).

IL-6 is a cytokine that is induced by IL-1β and tumour necrosis factor-α (TNFα) and can thus be considered a general measure of inflammation [28]. BDNF is a neurotrophic growth factor of importance in memory formation and neuroprotection [29, 30]. Protollin is an adjuvant made of *Shigella flexneri* 2a lipopolysaccharides (LPS) associated noncovalently to meningococcal outer membrane proteins (proteosomes), that has been proven to be safe for use in humans [31]. While LPS activates Toll-like receptor type 4 (TLR4), cd11, and cd14, proteosomes activate TLR2 [32]. Activation of TLR2 has been associated with increased phagocytosis in mice with sciatic nerve lesions [33]. Furthermore, intranasal application of Protollin was shown to prevent accumulation of Aβ in young transgenic mice expressing the human APP with the Swedish mutation and also to stimulate clearance of Aβ from the brain of aged mice of the same transgenic strain [34]. A correlation was found between the removal of Aβ and the level of microglial activation, as demonstrated by increased expression of the activation marker CD11b in conjunction with the removal of Aβ in animals treated with Protollin [34]. To investigate the effects of Protollin on cellular inflammatory markers, and the association of these markers with uptake of A$\beta_{1–42}$ by the human microglial cells, the expression of inducible nitric oxide synthase (iNOS), IL-1β, and the signalling type I receptor for IL-1β (IL-1RI) was analyzed in cells showing uptake of A$\beta_{1–42}$. iNOS is induced in inflammation and shown to be harmful for neurons due to the production of radical nitrogen species [35]. It has been shown to be associated with neurodegenerative disorders such as AD [36].

2. Materials and Methods

2.1. Chemicals. Protollin was provided by Glaxo-Smith Kline Biologicals, Laval, Quebec, Canada. A$\beta_{1–42}$ conjugated with HiLyteFluor488 or biotin was obtained from Anaspec (Fremont, USA). Dimethylsulfoxide (DMSO), Triton-X100, bovine serum albumin (BSA), 4′,6-diamidino-2-phenylindole (DAPI), and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma, Stockholm, Sweden. Normal donkey and goat serum and fluorescence mounting medium were from DakoPatts (Stockholm, Sweden). Streptavidin-7-amino-4-methyl-3-coumarinylacetic acid (AMCA) is from Jackson ImmunoResearch Europe Ltd (Suffolk, UK). Lactate deydrogenase (LDH) assay is from Roche (Stockholm, Sweden).

ELISA-kits for IL-6 and BDNF are from R&D systems (Abingdon, United Kingdom). IFNγ (Bachem, Weil am Rhein, Germany). Cell culture medium, phosphate-buffered saline (PBS), GlutaMaxII, foetal calf serum (FCS) and PBS-based enzyme-free cell dissociation buffer (Invitrogen, Stockholm, Sweden). Cell culture bottles and multiwell plates (BD Biosciences, Stockholm, Sweden).

2.2. Cell Cultures. Human microglial cells (CHME3) were obtained as a kind gift from Professor M. Tardieu (Neurologie pédiatrique, Hôpital Bicêtre, Assistance publique, Hôpitaux de Paris, Paris, France). CHME3 cells were cultured in T75 or T175 bottles in culture medium (DMEM/high glucose w/o sodium pyruvate supplemented with 2 mM L-glutamine or GlutaMaxII and 10% heat-inactivated FCS). The cells were subcultured at confluence using enzyme-free cell dissociation buffer after washing once with PBS without Mg^{2+} and Ca^{2+}.

2.3. Experimental Procedures. The CHME3 microglial cells were seeded in 48-well plates for analysis of cell viability (MTT) and cytotoxicity (LDH), in 6-well plates for flow-cytometry and analysis of substances released to the medium, or on glass coverslips in 24- or 48-well plates for immunocytochemistry. All experiments were performed at a confluence of ~60%. A$\beta_{1–42}$ was dissolved in DMSO and stored in darkness at +4°C until use at a final concentration of 1 μg/mL for all experiments except for a dose-response curve. In higher doses we observed aggregate-like precipitates of fluorescent A$\beta_{1–42}$ which were unwanted in this study. Before the addition of A$\beta_{1–42}$ or vehicle (DMSO), the cells were prestimulated for 24 hours with either Protollin (0.001, 0.01 and 0.1 μg/mL in serum-free culture medium) or cytokines (50 ng/mL IL-1β, 50 ng/mL IFNγ, or IL-1β + IFNγ). At 0, 24, 48, 72, and 96 hours after addition of A$\beta_{1–42}$ the cultures were analyzed for uptake of A$\beta_{1–42}$, expression of cellular markers, and secretory products. Uptake of fluorescent A$\beta_{1–42}$ by living cells was analysed in a Nikon TE600-inverted fluorescence microscope. Cell viability and cell death were also analysed.

2.4. Quantification of A$\beta_{1–42}$ Phagocytosis and Cellular Markers by Flow-Cytometry. After the experimental treatment, the CHME3 microglial cells were dissociated with PBS-based enzyme-free dissociation buffer as described above, and centrifuged at 1500 ×g for 10 minutes. The cells were then resuspended and fixed in 1% para-formaldehyde (PF) in PBS, for 40 minutes at room temperature, after which the fixative was diluted 20× by addition of PBS and the cells were centrifuged at 1500 ×g for 10 minutes. Fixation with PF renders cells permeable to PI and therefore all fixed cells will be stained with PI, allowing the distinction of cells from cell debris. Analysis was performed in a FACScalibur (BD) flow-cytometer. A detected event was defined as a cell if it was gated through the front-scatter (FSC) and side-scatter (SSC) plot gate in addition to being positive for PI. A cell positive for phagocytosis of A$\beta_{1–42}$ (A$\beta_{1–42}$+) was defined as a PI-positive cell that was also being positive for the fluorophore conjugated to A$\beta_{1–42}$ (HyliteFluor488). Negative controls were utilized to establish the limits of detection for positive signals.

To investigate the phenotype of the cells with regard to the presence of inflammatory markers, and the degree of colocalization of each marker with phagocytosed A$\beta_{1–42}$, the cells were stained with antibodies directed against human IL-1β (1 : 400; gift from Dr. Stefan Svensson, Statens Bakteriologiska Laboratorium, Stockholm, Sweden), IL-1RI (1 : 200; Amgen (Immunex Corporation) Thousand Oaks, USA), and iNOS (1 : 600; R&D systems, London, England). The cells were harvested and fixed as described above and subsequently an aliquot of the cell suspension was incubated with the primary antibodies, diluted in PBS containing 5% normal donkey serum and 0.1% Triton-X100. Omission of primary antibodies served as negative control to establish the limits of detection for positive signals. After incubation with primary antibodies overnight at +4°C, the cell suspension was washed by addition of PBS followed by centrifugation at 2500 ×g for 20 minutes. The cells were resuspended in PBS and incubated with donkey antigoat IgG-NL637 antibodies (1 : 500; R&D systems, London, England) and PI for 1 hour at room temperature. After incubation, the cell suspension was diluted with PBS and analyzed by flow-cytometry. A cell displaying immunoreactivity for an antibody was defined as a cell showing a stronger signal in this channel than the negative control. A cell displaying uptake of A$\beta_{1–42}$ (A$\beta_{1–42}$+) was defined as described above. The results were analysed from a scatter plot with fluorescence from the secondary antibody on the y-axis and fluorescence from HyliteFluor488 on the x-axis, divided into quadrants with borders based on the negative controls as described. The cells were thus being viewed in a two-way binary fashion with a cell being present in a certain quadrant thus indicating (a) positive for immunoreactivity to IL-1β(IL-1β+), IL-1RI (IL-1RI+), or iNOS (iNOS+) and for A$\beta_{1–42}$-uptake (A$\beta_{1–42}$+), (b) positive for immunoreactivity to one of these markers and negative for A$\beta_{1–42}$-uptake, (c) negative for immunoreactivity to the markers and positive for A$\beta_{1–42}$-uptake, or (d) negative for immunoreactivity to the markers and for A$\beta_{1–42}$-uptake. The parameters extracted from the data were (a) total proportion of the cells showing immunoreactivity to a marker, (b) immunoreactivity of cells negative for A$\beta_{1–42}$-uptake, and (c) immunoreactivity of cells positive for A$\beta_{1–42}$-uptake.

2.5. Immunocytochemistry and Staining of Fixed and Living Cells for Microscopy. To analyse the microglial cell expression of certain inflammatory markers and the localization of phagocytosed A$\beta_{1–42}$ by microscopy the culture medium was removed and the coverslips dried at 37°C for ~2 hours. The cells were fixed with 4% PF for 20 minutes at room temperature, washed in PBS, and incubated overnight at 4°C with antibodies against IL-1β (1 : 400), IL-1RI (1 : 200), and iNOS (1 : 600), respectively. The antibodies were diluted in PBS containing 5% normal donkey serum and 0.1% Triton-X100. After rinsing in PBS, the coverslips were incubated for 1 hour at room temperature with goat antirabbit-IgG conjugated with Cy2 (1 : 200), diluted in

PBS containing 5% normal goat serum, 0.1% Triton-X100, and 1 μg/mL PI. For visualization of biotinylated A$\beta_{1–42}$, streptavidin-AMCA was included in the secondary antibody solution (2 μg/mL). The coverslips were then washed with PBS, mounted with fluorescence mounting medium, and inspected in a Nikon E800 microscope. To investigate the targeting of A$\beta_{1–42}$ toward degradation, cells were incubated with HyliteFluor488-conjugated A$\beta_{1–42}$ and then fixed and incubated overnight at 4°C with mouse antibodies against human lysosome-associated membrane protein-2 (lamp-2, Millipore). After washing, the cells were incubated with donkey antimouse antibodies conjugated with Cy3 and with the nuclear stain DAPI and then mounted and inspected as described above.

2.6. Enzyme-Linked Immunosorbent Assay (ELISA). The levels of IL-6 and BDNF in the cell culture medium were analyzed with commercially available ELISA-kits according to the manufacturer's instructions. Analysis of optical density (OD) was performed in a TECAN Safire2 plate reader.

2.7. Cell Viability—MTT Assay. To analyse cell viability the culture medium was removed and after washing with serum- and phenol-free DMEM/high glucose with GlutaMaxII, MTT (0.3 mg/mL) was added and the cells incubated at 37°C for 1 hour. The crystals formed by the reaction were dissolved in DMSO and the OD was measured with a TECAN Safire2 plate reader (Tecan, Mölndal, Stockholm) at 592 nm with 620 nm as a reference wavelength.

2.8. Cell Death—LDH Assay. To analyse cell death the culture medium was removed and added to a 96-well plate and then incubated with LDH-reagent according to the manufacturer's instructions. The OD was measured at 492 nm with 620 nm as reference wavelength.

2.9. Statistics. The data were normalized to vehicle (MTT-assay, ELISA) A$\beta_{1–42}$ (flow-cytometry, for uptake) or positive control (LDH-assay), or not normalized (flow-cytometry, for association of cellular markers with the uptake of A$\beta_{1–42}$, correlation between variables). The results were analysed with Kruskal-Wallis nonparametric analysis of variance (ANOVA) and the data were then compared pairwise using the nonparametric Mann-Whitney U test with Bonferoni correction. Pairwise comparison of variable within experimental groups was performed with the Wilcoxon Matched Pairs Test. Correlations between variables were analysed with the Spearman's Rank Order Correlation test. All statistical analyses were performed in Statistica v8 (Statsoft).

3. Results

Human CHME3 microglial cells exposed to immunomodulatory substances were studied with regard to phagocytosis of A$\beta_{1–42}$ and expression of cellular markers (IL-1β, IL-1RI, and iNOS). As an indicator of an inflammatory response we analysed the secretion of IL-6 in the culture medium. To assess beneficial and neuroprotective activities the secretion of BDNF was determined. The levels of BDNF released into the medium varied between 8 and 300 pg/mL and the levels of IL-6 varied between 20 and 950 pg/mL under basal conditions (vehicle group at 24 h). Cell death and viability were also analysed.

The ability of CHME3 microglia to take up A$\beta_{1–42}$ was confirmed by inspection of living cells using phase contrast/fluorescence microscopy (Figure 1(a)), and fluorescence microscopy on the fixed cell suspension used for flow-cytometry (Figure 1(b)), and by confocal microscopy on fixed and living cells (Figure 2). The first signs of uptake (visible in inverted fluorescence microscope) by the microglial cells were observed approximately 4 hours after the addition of A$\beta_{1–42}$. Analysis of the intracellular content of A$\beta_{1–42}$ was performed by flow-cytometry at 24 and 96 hours after addition of different concentrations of A$\beta_{1–42}$ (Figure 3(a)). The uptake increased with increasing concentrations of A$\beta_{1–42}$. A 50-fold increase in the concentration of A$\beta_{1–42}$ from 0.1 to 5 μg/mL resulted in a 10-fold increase in the fraction of cells taking up A$\beta_{1–42}$ ($P < .05$).

3.1. Effects of A$\beta_{1–42}$ on Secretion of BDNF and IL-6. In dose-response experiments with 0.1, 0.5, 1, and 5 μg/mL of A$\beta_{1–42}$, we found that 5 μg/mL reduced the levels of BDNF in the culture medium by 50% compared to vehicle ($P < .01$, Figure 3(b)). A similar degree of decrease was seen for 5 μg/mL A$\beta_{1–42}$ when compared with the other concentrations of A$\beta_{1–42}$ ($P < .01$). At 96 hours, the concentrations of 0.1 and 0.5 μg/mL A$\beta_{1–42}$ slightly increased the secretion of BDNF ($P < .05$). No effect was seen on the secretion of IL-6 with the concentrations of A$\beta_{1–42}$ tested (Figure 3(b)).

3.2. Effects of IL-1β and IFNγ on Uptake of A$\beta_{1–42}$. To evaluate the potential of stimulating CHME3 microglia into phagocytosis by inflammation, the cells were incubated with the proinflammatory cytokines IL-1β and IFNγ. Pretreatment with IFNγ resulted in an increase in the proportion of cells showing A$\beta_{1–42}$ uptake (A$\beta_{1–42}$+ cells) with a median increase of 50% at 72 hours ($P < .01$, Figure 4, grey boxes), and the combined stimulation with IL-1β and IFNγ lead to a median increase of 66% at 72 hours ($P < .01$) as compared to controls (with A$\beta_{1–42}$ alone) and a 62% increase compared to IL-1β ($P = .05$). IL-1β alone had no significant effect on the A$\beta_{1–42}$ uptake. The observed effects appeared to linger at 96 hours although the differences were not significant.

3.3. Effects of IL-1β and IFNγ on Secretion of BDNF and IL-6. The levels of secreted BDNF were markedly reduced by the incubation with the proinflammatory cytokines IL-1β and IFNγ (Figure 5(a), white boxes). Thus, IFNγ decreased the median secretion of BDNF as compared to vehicle with 41% at 72 hours and with 44% at 96 hours ($P < .05$ at both time points). There was no effect of IL-1β alone, but the combination of IL-1β and IFNγ resulted in a significant, almost 50%, decrease in BDNF secretion at 72 and 96 hours ($P < .05$ at both time points) (Figure 5(a), white boxes).

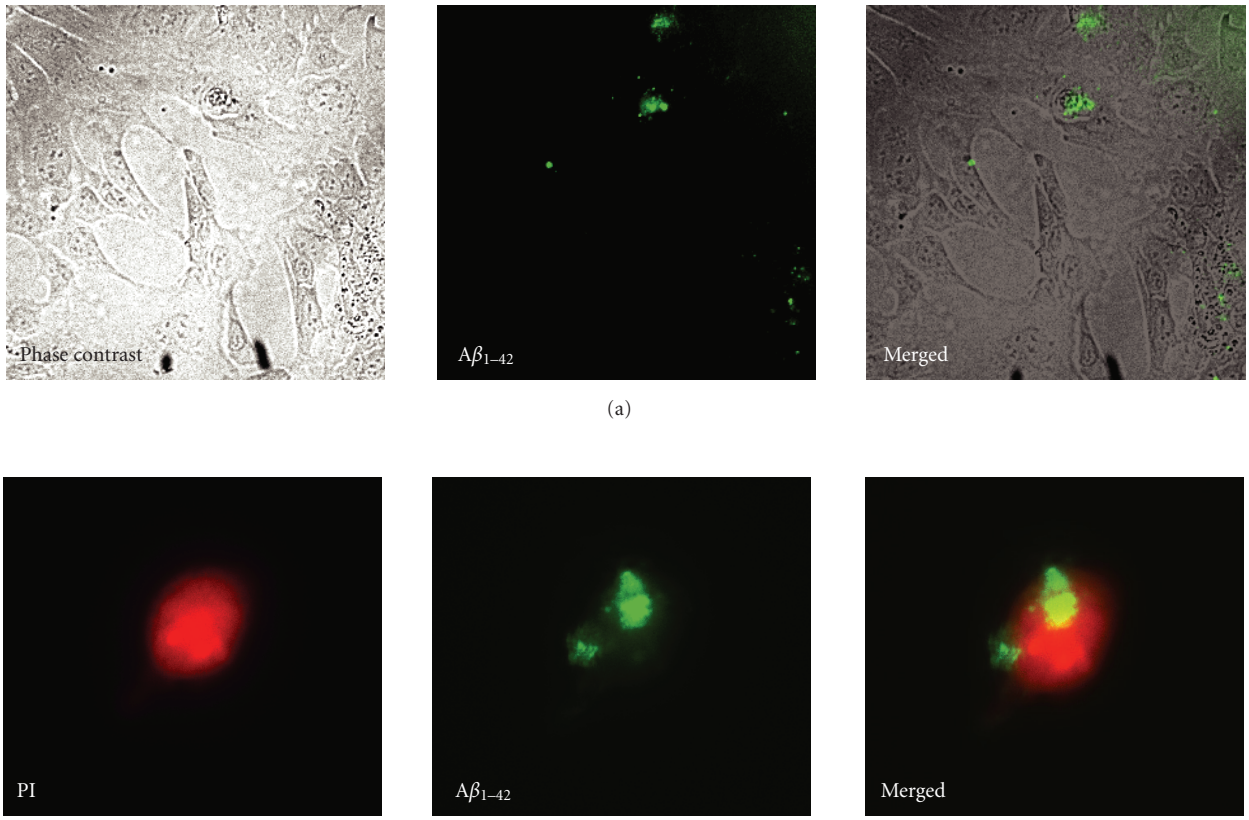


Figure 1: (a)-(b) Uptake of $A\beta_{1–42}$ in human microglial cells. The micrographs in (a) show living human CHME3 microglial cells in culture after incubation with $A\beta_{1–42}$, seen by phase contrast and fluorescence microscopy, and after merging of these. The micrographs in (b) show fixed CHME3 microglial cells in suspension after incubation with $A\beta_{1–42}$, seen by fluorescence microscopy with filters for propidium iodide (PI) staining and HiLyte488-conjugated $A\beta_{1–42}$, respectively, and the micrographs are merged in the 3rd micrograph. Magnifications 10× (a) and 40× (b).

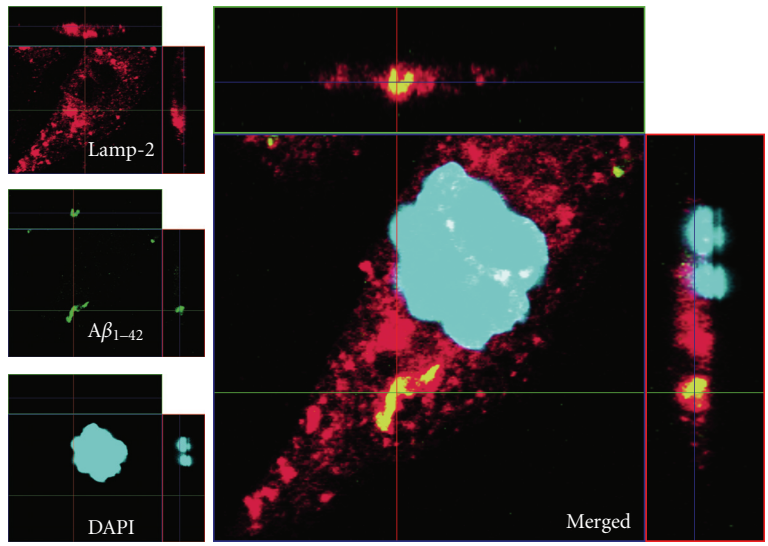
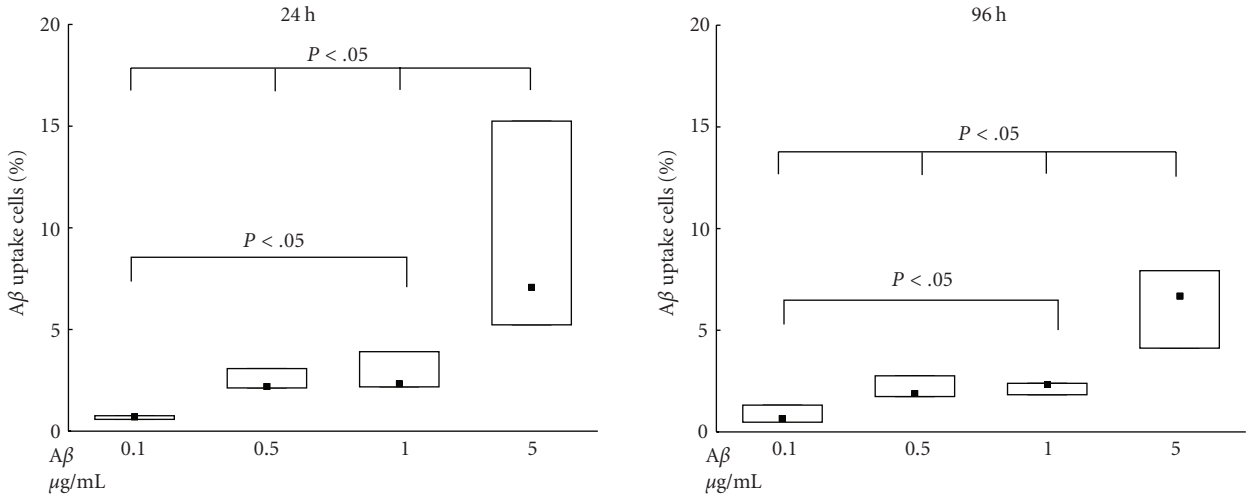


Figure 2: Uptake of $A\beta_{1–42}$ in human microglial cells. The confocal micrograph (63×) shows fixed CHME3 microglial cells demonstrating intracellular lysosomal location of HiLyte488-conjugated $A\beta_{1–42}$ (green filter). Lysosomes were visualized by staining with an antibody against lysosome-associated membrane protein-2 (lamp-2) and Cy3-conjugated secondary antibodies (red filter). Yellow colour thus indicates colocalization of lamp-2 and HiLyte488-conjugated $A\beta_{1–42}$.



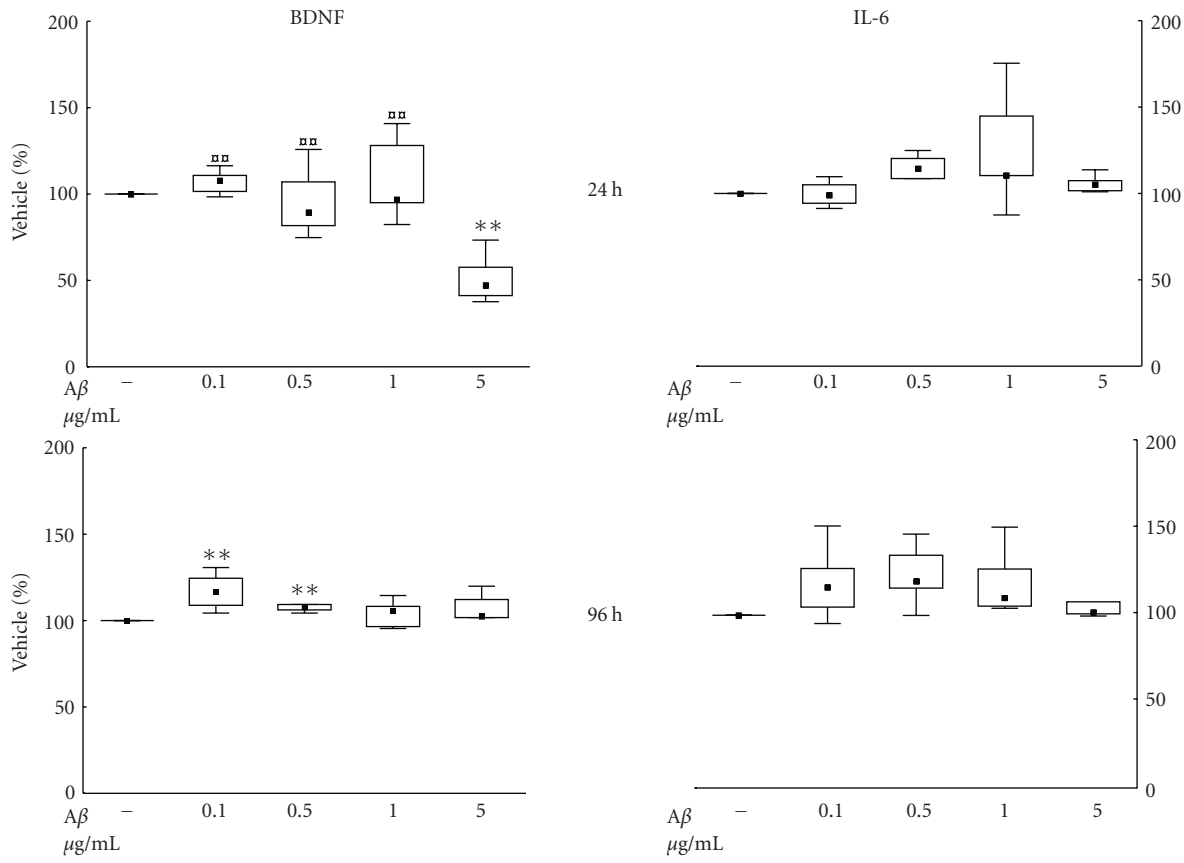


Figure 3: (a)-(b) Uptake of $A\beta_{1–42}$ (a) and the concurrent secretion of interleukin-6 (IL-6) and brain-derived neurotrophic factor (BDNF) (b) by human CHME3 microglial cells at 24 and 96 hours. The cells were incubated with concentrations of $A\beta_{1–42}$, ranging between 0.1 and 5 μg/mL, or vehicle. The cells were harvested and the medium collected at 24 and 96 hours. In (a), the data are expressed as the proportion of cells with intracellular $A\beta_{1–42}$, $n = 4$. In (b), the data are expressed as % of control (vehicle) set at 100% and shown as median ± percentiles (25%–75% and 10%–90%). There was a significant effect of the treatment on the uptake of $A\beta_{1–42}$ at 24 hours ($P = .0045$) and at 96 hours ($P = .0375$). The secretion of BDNF was also significantly altered by the treatment at 24 hours ($P = .0102$) and at 96 hours ($P = .0375$). Statistical difference from $A\beta_{1–42}$, 5 μg/mL, is indicated by ¤¤ ($P < .01$); statistical difference from vehicle is indicated by ** ($P < .01$).

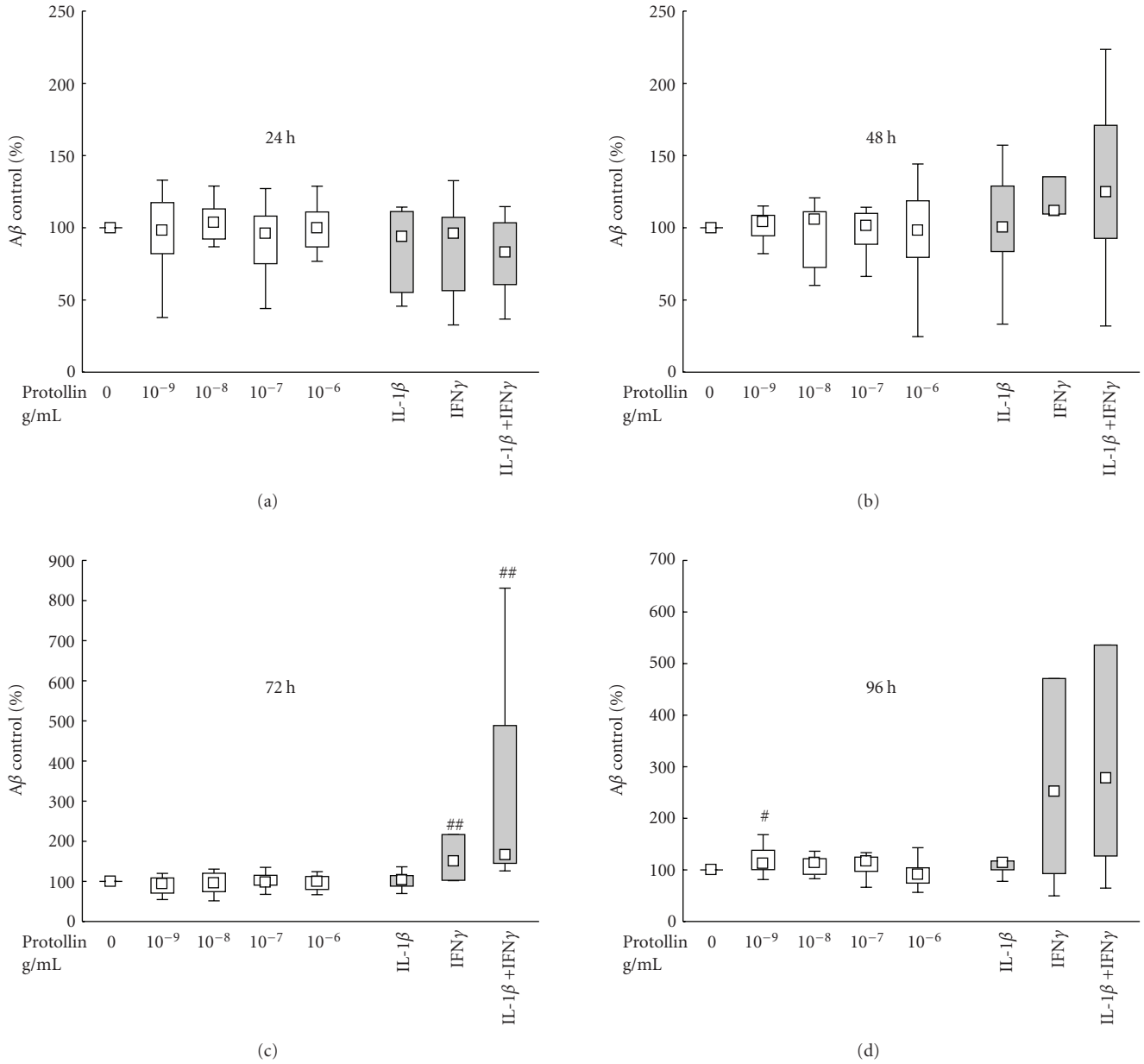


Figure 4: Effects of Protollin (white boxes) and interleukin-1β (IL-1β) and interferon-γ (IFNγ) (grey boxes) on the uptake of A$\beta_{1–42}$ in human CHME3 microglial cells. The cells were incubated with 1 μg/mL A$\beta_{1–42}$ following prestimulation for 24 hours with Protollin at 0.001–1 μg/mL, or 50 ng/mL IL-1β and 50 ng/mL IFNγ. The cells were harvested at 24, 48, 72, and 96 hours after addition of A$\beta_{1–42}$. The data are expressed as % uptake of control A$\beta_{1–42}$ set at 100%, and shown as median ± percentiles (25%–75% and 10%–90%), $n = 19$ (Protollin) or $n = 4$ (cytokines) for 24 and 48 hours, and $n = 17$ (Protollin) of $n = 6$ (cytokines) for 72 and 96 hours. A statistically significant effect of treatment was found by Kruskal-Wallis ANOVA at 96 hours when incubating microglia with Protollin ($P = .0157$) and at 72 hours when incubating with IL-1β and/or IFNγ ($P = .0022$). Statistical difference from control is indicated by $^{\#}(P < .05)$, $^{\#\#}(P < .01)$, and $^{\#\#\#}(P < .005)$ indicates statistical difference ($P < .01$) between IL-1β and IL-1β + IFNγ.

Treatment of the cells with proinflammatory cytokines resulted in a marked inflammatory response evidenced by a dramatic increase in IL-6 secretion (Figure 5(b), white boxes). At 0 hour (i.e., 24 hours after addition of the cytokines and at the time point of addition of A$\beta_{1–42}$, or in this case, vehicle), IL-1β and IL-1β + IFNγ stimulated the IL-6 secretion, producing levels that were 50 and 90 times higher than control (vehicle), respectively ($P < .05$ in both cases (Figure 5(b), white boxes). At 24 hours (i.e., after 48 hours incubation with the cytokines), IL-1β and IL-1β + IFNγ induced a 200- and 140-fold increase in IL-6 secretion, respectively, and the combination of IFNγ and IL-1β resulted in a 25× higher induction than that by IFNγ alone ($P < .05$). The incubation with IL-1β + IFNγ was still effective at 96 hours in increasing secreted levels of IL-6 ($P = .0108$). At this time point, IL-1β alone and IFNγ alone

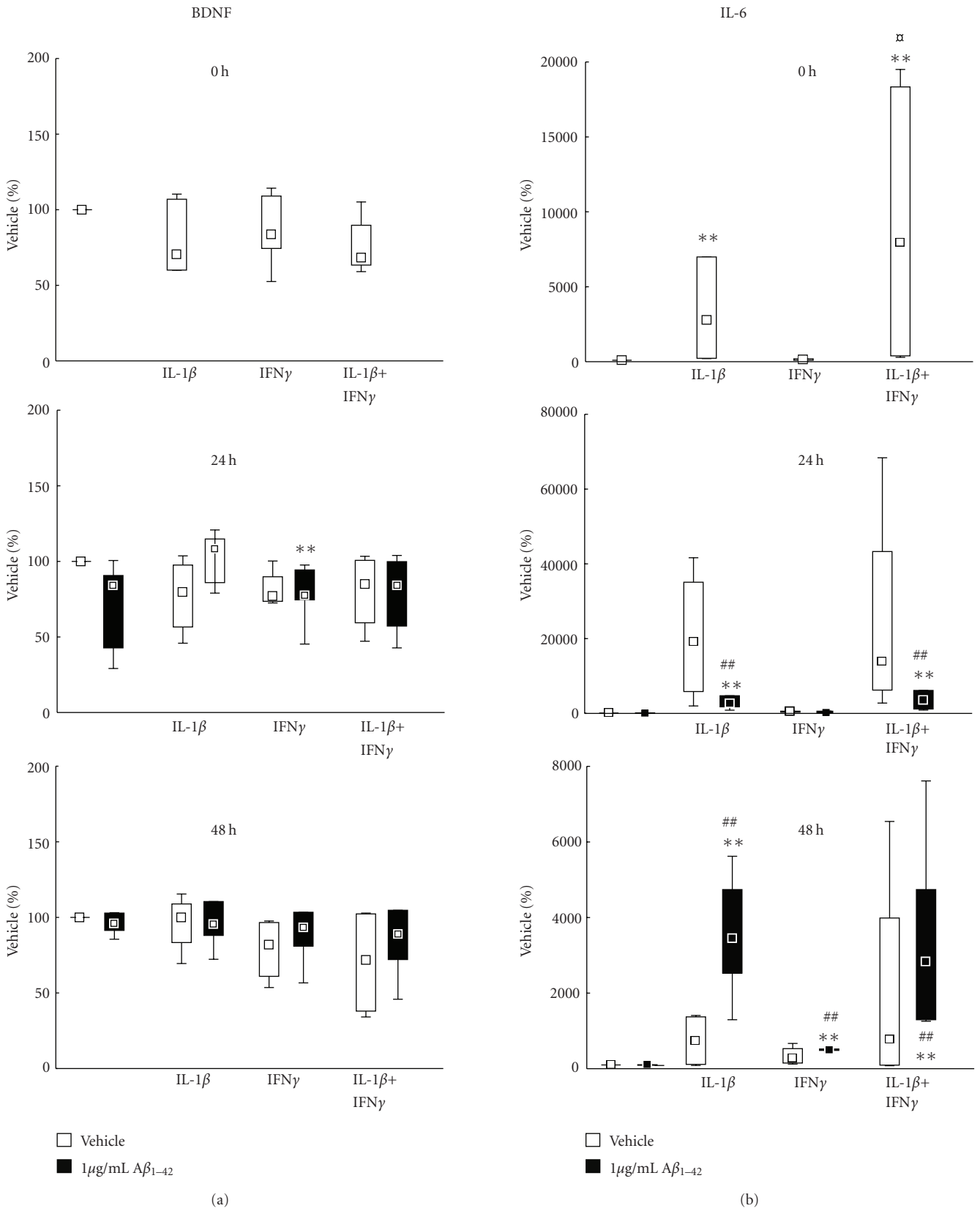


Figure 5: Continued.

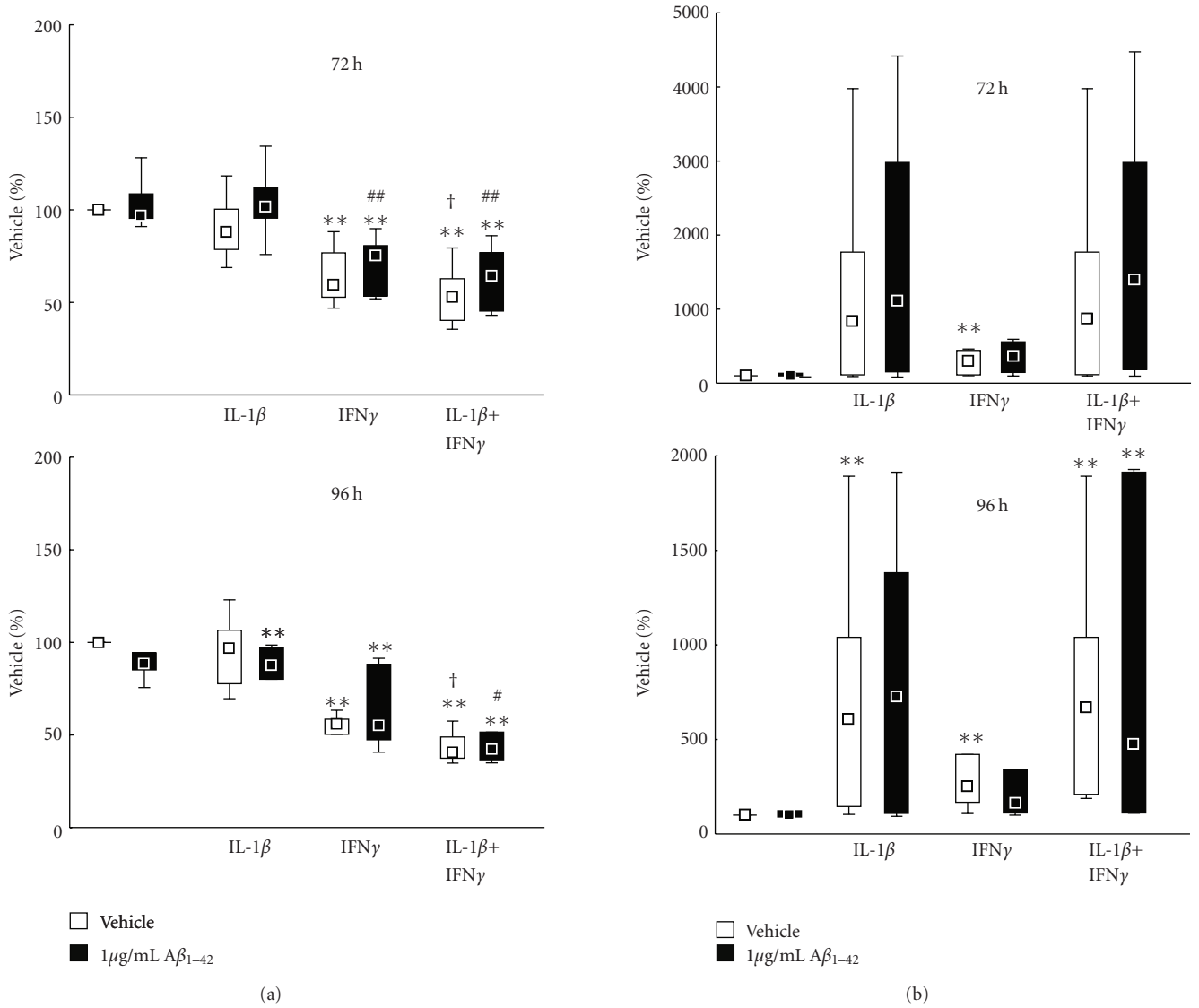


Figure 5: (a)-(b) Effects of incubation with interleukin-1β (IL-1β) and interferon-γ (IFNγ), added 24 hours before incubation with 1 μg/mL A$\beta_{1–42}$ (black boxes) or vehicle (white boxes), on secreted levels of IL-6 (a) and BDNF (b) from CHME3 microglial cells. The cells were incubated with 1 μg/mL HiLyte488-conjugated A$\beta_{1–42}$ following prestimulation for 24 hours with 50 ng/mL IL-1β and 50 ng/mL IFNγ. The cells were harvested at 24, 48, 72, and 96 hours after addition of A$\beta_{1–42}$. The data are expressed as % uptake of control vehicle set at 100% and shown as median ± percentiles (25%–75% and 10%–90%), $n = 4$ for 24 hours and 48 hours and $n = 6$ for 72 and 96 hours. A statistically significant effect of treatment was found by Kruskal-Wallis ANOVA at 24 hours ($P = .0087$), at 72 hours ($P = .004$), and at 96 hours ($P = .004$). Statistical difference from control is indicated by **($P < .01$); difference from A$\beta_{1–42}$ # is indicated by ($P < .05$) and $^{\#\#}$($P < .01$). † indicates statistical difference ($P < .05$) between IL-1β and IL-1β + IFNγ.

significantly increased the levels of IL-6 several-fold ($P < .05$ in both cases).

3.4. Effects of IL-1β and IFNγ in Combination with Aβ$_{1–42}$ on Secretion of BDNF and IL-6. Similarly to the effect seen for cytokines, the combined incubation of cytokines and A$\beta_{1–42}$ resulted in reduced secretion of BDNF (Figure 5(a), black boxes). There was no effect of A$\beta_{1–42}$ alone on secretion of BDNF in this series of experiments. At 24 hours, pretreatment with IFNγ resulted in a median reduction to 77% in BDNF secretion compared to vehicle (100%) ($P = .0086$). At 72 hours, the median reduction was 25% ($P < .01$) and at 96 hours it was 45% ($P < .01$) (Figure 5(a), black boxes). The combination of A$\beta_{1–42}$ with IFNγ pretreatment resulted in significantly lower secretion of BDNF than that observed after treatment with A$\beta_{1–42}$ alone at 72 hours ($P < .01$). Pretreatment with IL-1β decreased the median BDNF secretion to 88% of vehicle at 96 hours ($P < .01$). At 72 hours, pretreatment with IL-1β + IFNγ resulted in a significant decrease in BDNF secretion to 64% of vehicle secretion ($P < .01$) which was also lower than the secretion induced by A$\beta_{1–42}$ alone at this time point (97% of vehicle, $P < .01$). This effect was still present at 96 hours when pretreatment with IL-1β + IFNγ resulted in a decrease in

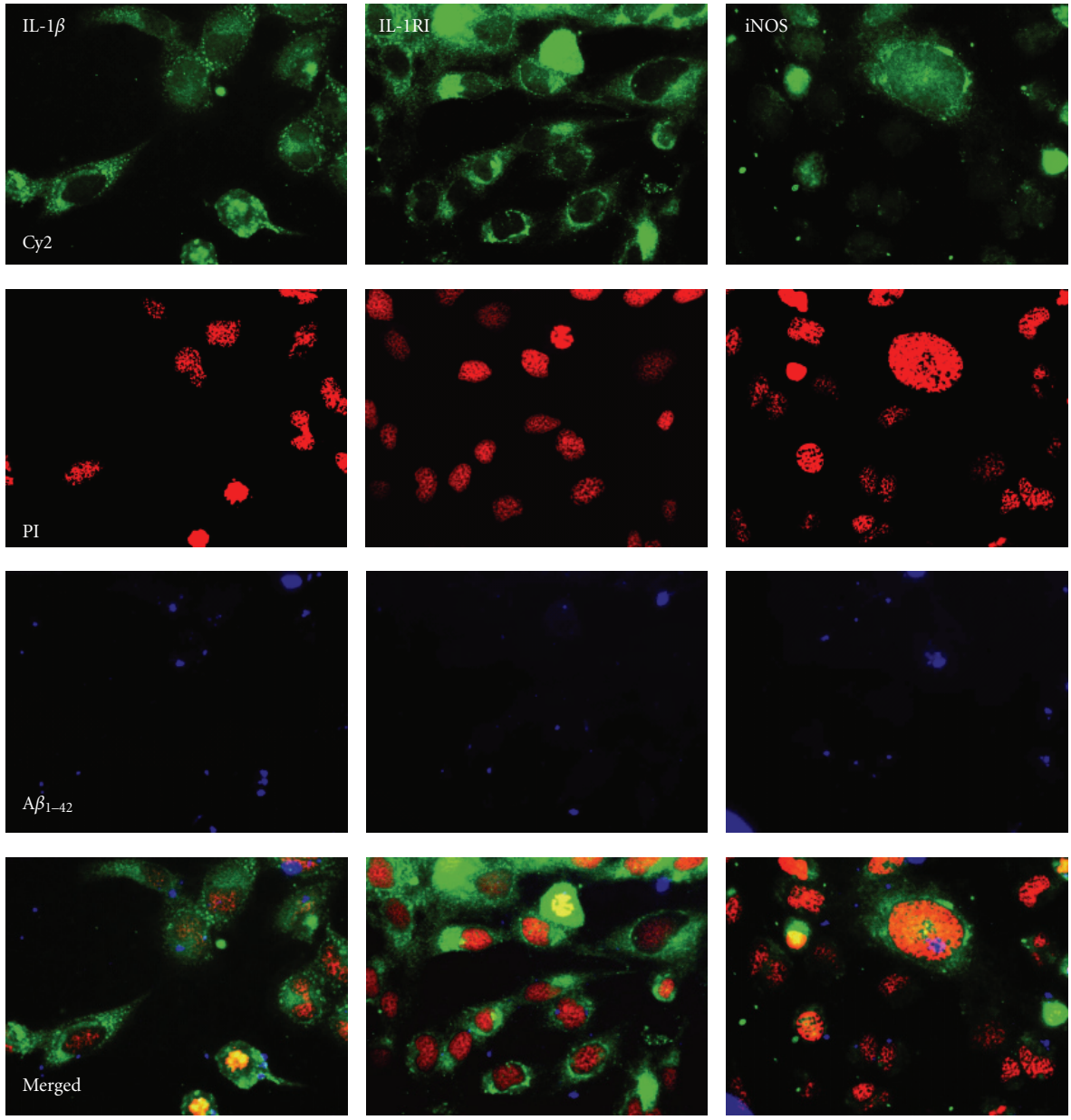


Figure 6: Uptake of $A\beta_{1–42}$ and expression of cellular markers in human microglial cells. The micrographs show human CHME3 microglial cells after incubation with biotinylated $A\beta_{1–42}$, after which they were fixed and stained with antibodies against interleukin-1β (IL-1β), IL-1 receptor type I (IL-1RI) and inducible nitric oxide synthase (iNOS), and subsequent incubation with Cy2-conjugated secondary antibodies. Cell nuclei were stained with propidium iodide (PI) and the biotinylated $A\beta_{1–42}$ was visualized with AMCA-conjugated streptavidin. All micrographs are in 20× magnification.

BDNF level to 43% of vehicle ($P < .01$), which also at this time point was lower than the secretion induced by $A\beta_{1–42}$ alone (88% of vehicle, $P = .017$). The pretreatment with IL-1β + IFNγ also reduced the secretion of BDNF compared with IL-1β at 72 hours ($P < .05$).

Treatment of the cells with cytokines before addition of $A\beta_{1–42}$ (Figure 5(b), black boxes) resulted in a marked increase in IL-6 secretion that paralleled the increase observed in the absence of $A\beta_{1–42}$ (Figure 5(b), white boxes). In this series of experiments there were no effects of $A\beta_{1–42}$ alone at any time point on the secretion of IL-6. The pretreatment of the CHME3 microglial cells with IL-1β or IL-1β + IFNγ had a strong stimulatory effect on the secretion of IL-6 as compared to vehicle and to $A\beta_{1–42}$ alone (Figure 5(b), black boxes). At 24 hours, IL-1β increased the median IL-6 secretion 25-fold compared with vehicle and $A\beta_{1–42}$ alone ($P < .01$ in both cases). A significant stimulatory effect of IL-1β pretreatment was seen at 48 hours with levels 35-fold increase as compared to vehicle and $A\beta_{1–42}$ alone ($P < .01$ in both cases). At 48 hours, a stimulatory effect

of IFNγ became apparent, with a 5-fold increase in IL-6 secretion compared with vehicle and A$\beta_{1–42}$ alone ($P < .01$ in both cases). Compared with vehicle and A$\beta_{1–42}$ alone, the combined pretreatment with IL-1β + IFNγ induced a 35-fold increase at 24 hours ($P < .01$ in both cases) and a 28-fold increase at 48 hours ($P < .01$ in both cases). A 5-fold increase was seen with the combined IL-1β + IFNγ treatment at 96 hours compared with vehicle ($P < .01$). Also, the combined stimulation with IFNγ and IL-1β prior to A$\beta_{1–42}$ resulted in an increase of almost 6 times in the mean secretion of IL-6 at 48 hours as compared to IFNγ alone ($P < .005$), but not compared to IL-1β.

3.5. Effects of Protollin on Uptake of A$\beta_{1–42}$. Pretreatment of the microglial cells with Protollin at 0.001 μg/mL was found to increase the median proportion of cells showing uptake of A$\beta_{1–42}$ to 115% ($P < .05$) as compared to control (A$\beta_{1–42}$ alone, 100%, Figure 4). This effect was seen at 96 hours, but no significant changes in any direction could be detected at the earlier time points.

3.6. Effects of Protollin, with and without A$\beta_{1–42}$, on Secretion of BDNF and IL-6. Treatment with Protollin alone, that is, prior to addition of vehicle, did not affect the secretion of BDNF (data not shown). The incubation with A$\beta_{1–42}$ alone resulted in a decrease in the secretion of BDNF in this series of experiments, that is, a reduction to 82% of vehicle at 24 hours ($P < .0000001$), to 77% of vehicle at 48 hours ($P < .005$) and to 95% of vehicle at 96 hours ($P < .05$).

In cultures pretreated with the lowest concentration of Protollin (0.001 μg/mL) followed by incubation with A$\beta_{1–42}$ there was a 22% reduction at the 24 hours time point as compared to vehicle ($P = .05$), whereas at the higher concentrations of Protollin and at later time points there was no significant differences in the secretion of BDNF in comparison with control conditions (no Protollin and no A$\beta_{1–42}$).

Pretreatment with Protollin followed by A$\beta_{1–42}$ or vehicle did not induce any significant effects on the levels of IL-6 in culture supernatants (data not shown).

3.7. Cellular Markers and Relation to Phagocytosis of A$\beta_{1–42}$. The uptake of A$\beta_{1–42}$ in cells expressing the inflammatory markers IL-1β, IL-1RI, and iNOS in the cultures treated with A$\beta_{1–42}$ following pretreatment with Protollin was demonstrated by immunocytochemistry (Figure 6). The proportion of cells positive for these markers was analysed by flow-cytometry, both after incubation with A$\beta_{1–42}$ alone and after pretreatment with Protollin, in order to assess the phenotype of the cells taking up A$\beta_{1–42}$ (A$\beta_{1–42}$+) (Figure 7).

3.8. IL-1β Immunoreactive Cells. In untreated (vehicle) cultures, the proportion of CHME3 microglial cells with immunoreactivity to IL-1β had a median of 2.9% at 24 hours. Incubation of the cells with 1 μg/mL A$\beta_{1–42}$ did not significantly affect the number of IL-1β immunoreactive cells compared with vehicle at any time point (Figure 7(a)).

The expression of IL-1β in A$\beta_{1–42}$+ cells was not significantly different from that in A$\beta_{1–42}$− cells according to the Wilcoxon Matched Pairs test, except at 72 hours when the population of A$\beta_{1–42}$+/IL-1β+ cells was larger than the A$\beta_{1–42}$−/IL-1β+ cell population (Figure 7(a)).

Pretreatment with Protollin increased the number of IL-1β+ cells displaying A$\beta_{1–42}$ uptake (Figure 7(a)). Significant differences were observed between A$\beta_{1–42}$+/IL-1β+ cells and A$\beta_{1–42}$−/IL-1β+ cells treated with Protollin at 24 hours in concentrations of 0.001 μg/mL ($P < .05$), 0.1 μg/mL ($P < .05$) and 1 μg/mL ($P < .05$), at 48 hours in concentrations of 0.1 μg/mL ($P < .05$) and 1 μg/mL ($P < .05$), and at 72 hours in a concentration of 0.1 μg/mL ($P < .05$).

3.9. IL-1RI Immunoreactive Cells. In untreated (vehicle) cultures the proportion of CHME3 microglial cells with immunoreactivity for IL-1RI (IL-1RI+) had a median value of 12.5% at 24 hours. Incubation with 1 μg/mL A$\beta_{1–42}$, with or without pretreatment with Protollin, did not significantly affect the number of IL-1RI immunoreactive cells at any time point (Figure 7(b)).

When incubated with A$\beta_{1–42}$ alone, the population of A$\beta_{1–42}$+/IL-1RI+ cells was significantly larger than the A$\beta_{1–42}$−/IL-1RI+ population at 24, 48, and 72 hours ($P < .05$, Figure 7(b)). When pretreated with Protollin, there was a significantly larger proportion of A$\beta_{1–42}$+ cells that were immunoreactive to IL-1RI (A$\beta_{1–42}$+/IL-1RI+) at 24 hours in concentrations of 0.001 μg/mL ($P < .05$), 0.1 μg/mL ($P < .05$) and 1 μg/mL ($P < .05$), at 48 hours in concentrations of 0.1 μg/mL ($P < .05$) and 1 μg/mL ($P < .05$) and at 72 hours in a concentration of 0.1 μg/mL ($P < .05$).

3.10. iNOS Immunoreactive Cells. In untreated (vehicle) cultures the median proportion of CHME3 microglial cells with immunoreactivity to iNOS (iNOS+) had a median of 16% at 24 hours under basal (vehicle) conditions. None of the treatments affected the total number of cells immunoreactive for iNOS (Figure 7(c)).

When incubated with A$\beta_{1–42}$ alone, the population of A$\beta_{1–42}$+/iNOS+ cells was significantly larger than the population of A$\beta_{1–42}$−/iNOS+ cells at all time points ($P = .0173$ at 24 hours, $P = .0172$ at 48 hours, $P = .0117$ at 72 hours, and $P = .05$ at 96 hours, Figure 7(c)). Also when cells were pretreated with Protollin, the A$\beta_{1–42}$+/iNOS+ population was significantly larger than the A$\beta_{1–42}$−/iNOS+ population, at all concentrations tested (0.001 μg/mL: $P = .0117$ at 24 hours, $P = .0357$ at 48 hours and $P = .025$ at 72 hours; 0.01 μg/mL : $P = .0117$ at 24 hours and $P = .0117$ at 48 hours; 0.1 μg/mL : $P = .0117$ at 24 hours, $P = .05$ at 48 hours and $P = .0251$ at 72 hours: and $P = .025$ at 72 hours: 0.1 μg/mL : $P = .0117$ at 24 hours, Figure 7(c)).

3.11. Correlation between Secretion of BDNF and Uptake of A$\beta_{1–42}$. We found a significant negative correlation ($P < .05$) between the levels of BDNF in culture supernatant and the proportion of A$\beta_{1–42}$+ cells at all time points in the experiments (Figure 8). The data were analysed by correlating the BDNF-levels with the proportion of A$\beta_{1–42}$+ cells in all the

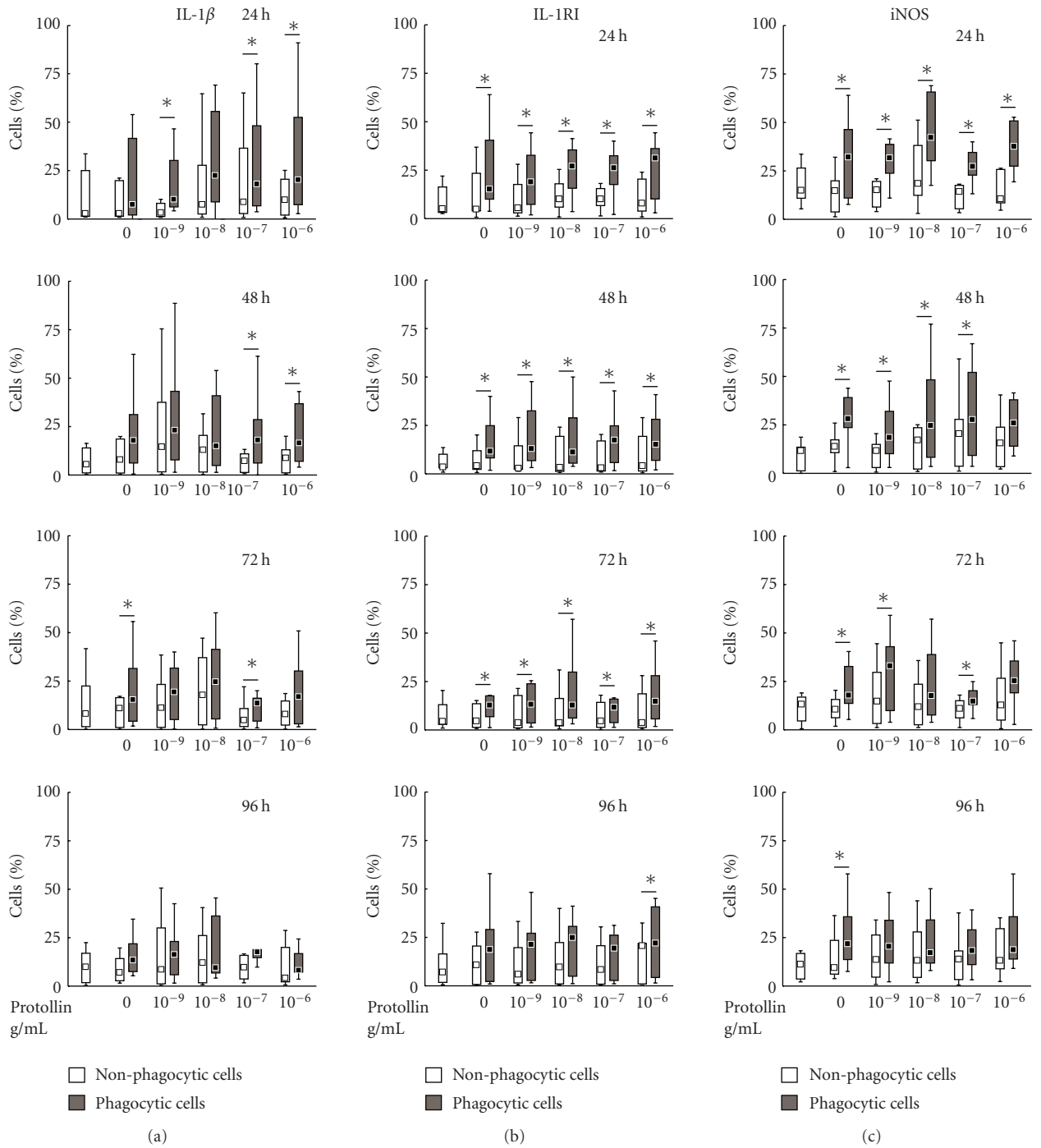


Figure 7: (a)–(c) Differential phenotype of human CHME3 microglia, in the populations of cells displaying ($A\beta_{1–42}$+, grey boxes) or not displaying ($A\beta_{1–42}$−, white boxes) phagocytosis of $A\beta_{1–42}$, with regard to immunoreactivity for interleukin-1β (IL-1β), inducible nitric oxide synthase (iNOS), and IL-1β receptor type I (IL-1RI), following pretreatment with Protollin (0.001–1 μg/mL). After 24–96 hours of exposure to $A\beta_{1–42}$, the cells were subjected to immunocytochemistry and analysed by flow-cytometry. The population of cells with immunoreactivity to the different markers within the $A\beta_{1–42}$+ cell population was compared with the corresponding population in the $A\beta_{1–42}$− cell population in each treatment group, using the Wilcoxon Matched Pairs Test. The data are shown as median ± percentiles (25%–75% and 10%–90%), $n = 7$. Statistical differences between the $A\beta_{1–42}$+ and $A\beta_{1–42}$− cells with regard to each marker are indicated by *($P < .05$).

treatments at one time point, and in one treatment at one time point, using Spearman's Rank Order Correlation test. There was a significant negative correlation in all analyses performed, except at 48 hours, when the treatment with 0.001 mg/mL Protollin was void of a significant correlation.

3.12. Cell Death and Viability. The effects of $A\beta_{1–42}$ and the immunomodulatory substances on cell death and viability were analysed by the LDH and MTT assays, respectively. Neither $A\beta_{1–42}$ nor Protollin, nor their combination, produced any significant effects on cell viability (data not shown).

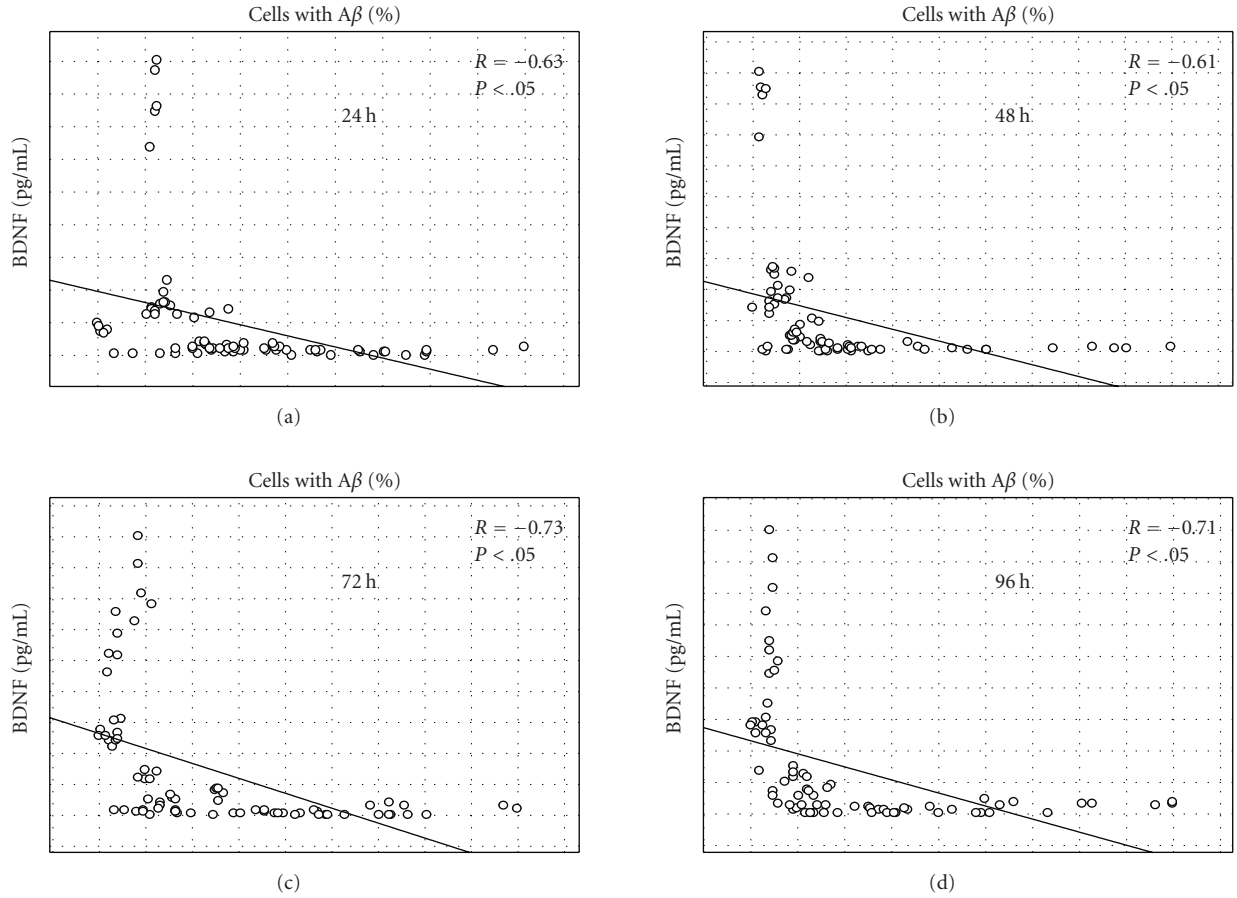


Figure 8: Correlation between the secretion of BDNF and the proportion of $A\beta_{1–42}$+ cells. The secretion of BDNF (pg/mL) and the proportion of $A\beta_{1–42}$+ cells in the Protollin series of experiments were analysed at each time point using Spearman's Rank Order Correlations test. A significant negative correlation was found at all time points. A statistical significant correlation is indicated by * ($P < .05$).

The incubation with IFNγ or IL-1β + IFNγ decreased the signal from the MTT assay significantly to almost 50% of vehicle starting at 48 hours ($P < .05$), and at 72 ($P < .005$) and 96 hours ($P < .005$) (Figure 9). This effect was seen both when the microglia were stimulated with the cytokines alone and when they were added before $A\beta_{1–42}$.

There were no significant differences in the LDH-activity in the medium from any of the treatments (not shown). Inspection with microscope, however, showed signs of cell death in treatments associated with a significant decrease in MTT signal.

4. Discussion

In this study, a human microglial cell line was used to model phagocytosis of Aβ and to evaluate the effects of different substances. The ability to analyse the effects of different substances with a human microglial cell line, which can be cultured in significant quantities, and the cellular reactions to pathological factors, such as Aβ, is a valuable tool in studies of human CNS-pathologies. The CHME3 cell line was established by Professor Tardieu (see [37]). This cell line responds to stimulation with LPS by increased secretion of IL-6 [38], a cytokine also secreted under basal conditions. We have found that CHME3 microglial cells also secrete low, but detectable, levels of IL-1β and TNFα as well as other cytokines (unpublished observations).

Phagocytosis of $A\beta_{1–42}$ was established using fluorophore-labelled $A\beta_{1–42}$. The uptake was first detectable at 4 hours after the addition as seen by microscopical analysis of living cells. Phagocytosis of $A\beta_{1–42}$ was differentiated from unspecific adherence to cell membranes by confocal microscopy which confirmed the lysosomal location of $A\beta_{1–42}$. In terms of inflammatory response, the incubation with $A\beta_{1–42}$ at the concentration used (1 μg/mL) did not affect the basal secretion of IL-6, in accordance with studies on primary human embryonic microglia in which $A\beta_{1–42}$ failed to increase transcription of the IL-6 gene and $A\beta_{25–35}$ had no effect on IL-6 secretion [39]. Similarly, the secretion of IL-6 from rat microglial cells was not altered upon incubation with 75 μM $A\beta_{1–42}$, a concentration 150-fold the concentration used in this study [18]. In contrast, primary mouse microglia have been shown to respond with markedly increased IL-6 secretion upon stimulation with $A\beta_{1–42}$ in a concentration similar to that in the present study [40], indicating a species difference. In our previous studies on the CHME3 microglia a marked increase in the secreted levels of IL-6 could be seen upon incubation with $A\beta_{1–40}$ [38],

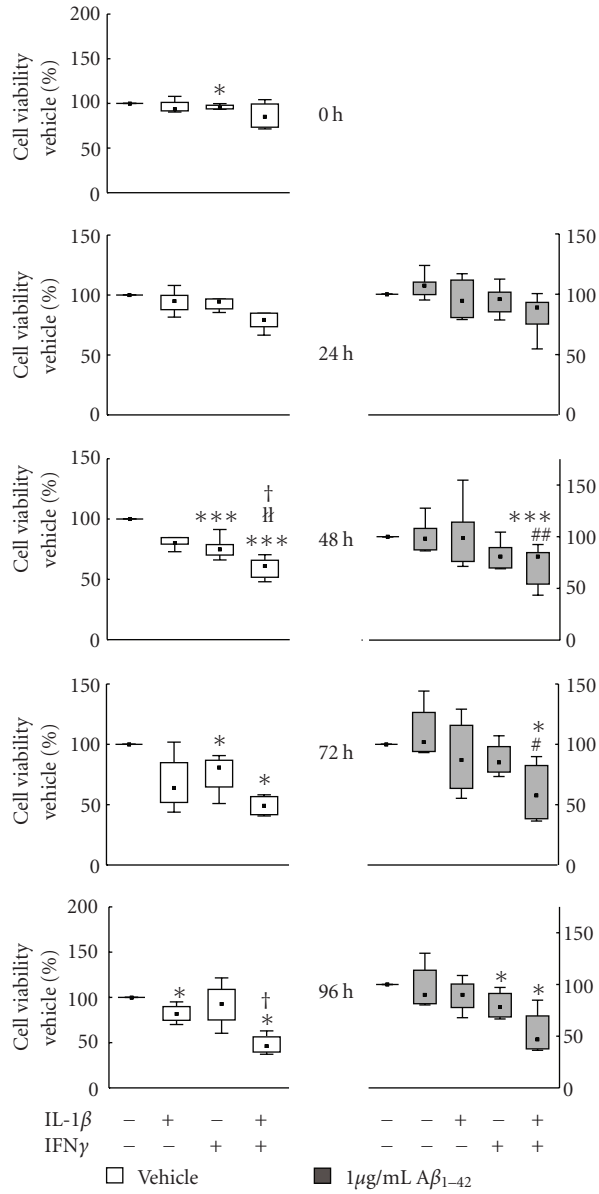


Figure 9: Effects of interleukin-1β (IL-1β) and interferon-γ (IFNγ) on cell viability in human CHME3 microglial cells treated with A$\beta_{1–42}$ (grey boxes) or vehicle (white boxes). The cells were preincubated with 50 ng/mL IL-1β and 50 ng/mL IFNγ for 24 hours prior to addition of A$\beta_{1–42}$ (1 μg/mL). At 24, 48, 72, and 96 hours after addition of A$\beta_{1–42}$ the viability of the cultures was assessed with the MTT assay. The data are expressed as % of control (vehicle) set at 100% and shown as median ± percentiles (25%–75% and 10%–90%), $n = 4$ (24, 48 h), $n = 6$ (72, 96 h). Statistical difference from vehicle is indicated by $^{*}(P < .05)$ and $^{***}(P < .005)$, and statistical difference from A$\beta_{1–42}$ is indicated by $^{\#}(P < .05)$ and $^{\#\#}(P < .01)$. $^{ǂ}(P < .01)$ indicates statistical difference between IFNγ and IL-1β + IFNγ, and $^{\dagger}(P < .05)$ indicate differences between IL-1β and IL-1β + IFNγ.

suggesting a difference in the immune-activating properties of the two forms of Aβ.

We show here that the human microglial cell line produces and secretes BDNF, in agreement with studies on human *post mortem* tissue [41]. Studies in different injury models in animals have shown the induction of BDNF production in microglia [42], supporting a view of BDNF as a glial response to neuronal injury serving to help neurons to recover. In this context it is remarkable that the incubation with A$\beta_{1–42}$ reduced the secretion of BDNF, which to our knowledge is the first time that A$\beta_{1–42}$ has been shown to exert this effect on any cell type. This adds to the negative effects of A$\beta_{1–42}$ and indicates a further reason for limiting the amyloidosis in AD. Evidence for detrimental interference by A$\beta_{1–42}$ on BDNF signalling in neurons has been provided previously [43]. A reduced secretion and an impaired signalling of BDNF may contribute to the cell death and impaired neuronal function in AD. In fact, decreased

levels of BDNF have been observed in the CSF of AD patients, and at later stages of the disease this reduction correlated with the severity of impairment [44].

Interestingly, a marked decrease in basal BDNF secretion was observed also upon incubation of the microglial cells with IFNγ, an effect that was also observed upon coincubation with IL-1β. In contrast, studies on rodent microglia showed an increase in the levels of BDNF in association with induction of inflammation [42], again suggesting species differences in microglial responses. Furthermore, studies on rat astrocytes indicated a stimulating effect on BDNF production by TNFα mediated by NFκB [45]. In the present study IL-1β, which is also known to activate NFκB [46], caused a mild inhibitory effect on BDNF secretion at 96 hours.

To analyse the possibility to stimulate the phagocytosis of A$\beta_{1–42}$, we analysed the effects of different immunomodulatory substances. Pretreatment of the human microglial cells with IFNγ, alone or together with IL-1β, resulted in a significant increase in the proportion of cells with an uptake of A$\beta_{1–42}$. This was accompanied by a pronounced reduction in BDNF secretion, as well as decreased cell viability. The results indicate that proinflammatory factors may be able to stimulate A$\beta_{1–42}$ phagocytosis. However, the stimulation by IFNγ was seen in the later time points and it may be speculated that the acute inflammation at the start of the experiment had expired at this stage with presumably only low levels of the added cytokines still present in the medium. The effects observed may thus be due to factors induced by and secondary to IFNγ. The effect of long-term, chronic exposure to inflammatory stimulation may be different. The influence of inflammation on A$\beta_{1–42}$ phagocytosis and clearance is a complex matter, where factors such as age and species may be pivotal. Even more complexity is added by the effects of A$\beta_{1–42}$ itself on inflammation, including the findings that the 40 and 42 amino acid peptides, and the aggregational form of A$\beta_{1–42}$ (monomers, oligomers, protofibrils, etc.), induce different inflammatory responses as seen in studies on rat microglia [18]. Furthermore, the shorter and longer species of Aβ have not been characterized with regard to their influence on glia. In the present study we prepared A$\beta_{1–42}$ by dissolving the lyophilized peptide in pure DMSO, and therefore it is reasonable to assume that soluble monomers or oligomers dominate at the start of the experiments. Interestingly, we could not detect any significant difference in the proportion of A$\beta_{1–42}$+ cells with time (data not shown). Factors influencing this proportion may be the rate of phagocytosis, degradation of the peptide, or changes in cell number. Our data show an ongoing cell proliferation continuing to the end of the experiment. This fact, taken together with a stable A$\beta_{1–42}$+ cell proportion with time, suggests that phagocytosis is continuous. An exception was seen upon treatment with IFNγ, in which the A$\beta_{1–42}$+ proportion increased significantly simultaneous with a reduction in cell viability.

Protollin, a proteosome-based adjuvant with immunomodulatory activities, was found to modestly increase A$\beta_{1–42}$ uptake by the human CHME3 microglia at 96 hours after addition of A$\beta_{1–42}$, but not at the earlier time points and only at the lowest concentration tested (0.001 μg/mL). This potentially stimulatory effect of Protollin on A$\beta_{1–42}$ uptake is concordant with results from in vivo studies on Protollin in an AD mouse model [34], showing clearance of amyloid plaques and improved cognitive performance upon intranasal administration of Protollin. The limited effect of Protollin in the present in vitro experiments as compared to the in vivo studies may have several explanations. In the mouse in vivo model, the stimulatory effect of Protollin on Aβ-uptake appears to be mediated via activation of monocytes in the periphery that migrate to the brain and phagocytose Aβ, rather than direct activation of microglial cells resident in the brain [34]. Also, there was no evidence from the in vivo studies that Protollin translocates to the brain following nasal administration [34]. The modest stimulatory effect of Protollin on A$\beta_{1–42}$ uptake by microglial cells in the present study may reflect the absence of accessory cells in the in vitro cultures, that is, cells that Protollin directly activates in the periphery in vivo. Alternatively, considering that the effect of Protollin did not become apparent until the later part of the experiment, it may also be speculated that activation of microglial cell precursors by Protollin in the periphery results in the secretion of factors in a para/autocrine fashion, that with time build up to concentrations that stimulate phagocytosis. Species differences should also be considered. The response repertoire of glial cells appears to be different in human as compared with murine cells [47, 48].

Interestingly, the pretreatment with Protollin appeared to ameliorate the A$\beta_{1–42}$-induced decrease in BDNF secretion. This highlights a therapeutic target: stimulation of glial cells for the production of beneficial and neuroprotective molecules. As indicated above, beneficial effects of Protollin in the context of immunotherapy may be elicited through stimulation of peripheral monocytes [34], directly, or indirectly through interaction with other immunocompetent cells. The present data, for example, on BDNF, suggest that Protollin or similarly acting substances may also stimulate beneficial effects on glial cells within the brain.

To our knowledge, no studies on human cells have until now investigated the effects of TLR2 activation on BDNF secretion. A strong negative correlation between the A$\beta_{1–42}$+ cell proportion and the secretion of BDNF was also observed. This result can be due to a negative effect on phagocytosis by BDNF or a decrease in BDNF secretion by cells performing phagocytosis. In a previous study BDNF was found to stimulate phagocytosis [49]. Although the experiments were performed on mouse peritoneal macrophages, the results support the latter explanation for the negative correlation between BDNF levels and phagocytosis.

Analysis with flow-cytometry showed that cells displaying phagocytosis of A$\beta_{1–42}$ had a significantly higher degree of expression of IL-1RI and iNOS, indicating that phagocytosis of A$\beta_{1–42}$ was associated with an inflammatory phenotype. Similarly, in a mouse AD-model, the expression of CD11b, a microglial activation marker that has been used as an indicator of harmful inflammation in several studies, was associated with removal of Aβ [34].

Protollin appeared to decrease the proportion of iNOS+ cells displaying phagocytosis of A$\beta_{1–42}$, although the total

number of iNOS+ cells remained unchanged. This proportion was significantly higher at all time points when incubated with $A\beta_{1–42}$ alone. At 96 hours, the pretreatment with Protollin abolished this difference. A reduction in the levels of iNOS is beneficial due to the contribution of this enzyme to oxidative stress, supporting beneficial effects of Protollin.

The decrease in cell viability accompanying the induction of Aβ phagocytosis by IFNγ and IL-1β suggests microglial cell death. This was confirmed by microscopical inspection of the cultures. In spite of this, there was no detectable increase in the LDH-activity. However, a decrease in cell number as indicated by the MTT-assay could mask an increase in cell death as measured by the LDH-assay since fewer cells are available to release LDH into the medium. IFNγ has been shown to induce cell death in murine microglia, concomittant with an upregulation of the expression of Fas and FasL [50]. In addition, IL-1β and IFNγ are known to be involved in the expression and activation of iNOS [51, 52], which in turn may lead to oxidative stress.

In conclusion, we provide a model suitable for testing candidates for stimulating the phagocytosis of $A\beta_{1–42}$ by human microglial cells. The capacity to withstand serum-withdrawal for long periods of time and the high rate of proliferation makes the human CHME3 cell line suitable for this type of studies. Expanding primary cultures of microglia for large experimental series is not trivial. The data presented indicate differences between human microglial cells and murine glia, as described in other studies. In the present study, we show that the immunomodulatory substance Protollin affects the proportion of cells phagocytosing Aβ and their expression of inflammatory markers. Pretreatment with IFNγ had a robust stimulatory effect on phagocytosis of $A\beta_{1–42}$ at later time points, suggesting influence of secondary factors induced by this cytokine. In addition, we present data suggesting a neuropathological role for IFNγ by decreasing BDNF levels. Importantly, we show a strong negative effect of $A\beta_{1–42}$ on BDNF secretion. If phagocytic cells indeed have a decreased secretion of BDNF, as indicated by the negative correlation between phagocytosis and BDNF, this suggests an interesting parameter for future evaluation of potential drugs. The results present a scenario in which inflammation increases phagocytosis of $A\beta_{1–42}$, induces microglial cell death, and reduces secretion of BDNF. The reduction in BDNF is unwanted, since it deprives the brain of an important neuroprotective and plasticity-promoting factor. Considering the presence of inflammation in AD, it may be suggested that the combined effect of Aβ, IL-1β, and IFNγ on the secretion of BDNF from microglia may contribute to the neuronal pathology in AD. We hope that this and future studies can help develop directed and controlled activation of differential pro- and anti-inflammatory responses for therapeutic use.

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References

[1] V. M.-Y. Lee, "Biomedicine: tauists and βaptists united—well almost!," *Science*, vol. 293, no. 5534, pp. 1446–1447, 2001.

[2] J. Hardy and D. J. Selkoe, "The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics," *Science*, vol. 297, no. 5580, pp. 353–356, 2002.

[3] A. Guntert, H. Dobeli, and B. Bohrmann, "High sensitivity analysis of amyloid-β peptide composition in amyloid deposits from human and PS2APP mouse brain," *Neuroscience*, vol. 143, no. 2, pp. 461–475, 2006.

[4] Y.-M. Kuo, M. R. Emmerling, C. Vigo-Pelfrey, et al., "Water-soluble Aβ (N-40, N-42) oligomers in normal and Alzheimer disease brains," *Journal of Biological Chemistry*, vol. 271, no. 8, pp. 4077–4081, 1996.

[5] J. W. Deitmer, "Strategies for metabolic exchange between glial cells and neurons," *Respiration Physiology*, vol. 129, no. 1-2, pp. 71–81, 2001.

[6] W. Noske, H. Lentzen, K. Lange, and K. Keller, "Phagocytotic activity of glial cells in culture," *Experimental Cell Research*, vol. 142, no. 2, pp. 437–445, 1982.

[7] H. Akiyama, S. Barger, S. Barnum, et al., "Inflammation and Alzheimer's disease," *Neurobiology of Aging*, vol. 21, no. 3, pp. 383–421, 2000.

[8] E. G. McGeer and P. L. McGeer, "Inflammatory processes in Alzheimer's disease," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 27, no. 5, pp. 741–749, 2003.

[9] C. F. Ide, J. L. Scripter, B. W. Coltman, R. S. Doston, D. C. Snyder, and A. Jelaso, "Cellular and molecular correlates to plasticity during recovery from injury in the developing mammalian brain," *Progress in Brain Research*, vol. 108, pp. 365–377, 1996.

[10] S. Itagaki, P. L. McGeer, H. Akiyama, S. Zhu, and D. Selkoe, "Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease," *Journal of Neuroimmunology*, vol. 24, no. 3, pp. 173–182, 1989.

[11] W. S. T. Griffin, L. C. Stanley, C. Ling, et al., "Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 19, pp. 7611–7615, 1989.

[12] M. Huell, S. Strauss, B. Volk, M. Berger, and J. Bauer, "Interleukin-6 is present in early stages of plaque formation and is restricted to the brains of Alzheimer's disease patients," *Acta Neuropathologica*, vol. 89, no. 6, pp. 544–551, 1995.

[13] D. Blum-Degena, T. Muller, W. Kuhn, M. Gerlach, H. Przuntek, and P. Riederer, "Interleukin-1β and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients," *Neuroscience Letters*, vol. 202, no. 1-2, pp. 17–20, 1995.

[14] R. Cacabelos, M. Barquero, P. Garcia, X. A. Alvarez, and E. Varela de Seijas, "Cerebrospinal fluid interleukin-1β (IL-1β) in Alzheimer's disease and neurological disorders," *Methods and Findings in Experimental and Clinical Pharmacology*, vol. 13, no. 7, pp. 455–458, 1991.

[15] D. M. Araujo and C. W. Cotman, "β-amyloid stimulates glial cells in vitro to produce growth factors that accumulate in

senile plaques in Alzheimer's disease," *Brain Research*, vol. 569, pp. 141–145, 1992.

[16] E. N. Benveniste, V. T. Nguyen, and G. M. O'Keefe, "Immunological aspects of microglia: relevance to Alzheimer's disease," *Neurochemistry International*, vol. 39, no. 5-6, pp. 381–391, 2001.

[17] P. Garcao, C. R. Oliveira, and P. Agostinho, "Comparative study of microglia activation induced by amyloid-β and prion peptides: role in neurodegeneration," *Journal of Neuroscience Research*, vol. 84, no. 1, pp. 182–193, 2006.

[18] C. Lindberg, M.-L. B. Selenica, A. Westlind-Danielsson, and M. Schultzberg, "β-amyloid protein structure determines the nature of cytokine release from rat microglia," *Journal of Molecular Neuroscience*, vol. 27, no. 1, pp. 1–12, 2005.

[19] D. J. Selkoe, "Cell biology of the amyloid β-protein precursor and the mechanism of Alzheimer's disease," *Annual Review of Cell and Developmental Biology*, vol. 10, pp. 373–403, 1994.

[20] J. T. Rogers, L. M. Leiter, J. McPhee, et al., "Translation of the Alzheimer amyloid precursor protein mRNA is up-regulated by interleukin-1 through 5′-untranslated region sequences," *Journal of Biological Chemistry*, vol. 274, no. 10, pp. 6421–6431, 1999.

[21] M. Grilli, M. Ribola, A. Alberici, A. Valerio, M. Memo, and P. Spano, "Identification and characterization of a κB/Rel binding site in the regulatory region of the amyloid precursor protein gene," *Journal of Biological Chemistry*, vol. 270, no. 45, pp. 26774–26777, 1995.

[22] I. Blasko, R. Veerhuis, M. Stampfer-Kountchev, M. Saurwein-Teissl, P. Eikelenboom, and B. Grubeck-Loebenstein, "Costimulatory effects of interferon-γ and interleukin-1β or tumor necrosis factor β on the synthesis of Aβ1-40 and Aβ1-42 by human astrocytes," *Neurobiology of Disease*, vol. 7, no. 6, pp. 682–689, 2000.

[23] R. E. Mrak and W. S. Griffin, "Interleukin-1, neuroinflammation, and Alzheimer's disease," *Neurobiology of Aging*, vol. 22, pp. 903–908, 2001.

[24] D. Schenk, R. Barbour, W. Dunn, et al., "Immunization with amyloid-β attenuates Alzheimer disease-like pathology in the PDAPP mouse," *Nature*, vol. 400, no. 6740, pp. 173–177, 1999.

[25] J.-M. Orgogozo, S. Gilman, J.-F. Dartigues, et al., "Subacute meningoencephalitis in a subset of patients with AD after Aβ_{1-42} immunization," *Neurology*, vol. 61, no. 1, pp. 46–54, 2003.

[26] R. von Bernhardi, G. Ramirez, R. Toro, and J. Eugenin, "Proinflammatory conditions promote neuronal damage mediated by amyloid precursor protein and decrease its phagocytosis and degradation by microglial cells in culture," *Neurobiology of Disease*, vol. 26, no. 1, pp. 153–164, 2007.

[27] J. Koenigsknecht-Talboo and G. E. Landreth, "Microglial phagocytosis induced by fibrillar β-amyloid and IgGs are differentially regulated by proinflammatory cytokines," *Journal of Neuroscience*, vol. 25, no. 36, pp. 8240–8249, 2005.

[28] T. Bartfai, M. Sanchez-Alavez, S. Andell-Jonsson, et al., "Interleukin-1 system in CNS stress: seizures, fever, and neurotrauma," *Annals of the New York Academy of Sciences*, vol. 1113, pp. 173–177, 2007.

[29] T. Beck, D. Lindholm, E. Castren, and A. Wree, "Brain-derived neurotrophic factor protects against ischemic cell damage in rat hippocampus," *Journal of Cerebral Blood Flow and Metabolism*, vol. 14, no. 4, pp. 689–692, 1994.

[30] B. Xu, W. Gottschalk, A. Chow, et al., "The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving trkB," *Journal of Neuroscience*, vol. 20, no. 18, pp. 6888–6897, 2000.

[31] L. F. Fries, A. D. Montemarano, C. P. Mallett, D. N. Taylor, T. L. Hale, and G. H. Lowell, "Safety and immunogenicity of a proteosome-Shigella flexneri 2a lipopolysaccharide vaccine administered intranasally to healthy adults," *Infection and Immunity*, vol. 69, no. 7, pp. 4545–4553, 2001.

[32] S. M. Chabot, T. S. Chernin, M. Shawi, et al., "TLR2 activation by proteosomes promotes uptake of particulate vaccines at mucosal surfaces," *Vaccine*, vol. 25, no. 29, pp. 5348–5358, 2007.

[33] A. Boivin, I. Pineau, B. Barrette, et al., "Toll-like receptor signaling is critical for Wallerian degeneration and functional recovery after peripheral nerve injury," *Journal of Neuroscience*, vol. 27, no. 46, pp. 12565–12576, 2007.

[34] D. Frenkel, L. Puckett, S. Petrovic, et al., "A nasal proteosome adjuvant activates microglia and prevents amyloid deposition," *Annals of Neurology*, vol. 63, no. 5, pp. 591–601, 2008.

[35] A. F. Samdani, T. M. Dawson, and V. L. Dawson, "Nitric oxide synthase in models of focal ischemia," *Stroke*, vol. 28, no. 6, pp. 1283–1288, 1997.

[36] S. C. Lee, M.-L. Zhao, A. Hirano, and D. W. Dickson, "Inducible nitric oxide synthase immunoreactivity in the Alzheimer disease hippocampus: association with Hirano bodies, neurofibrillary tangles, and senile plaques," *Journal of Neuropathology and Experimental Neurology*, vol. 58, no. 11, pp. 1163–1169, 1999.

[37] N. Janabi, S. Peudenier, B. Héron, K. H. Ng, and M. Tardieu, "Establishment of human microglial cell lines after transfection of primary cultures of embryonic microglial cells with the SV40 large T antigen," *Neuroscience Letters*, vol. 195, no. 2, pp. 105–108, 1995.

[38] C. Lindberg, E. Hjorth, C. Post, B. Winblad, and M. Schultzberg, "Cytokine production by a human microglial cell line: effects of βamyloid and α-melanocyte-stimulating hormone," *Neurotoxicity Research*, vol. 8, no. 3-4, pp. 267–276, 2005.

[39] Y. B. Lee, A. Nagai, and S. U. Kim, "Cytokines, chemokines, and cytokine receptors in human microglia," *Journal of Neuroscience Research*, vol. 69, no. 1, pp. 94–103, 2002.

[40] A. M. Szczepanik, S. Funes, W. Petko, and G. E. Ringheim, "IL-4, IL-10 and IL-13 modulate Aβ_{1-42}-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line," *Journal of Neuroimmunology*, vol. 113, no. 1, pp. 49–62, 2001.

[41] C. Knott, G. Stern, A. Kingsbury, A. A. Welcher, and G. P. Wilkin, "Elevated glial brain-derived neurotrophic factor in Parkinson's diseased nigra," *Parkinsonism and Related Disorders*, vol. 8, no. 5, pp. 329–341, 2002.

[42] A. Y. Lai and K. G. Todd, "Differential regulation of trophic and proinflammatory microglial effectors is dependent on severity of neuronal injury," *Glia*, vol. 56, no. 3, pp. 259–270, 2008.

[43] L. Tong, R. Balazs, P. L. Thornton, and C. W. Cotman, "β-amyloid peptide at sublethal concentrations downregulates brain-derived neurotrophic factor functions in cultured cortical neurons," *Journal of Neuroscience*, vol. 24, no. 30, pp. 6799–6809, 2004.

[44] C. Laske, E. Stransky, T. Leyhe, et al., "BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls," *Journal of Psychiatric Research*, vol. 41, no. 5, pp. 387–394, 2007.

[45] R. N. Saha, X. Liu, and K. Pahan, "Up-regulation of BDNF in astrocytes by TNF-α: a case for the neuroprotective role of cytokine," *Journal of Neuroimmune Pharmacology*, vol. 1, no. 3, pp. 212–222, 2006.

[46] F. Shirakawa, M. Chedid, J. Suttles, B. A. Pollok, and S. B. Mizel, "Interleukin 1 and cyclic AMP induce κ immunoglobulin light-chain expression via activation of an NF-κB-like DNA-binding protein," *Molecular and Cellular Biology*, vol. 9, no. 3, pp. 959–964, 1989.

[47] C. Colton, S. Wilt, D. Gilbert, O. Chernyshev, J. Snell, and M. Dubois-Dalcq, "Species differences in the generation of reactive oxygen species by microglia," *Molecular and Chemical Neuropathology*, vol. 28, no. 1–3, pp. 15–20, 1996.

[48] S. C. Lee and C. F. Brosnan, "Cytokine regulation of iNOS expression in human glial cells," *Methods*, vol. 10, no. 1, pp. 31–37, 1996.

[49] T. Asami, T. Ito, H. Fukumitsu, H. Nomoto, Y. Furukawa, and S. Furukawa, "Autocrine activation of cultured macrophages by brain-derived neurotrophic factor," *Biochemical and Biophysical Research Communications*, vol. 344, no. 3, pp. 941–947, 2006.

[50] B. Badie, J. Schartner, J. Vorpahl, and K. Preston, "Interferon-γ induces apoptosis and augments the expression of Fas and Fas ligand by microglia in vitro," *Experimental Neurology*, vol. 162, no. 2, pp. 290–296, 2000.

[51] K. T. Akama and L. J. van Eldik, "β-amyloid stimulation of inducible nitric-oxide synthase in astrocytes is interleukin-1β- and tumor necrosis factor-α (TNFα)-dependent, and involves a TNFα receptor-associated factor- and NFκB-inducing kinase-dependent signaling mechanism," *Journal of Biological Chemistry*, vol. 275, no. 11, pp. 7918–7924, 2000.

[52] H.-M. Kim, E.-H. Lee, T.-K. Shin, C.-K. Chung, and N.-H. An, "Inhibition of the induction of the inducible nitric oxide synthase in murine brain microglial cells by sodium salicylate," *Immunology*, vol. 95, no. 3, pp. 389–394, 1998.

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Review Article

Amyloid-Beta Peptide, Oxidative Stress and Inflammation in Alzheimer's Disease: Potential Neuroprotective Effects of Omega-3 Polyunsaturated Fatty Acids

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Alzheimer's disease is the most common form of dementia in the elderly and is a progressive neurodegenerative disorder characterised by a decline in cognitive function and also profound alterations in mood and behaviour. The pathology of the disease is characterised by the presence of extracellular amyloid peptide deposits and intracellular neurofibrillary tangles in the brain. Although many hypotheses have been put forward for the aetiology of the disease, increased inflammation and oxidative stress appear key to be features contributing to the pathology. The omega-3 polyunsaturated fats, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have well-characterised effects on inflammation and may have neuroprotective effects in a number of neurodegenerative conditions including Alzheimer's disease. The aims of this paper are to review the neuroprotective effects of EPA and DHA in Alzheimer's disease, with special emphasis on their role in modulating oxidative stress and inflammation and also examine their potential as therapeutic agents.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly. It is a progressive neurodegenerative disorder characterised by a decline in cognitive function and also profound alterations in mood and behaviour [1]. The pathology of the disease is characterised by the presence of extracellular amyloid peptide deposits, soluble amyloid β-protein and hyperphosphorylated tau protein leading to the formation of intracellular neurofibrillary tangles in the brain. The aetiology and pathogenesis of the disease are currently poorly understood, and the present management is to a large extent symptomatic and focused on ameliorating the cognitive deficits [2]. However, although many hypotheses have been put forward for the aetiology of the disease, increased inflammation [3] and oxidative stress [4] appear to be key features contributing to the pathology of the disease.

The omega-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have well-characterised effects on inflammation [5] and may have neuroprotective effects in a number of neurodegenerative conditions including AD [6]. The purpose of this paper is to review the neuroprotective effects of EPA and DHA in AD with emphasis on their potential for modulating amyloidosis and the increased oxidative stress and inflammation seen with AD and explore their potential application as therapeutic agents.

2. Amyloid β-Peptide

A major hallmark of AD is the overproduction of amyloid β-peptide (Aβ), which results in the formation of plaques. Aβ peptides are produced by successive proteolysis of amyloid precursor protein (APP) by β-site APP-cleaving enzyme-1 (BACE1) followed by γ-secretase [7–9]. This cleavage is imprecise and produces Aβ variants, which include those ending at residues 40 (Aβ_{40}) and 42 (Aβ_{42}) [10]. The Aβ_{42} is deposited earliest and most abundantly in plaques [11]. Aβ has been shown to induce lipid peroxidation in brain cell membranes and increase production of the lipid peroxidation products 4-hydroxynonenal and acrolein, and this may in part account for neurodegeneration in AD brain [12].

Several groups have investigated the role of DHA-enriched diets in animal models of AD and amyloidosis. In a series of studies Hashimoto and colleagues pretreated rats with DHA (300 mg/kg per day for 12 weeks) before an infusion of $A\beta_{1-40}$ [13–15]. DHA had a significantly protective effect against the decrease in learning ability and reduced the oxidative stress induced by the Aβ infusion in the cerebral cortex and hippocampus. Pretreatment with DHA also prevented Aβ-induced impairment of an avoidance ability-related memory function. This group also investigated the protective effects of the DHA pretreatment before Aβ infusion on synaptosomal membranes properties. DHA content significantly increased along with both lateral and rotational membrane fluidity, whereas the cholesterol to phospholipid molar ratio and lipid peroxidation decreased.

Elevated cholesterol increases Aβ levels in both *in vitro* and *in vivo* models of AD, and the generation, accumulation, and clearance of Aβ are regulated by cholesterol [16–18], suggesting an important role for cholesterol in the pathogenesis of AD. Indeed, cholesterol is now a recognized risk factor in the pathogenesis of AD [18]. Furthermore, Aβ generation may be determined by dynamic interactions of APP with lipid rafts, since APP inside rafts undergoes cleavage by β-secretase, whereas APP outside rafts undergoes cleavage by α-secretase [19]. Lipid rafts are lateral assemblies of sphingolipids and cholesterol within the membrane [20], and it has been suggested that age-related increases in cholesterol in lipid rafts provides a cooperative environment for accumulation of Aβ in plasma membranes [21]. It is therefore of interest to note that omega-3 PUFAs reduce the level of cholesterol in neuronal membranes [22], moreover cholesterol has a low affinity for DHA-containing phospholipids and therefore alterations in the level of membrane DHA will affect the formation of lipid rafts [23, 24]. It may be that the effects of DHA on cholesterol levels and lipid raft formation represent an important, but as-yet relatively unexplored, neuroprotective mechanism.

Recent work by Green and coworkers has shown that DHA reduces the levels of soluble and intraneuronal Aβ and somatodendritic tau protein in the 3xTg AD mouse model [25]. The reduction was attributed to a decrease in the steady-state levels of presenilin 1. Importantly, when DHA was combined with either arachidonic or docosapentaenoic acids (both omega-6 PUFAs) the efficacy of DHA diminished over time, with the effects lost by 9 months. However, the additional presence of docosapentaenoic acid in the diet reduced levels of early-stage phospho-tau epitopes, which correlated with the positive outcome of a reduction in phosphorylated (activated) c-Jun N-terminal kinase, a putative tau kinase. It may be that the interrelationship between omega-6 and omega-3 PUFAs is a further important but neglected area of research. DHA has also been shown to significantly increase levels of the sorting protein LR11/SorLA in primary rat neurons, aged nontransgenic mice, and aged DHA-depleted APPsw AD mice [26]. This increase reduces the trafficking of the amyloid precursor protein to secretases involved in the β-amyloidogenic pathway, and reduced LR11/SorLA expression is strongly correlated with AD neuropathology [27].

DHA also attenuates Aβ secretion in cytokine-stressed human neural cells, and this is accompanied by formation of neuroprotectin D1 [28]. 10,17S-docosatriene, also known as neuroprotectin D1, is a metabolite of DHA, which has been shown to have potent anti-inflammatory and neuroprotective effects in neural systems [29] and stroke [30]. DHA and neuroprotectin D1 were reduced in the hippocampus cornu ammonis region 1 (CA1) of AD brains, but not in the thalamus or occipital lobes of the same brains. Furthermore, expression of cytosolic phospholipase A_2 and 15-lipoxygenase, which are key enzymes in neuroprotectin D1 biosynthesis, was altered in AD hippocampus. Neuroprotectin D1 also repressed the $A\beta_{42}$-triggered activation of proinflammatory genes and upregulated the antiapoptotic genes encoding Bcl-2, Bcl-xl, and Bfl-1(A1). The soluble amyloid precursor protein-alpha (APP-α) stimulated the biosynthesis of neuroprotectin D1 from DHA. These results suggest that the beneficial effects of DHA may in part also be mediated via the production of neuroprotectin D1, which induces anti-apoptotic and neuroprotective gene expression and consequently suppresses $A\beta_{42}$-induced neurotoxicity.

Positive effects of DHA treatment have not however been universally reported and a recent study by Arendash and co-workers found that a high omega-3 PUFA diet provided no significant benefit in terms of decreasing the levels of soluble/insoluble hippocampal Aβ levels or improving cognitive performance in neither amyloid precursor protein (APP)-sw and PS1 double transgenic or wild-type mice [31]. The authors did find that higher cortical levels of omega-6 PUFA in both the transgenic and wild type mice were associated with impaired cognitive function, as measured by the radial arm water maze and Morris water maze tests. It may be that if DHA is acting via a reduction in the steady-state levels of presenilin 1, as suggested by Green and co-workers [25], the overexpression of presenilin 1 in the transgenic mouse model used in this study overwhelms the capacity of DHA, and therefore this model may not accurately reflect the potential effects in patients, especially those affected by sporadic AD. Furthermore, the omega-3 PUFA experimental diet contained high levels of EPA, which may have antagonised the effects of DHA (4.7% EPA and 5.7% DHA expressed as % total fat) by for example competing for enzymes in the neuroprotectin D1 biosynthetic pathway.

An additional mechanism leading to a decrease in Aβ levels has been suggested by a study investigating the effect of omega-3 PUFA enrichment on gene expression in aged rats [32]. In this study, there was a 10-fold increase in transthyretin transcription following treatment, and since transthyretin is an Aβ protein scavenger [33], the authors concluded that the omega-3 PUFA-induced expression could potentially prevent amyloid aggregate formation. Indeed Serot and co-workers found an inverse relationship between transthyretin levels in cerebrospinal fluid and the severity of dementia in AD patients [34].

Several lines of evidence indicate that alterations in retinoid signalling lead to Aβ accumulation. For example, vitamin A deprivation results in deposition of Aβ in the cerebral blood vessels and downregulation of the retinoic

acid receptor, RARα in adult rat forebrain [35], and APP/presenilin 1 double mutant transgenic mice treated for 8 weeks with retinoic acid show significantly decreased Aβ deposition, tau phosphorylation, activation of microglia and astrocytes, attenuated neuronal degeneration and improved spatial memory compared to controls [36]. Retinoic acid regulates gene expression via its nuclear receptors: the retinoic acid receptors (RARs) and retinoid X receptors (RXRs) [37]. DHA and EPA have been reported to act as endogenous ligands of RXRs [38–40], and omega-3 PUFA supplementation has recently been shown to reverse age-related decreases in the levels of RARα, RXRα, and RXRβ in the aged rat forebrain [41]. It may be that omega-3 PUFAs are acting at a fundamental level of cell regulation by controlling gene expression via these receptors.

Omega-3 PUFA may also be acting at proliferator-activated receptors (PPARs), in particular PPARγ. PPARs are involved in the control of the expression of a variety of genes involved in lipid energy metabolism and inflammation [42]. *In vitro* Aβ uptake and clearance from glial and neuronal medium is increased by PPARγ [43]. In models of AD PPARγ agonists reduce BACE1 transcription and expression in APP transgenic mice [44, 45], PPARγ agonist and ibuprofen treatment reduce the expression of BACE1 and Aβ_{42} amyloid deposits in the hippocampus and cortex of APPV717I mice [46], and PPARγ agonists protect neurons against Aβ-induced mitochondrial damage, apoptosis, and oxidative stress [47]. Furthermore, treatment with PPARγ agonists significantly improves measures of cognition in mild AD and mild cognitively impaired subjects compared to a placebo [48]. PPARγ's are known to bind to and be activated by EPA, DHA, and DHA metabolites [49–51] and omega-3 PUFA supplementation has also been shown to reverse age-related decreases in the levels of PPARγ in the aged rat forebrain [41].

There are a number of inflammatory events that occur in the brain as a response to the presence of Aβ. The key event appears to be the presence of activated microglia in the vicinity of the Aβ-containing plaques [52]. Microglial activation results in the sustained production of proinflammatory cytokines, growth factors, complement molecules, and adhesion molecules [53]. It has also been demonstrated that exposure of microglia to β-amyloid fibrils leads to the production of reactive oxygen species and neurotoxins [54] and activated microglia generate and release large numbers of superoxide ions [52], thereby increasing oxidative stress. The remainder of this paper will focus on this increased oxidative stress and inflammation and the potential role of omega-3 PUFA.

3. Oxidative Stress and Lipid Peroxidation

The importance of increased oxidative stress, lipid peroxidation, and lipid peroxidation products has consistently been shown in the pathogenesis of AD [4]. For example, increased lipid peroxidation has been detected in the frontal, temporal, parietal, and occipital cortices of AD patients [55]. Moreover, significantly increased levels of the lipid peroxidation products 4-hydroxynonenal and acrolein, are found in the hippocampus/parahippocampal gyrus, superior and middle temporal gyrus, and cerebellum of subjects with mild cognitive impairment and early AD compared to age-matched controls [56], suggesting that lipid peroxidation occurs as an early event in the pathogenesis of AD. This is consistent with evidence from animal models of AD, where increased oxidative stress is also found in the APPsw (Tg2576) transgenic mouse model of AD amyloidosis. Cerebral cortical and hippocampal homogenates were found to have higher levels of lipid peroxidation than those from wild-type mice, and lipid peroxidation preceded amyloid plaque formation [57].

Isoprostanes are prostaglandin-like compounds formed *in situ* by nonenzymatic free radical-catalysed lipid peroxidation [58]. F_2-isoprostanes are derived from the omega-6 PUFA, arachidonic acid, F_3-isoprostanes from EPA and F_4-isoprostanes, also called neuroprostanes, from DHA [59–61]. Measurement of isoprostanes provides a sensitive marker of *in vivo* lipid peroxidation [62–64]. Measurements comparing the level of isoprostanes and neuroprostanes between AD and matched controls in different regions of postmortem brains show a PUFA-specific pattern of lipid peroxidation. There is an increase in esterified neuroprostanes in the occipital and temporal lobes, whereas F_2-isoprostanes in these regions are unchanged [65]. Similar results were found by Reich and coworkers, in the superior and middle temporal gyri, hippocampus, inferior parietal lobule, and cerebral cortex [66]. There were increased levels of neuroprostanes while F_2-isoprostanes levels were unchanged. These results show a selective pattern of lipid peroxidation occuring in AD, whereby DHA appears especially vulnerable and arachidonic acid is unaffected.

These results may reflect more than simply the regional distribution of PUFA, since grey matter, where DHA is more abundant, has a significantly greater susceptibility to oxidative stress than white matter [64]. Levels of F_2-isoprostanes and neuroprostanes was measured in the grey and white matter of brains from rats at intervals between 4 and 100 weeks of age. The level of neuroprostanes were consistently 20-fold greater than F_2-isoprostanes, whereas DHA content was only two-fold greater than arachidonic acid, suggesting that DHA is especially prone to oxidative stress.

PUFAs are particularly susceptible to lipid peroxidation and as such are a potential abundant source of destructive peroxidation products. The *in vitro* peroxidisability of unsaturated fatty acids is linearly dependent upon the number of bis-allylic positions, such that the peroxidisability of DHA is five times greater than that of the omega-6 PUFA linoleic acid, which contains only two double bonds [67]. This increased susceptibility would help to explain the vulnerability of membrane DHA to lipid peroxidation and may also suggest that elevating omega-3 PUFA intake *in situ*ations of increased oxidative stress, such as AD, would further increase production of toxic peroxidation products.

Although some studies have shown increased lipid peroxidation following dietary supplementation with DHA and EPA in plasma, liver, and kidney [68–73] others have shown that EPA and DHA enriched diets do not increase levels of lipid peroxidation [74, 75]. Indeed, rather than

increasing lipid peroxidation DHA has been shown to be neuroprotective by actually decreasing lipid peroxidation in the brain [76–78]. For example, rats being fed a diet enriched with DHA showed a significantly reduced level of cerebral lipid peroxide compared to controls [78], and the level of peroxidation was inversely related to the cerebral DHA/arachidonic acid ratio [77]. Furthermore, Tg2576 transgenic mice being fed a DHA deficient diet have significantly elevated levels of oxidised proteins compared to the control group, whereas the levels of oxidised proteins were significantly reduced in a DHA supplemented group [79].

Overall, these results suggest that DHA may function in an antioxidant role, at least in the brain. Yavin and colleagues have suggested a number of potential mechanisms for these observed antioxidant effects [80]. Membrane bound DHA may act as a trap for reactive oxygen species, DHA may be able to enhance the activity of endogenous antioxidant enzymes, or phosphatidylethanolamine plasmalogens containing DHA may contain intrinsic antioxidant properties. DHA has also been shown to induce antioxidant defences by enhancing cerebral activities of catalase, glutathione peroxidase, and levels of glutathione [81]. Furthermore, work by Green and colleagues suggests that DHA may reduce reactive oxygen species production by increasing nitric oxide production, which may decrease the cellular oxygen pool and consequently reduce the amount of reactive oxygen species and generate lipid peroxides [82].

4. Fatty Acid Composition

The increased lipid peroxidation and concomitant damage to membrane PUFA have the potential to significantly reduce the brain levels of PUFA and specifically DHA. However, a recent review found that low DHA is not consistently observed in plasma or brain of AD patients [83]. Although the authors did note wide variability between studies and suggested that it may be too early to draw any reliable conclusions. They did however report AD to be consistently associated with significantly lower phosphatidylethanolamine and phosphatidylcholine concentrations in the frontal cortex and hippocampus and the DHA content of hippocampal phosphatidylethanolamine is significantly lower compared to age-matched controls.

DHA is particularly enriched in phosphatidylethanolamine [84], and this is therefore an important cellular store of DHA. Furthermore, in the hippocampal CA1 region of AD patients unesterified DHA and neuroprotectin D1 levels are reported to be about one-half and one-twentieth of those in age-matched controls, respectively [28]. It may therefore be hypothesised that although DHA decreases do not appear widespread or large in magnitude, these selective decreases in the hippocampus may have profound effects on specific cellular pools and the subsequent production of key DHA metabolites.

5. Inflammation

In comparison with nondemented elderly subjects virtually all the inflammatory cytokines and chemokines so far investigated appear to be upregulated in AD patients, including IL-1β, IL-6, TNF-α, IL-8, transforming growth factor-β (TGF-β), and macrophage inflammatory protein-1α (MIP-1α), for reviews see [3, 85]. The most notable proinflammatory cytokines produced by microglial cells, IL-1α and IL-1β are found throughout the brain of AD patients at autopsy [86]. IL-1β induces formation of reactive oxygen species, causing lipid peroxidation, and depletes membrane PUFA levels [87, 88]. IL-1β also triggers microglial activation and increases expression of amyloid precursor protein [89, 90]. Age-related increases in IL-1β are coupled with increased activity of MAPK, c-jun-N-terminal kinases (JNK, a stress activated MAP kinase), and p38 kinase and enhanced caspase-3 activity (an effector of apoptosis) [91]. MAP kinases mediate many specific cellular responses, including apoptosis and stress responses.

EPA supplementation has been reported to decrease the production of proinflammatory cytokines in rodents. For example, EPA reversed the age-related increases in IL-1β concentration and activation of p38 and caspase-3 [92]. The EPA supplementation also restored age-related decreases in arachidonic acid and DHA concentrations and decreased reactive oxygen species accumulation. It has also recently been shown that IL-1β reduces acetylcholine release in rats, which correlates with memory deficits, and EPA supplementation prevents the effects of IL-1β and significantly improves memory [93]. Degeneration of cholinergic neurons is thought to contribute to cognitive impairments in AD [94].

EPA and DHA are the precursors of a diverse array of second messengers, called resolvins and docosanoids, with potent anti-inflammatory and proresolving actions [30, 95, 96]. Resolution of inflammation has traditionally been thought of as a passive process; however, recent evidence suggests that resolution is an active process involving the biosynthesis of local lipid derived mediators within the resolution phase, called resolvins [96]. The D class resolvins, derived from DHA block TNF-α-induced IL-1β transcripts in brain [95] and resolvin E1 (RvE1), produced from EPA, reduces IL-12 production via the ChemR23 receptor [97]. 10,17S-docosatriene, also known as neuroprotectin D1 is produced from DHA [30] and potently blocks the generation of both TNF-α and IFN-c by stimulated T cells [98]. Together, these data suggest that the omega-3 PUFA metabolites have the potential to decrease inflammation by decreasing inflammatory cytokine production.

Most human dietary supplementation studies investigating the effects of omega-3 PUFA on cytokine production have typically examined the effects of high EPA rather than DHA preparations [99–105]. EPA and DHA have differing effects on inflammatory response and they should not be considered as having mechanistic equivalence [106, 107]. Furthermore, EPA and DHA produce distinct metabolites, and it may be that DHA is the more potent immunological modulator via production of neuroprotectin D1. A recent trial supplemented 174 AD patients with a high DHA preparation (1.7 g DHA and 0.6 g EPA or placebo for 6 months) [108]. The DHA/EPA supplemented patients had significantly reduced IL-1β, and IL-6 release from lipopolysaccharide stimulated peripheral blood

mononuclear cells compared to the placebo group, although TNF-α, IL-8, IL-10 and granulocyte colony-stimulating factor were not affected.

The mechanism by which DHA and EPA are able to attenuate inflammatory processes has yet to be elucidated; however it is likely that these observed effects are mediated at the level of gene regulation by modifying the actions of transcription factors, with nuclear factor-kappa B (NF-κB) a being potential candidate. NF-κB has many diverse functions in the nervous system and regulates a large number of genes encoding many inflammatory cytokines, such as IL-1β, IL-6, INF-α, and MCP-1; however, it also regulates anti- and proapoptotic proteins including Bcl-2, Bcl-xl, Bcl-xs, Bax, cyclooygenase-2, and inducible nitric oxide synthase [109]. NF-κB activation occurs following a signalling cascade initiated by external inflammatory stimuli and involves the phosphorylation and subsequent degradation of the IκB inhibitory subunit, which allows NF-κB translocation to the nucleus and the subsequent activation of expression of target genes [109].

The role of NF-κB appears to be determined by cell type and timing of activation. For example, activation of NF-κB in neurons associated with amyloid deposits is currently thought to be neuroprotective, whereas induction of NF-κB in glia may be neurotoxic. For reviews see [109–111]. EPA or omega-3 PUFA supplemented media decreases lipopolysaccharide-induced activation of NF-κB and TNF-α levels in cultured macrophages [112, 113] and monocytes [114], importantly, DHA suppresses IL-6 production and activation of NF-κB in lipopolysaccharide/interferon-γ stimulated glial cells [115], suggesting omega-3 PUFAs may have direct effects on inflammatory cytokine production via effects on the NF-κB signalling pathway. However, their site of action in this pathway and specific effects in neurons are yet to be determined.

6. Conclusions

Although there are strong correlations between low tissue levels of omega-3 PUFA and increased risk of AD, and low dietary intakes of omega-3 PUFA and cognitive decline and AD [116, 117], the results of dietary intervention studies have so far failed to live up to expectations raised by the preclinical and epidemiological studies. In the first study to look at the effects of omega-3 PUFA in AD, Yehuda and colleagues conducted a 4 week double-blind trial with 100 AD patients [118]. The supplemented group showed improvements in mood, cooperativity, short-term memory, appetite, sleep, and spatial orientation, whereas no improvements were seen in the placebo group. A subsequent pilot study investigated the effects of 500 mg EPA given twice daily for 12 weeks in patients with AD [119]. No differences were found and the authors concluded that EPA had no effects on cognition. A larger study in 174 patients found that administration of 1.7 g of DHA and 0.6 g of EPA per day for 6 months in patients with mild to moderate AD did not delay the rate of cognitive decline [120], although, positive effects were observed in a small subgroup of patients with very mild AD (MMSE >27 points).

Therefore, current evidence appears to indicate that the beneficial effects of omega-3 PUFAs are more related to limiting the progression of cognitive decline, as most clinical trials have so far failed to demonstrate the efficacy of omega-3 PUFA treatment after the onset of AD symptoms. However, it should be noted that preclinical studies have focused on the effects of DHA and its metabolites, whereas clinical studies have investigated EPA, or may have used insufficient doses of DHA. In brain tissue, DHA-derived metabolites promote resolution and protect neural cells from neurodegeneration [121] and may therefore be the more important omega-3 PUFA in modulating the secretion of cytokines and inhibiting neuroinflammation and oxidative stress in AD. Furthermore, recent evidence suggests that the beneficial effects of omega-3 PUFA on reducing the risk of dementia and AD may be reduced by the presence of the apolipoprotein E ε4 (*APOE* ε4) allele [122–124]. It is hoped that future clinical studies will help clarify these issues and the relative roles and utility of DHA and EPA in the treatment of AD.

References

[1] D. J. Selkoe, "Alzheimer's disease: genes, proteins, and therapy," *Physiological Reviews*, vol. 81, no. 2, pp. 741–766, 2001.

[2] M. F. Murphy, J. J. Sramek, N. M. Kurtz, A. Carta, and N. R. Cutler, *Alzheimer's Disease: Optimizing the Development of the Next Generation of Therapeutic Compounds*, Greenwich Medical Media, London, UK, 1998.

[3] H. Akiyama, S. Barger, S. Barnum, B. Bradt, J. Bauer, G. M. Cole, N. R. Cooper, P. Eikelenboom, M. Emmerling, B. L. Fiebich, C. E. Finch, S. Frautschy, W. S. T. Griffin, H. Hampel, M. Hull, G. Landreth, L.-F. Lue, R. Mrak, I. R. MacKenzie, P. L. McGeer, M. K. O'Banion, J. Pachter, G. Pasinetti, C. Plata-Salaman, J. Rogers, R. Rydel, Y. Shen, W. Streit, R. Strohmeyer, I. Tooyoma, F. L. Van Muiswinkel, R. Veerhuis, D. Walker, S. Webster, B. Wegrzyniak, G. Wenk, and T. Wyss-Coray, "Inflammation and Alzheimer's disease," *Neurobiology of Aging*, vol. 21, no. 3, pp. 383–421, 2000.

[4] W. R. Markesbery, "Oxidative stress hypothesis in Alzheimer's disease," *Free Radical Biology and Medicine*, vol. 23, no. 1, pp. 134–147, 1997.

[5] P. C. Calder, "Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale," *Biochimie*, vol. 91, pp. 791–795, 2009.

[6] S. C. Dyall and A. T. Michael-Titus, "Neurological benefits of omega-3 fatty acids," *NeuroMolecular Medicine*, vol. 10, no. 10, pp. 219–235, 2008.

[7] B. De Strooper, P. Saftig, K. Craessaerts, H. Vanderstichele, G. Guhde, W. Annaert, K. Von Figura, and F. Van Leuven, "Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein," *Nature*, vol. 391, no. 6665, pp. 387–390, 1998.

[8] D. Edbauer, E. Winkler, J. T. Regula, B. Pesold, H. Steiner, and C. Haass, "Reconstitution of γ-secretase activity," *Nature Cell Biology*, vol. 5, no. 5, pp. 486–488, 2003.

[9] R. Vassar, B. D. Bennett, S. Babu-Khan, S. Kahn, E. A. Mendiaz, P. Denis, D. B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M. A. Jarosinski, A. L. Biere, E. Curran, T. Burgess, J.-C. Louis, F. Collins, J. Treanor, G. Rogers, and M. Citron, "β-Secretase cleavage of Alzheimer's amyloid precursor protein by the

transmembrane aspartic protease BACE," *Science*, vol. 286, no. 5440, pp. 735–741, 1999.

[10] S. L. Cole and R. Vassar, "The role of amyloid precursor protein processing by BACE1, the β-secretase, in Alzheimer disease pathophysiology," *Journal of Biological Chemistry*, vol. 283, no. 44, pp. 29621–29625, 2008.

[11] D. M .A. Mann, T. Iwatsubo, N. J. Cairns, et al., "Amyloid β protein (Aβ) deposition in chromosome 14-linked Alzheimer's disease: predominance of Aβ42(43)," *Annals of Neurology*, vol. 40, no. 2, pp. 149–156, 1996.

[12] D. A. Butterfield and C. M. Lauderback, "Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress," *Free Radical Biology and Medicine*, vol. 32, no. 11, pp. 1050–1060, 2002.

[13] M. Hashimoto, S. Hossain, T. Shimada, K. Sugioka, H. Yamasaki, Y. Fuj II, Y. Ishibashi, J.-I. Oka, and O. Shido, "Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats," *Journal of Neurochemistry*, vol. 81, no. 5, pp. 1084–1091, 2002.

[14] M. Hashimoto, S. Hossain, H. Agdul, and O. Shido, "Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid β-infused rats relates to the decreases of amyloid β and cholesterol levels in detergent-insoluble membrane fractions," *Biochimica et Biophysica Acta*, vol. 1738, no. 1–3, pp. 91–98, 2005.

[15] M. Hashimoto, S. Hossain, T. Shimada, and O. Shido, "Docosahexaenoic acid-induced protective effect against impaired learning in amyloid β-infused rats is associated with increased synaptosomal membrane fluidity," *Clinical and Experimental Pharmacology and Physiology*, vol. 33, no. 10, pp. 934–939, 2006.

[16] M. Simons, P. Keller, B. de Strooper, K. Beyreuther, C. G. Dotti, and K. Simons, "Cholesterol depletion inhibits the generation of β-amyloid in hippocampal neurons," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 11, pp. 6460–6464, 1998.

[17] W. G. Wood, F. Schroeder, U. Igbavboa, N. A. Avdulov, and S. V. Chochina, "Brain membrane cholesterol domains, aging and amyloid beta-peptides," *Neurobiology of Aging*, vol. 23, no. 5, pp. 685–694, 2002.

[18] L. Puglielli, R. E. Tanzi, and D. M. Kovacs, "Alzheimer's disease: the cholesterol connection," *Nature Neuroscience*, vol. 6, no. 4, pp. 345–351, 2003.

[19] R. Ehehalt, P. Keller, C. Haass, C. Thiele, and K. Simons, "Amyloidogenic processing of the Alzheimer β-amyloid precursor protein depends on lipid rafts," *Journal of Cell Biology*, vol. 160, no. 1, pp. 113–123, 2003.

[20] K. Simons and D. Toomre, "Lipid rafts and signal transduction," *Nature Reviews Molecular Cell Biology*, vol. 1, no. 1, pp. 31–39, 2000.

[21] W. G. Wood, F. Schroeder, U. Igbavboa, N. A. Avdulov, and S. V. Chochina, "Brain membrane cholesterol domains, aging and amyloid beta-peptides," *Neurobiology of Aging*, vol. 23, no. 5, pp. 685–694, 2002.

[22] S. Yehuda, S. Rabinovitz, R. L. Carasso, and D. I. Mostofsky, "The role of polyunsaturated fatty acids in restoring the aging neuronal membrane," *Neurobiology of Aging*, vol. 23, no. 5, pp. 843–853, 2002.

[23] S. R. Shaikh, M. R. Brzustowicz, N. Gustafson, W. Stillwell, and S. R. Wassall, "Monounsaturated PE does not phase-separate from the lipid raft molecules sphingomyelin and cholesterol: role for polyunsaturation?" *Biochemistry*, vol. 41, no. 34, pp. 10593–10602, 2002.

[24] S. R. Shaikh, V. Cherezov, M. Caffrey, W. Stillwell, and S. R. Wassall, "Interaction of cholesterol with a docosahexaenoic acid-containing phosphatidylethanolamine: trigger for microdomain/raft formation?" *Biochemistry*, vol. 42, no. 41, pp. 12028–12037, 2003.

[25] K. N. Green, H. Martinez-Coria, H. Khashwji, E. B. Hall, K. A. Yurko-Mauro, L. Ellis, and F. M. LaFerla, "Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-β and tau pathology via a mechanism involving presenilin 1 levels," *Journal of Neuroscience*, vol. 27, no. 16, pp. 4385–4395, 2007.

[26] Q.-L. Ma, B. Teter, O. J. Ubeda, T. Morihara, D. Dhoot, M. D. Nyby, M. L. Tuck, S. A. Frautschy, and G. M. Cole, "Omega-3 fatty acid docosahexaenoic acid increases SorLA/LR11, a sorting protein with reduced expression in sporadic Alzheimer's disease (AD): relevance to AD prevention," *Journal of Neuroscience*, vol. 27, no. 52, pp. 14299–14307, 2007.

[27] K. Offe, S. E. Dodson, J. T. Shoemaker, J. J. Fritz, M. Gearing, A. I. Levey, and J. J. Lah, "The lipoprotein receptor LR11 regulates amyloid β production and amyloid precursor protein traffic in endosomal compartments," *Journal of Neuroscience*, vol. 26, no. 5, pp. 1596–1603, 2006.

[28] W. J. Lukiw, J.-G. Cui, V. L. Marcheselli, M. Bodker, A. Botkjaer, K. Gotlinger, C. N. Serhan, and N. G. Bazan, "A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease," *Journal of Clinical Investigation*, vol. 115, no. 10, pp. 2774–2783, 2005.

[29] P. K. Mukherjee, V. L. Marcheselli, C. N. Serhan, and N. G. Bazan, "Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 22, pp. 8491–8496, 2004.

[30] V. L. Marcheselli, S. Hong, W. J. Lukiw, X. H. Tian, K. Gronert, A. Musto, M. Hardy, J. M. Gimenez, N. Chiang, C. N. Serhan, and N. G. Bazan, "Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression," *Journal of Biological Chemistry*, vol. 278, no. 44, pp. 43807–43817, 2003.

[31] G. W. Arendash, M. T. Jensen, N. Salem Jr., N. Hussein, J. Cracchiolo, A. Dickson, R. Leighty, and H. Potter, "A diet high in omega-3 fatty acids does not improve or protect cognitive performance in Alzheimer's transgenic mice," *Neuroscience*, vol. 149, no. 2, pp. 286–302, 2007.

[32] G. Barceló-Coblijn, K. Kitajka, L. G. Puskás, E. Hogyes, A. Zvara, L. Hackler Jr., and T. Farkas, "Gene expression and molecular composition of phospholipids in rat brain in relation to dietary n-6 to n-3 fatty acid ratio," *Biochimica et Biophysica Acta* , vol. 1632, no. 1–3, pp. 72–79, 2003.

[33] C. D. Link, "Expression of human β-amyloid peptide in transgenic *Caenorhabditis elegans*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 20, pp. 9368–9372, 1995.

[34] J.-M. Serot, D. Christmann, T. Dubost, and M. Couturier, "Cerebrospinal fluid transthyretin: aging and late onset Alzheimer's disease," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 63, no. 4, pp. 506–508, 1997.

[35] J. P. T. Corcoran, P. L. So, and M. Maden, "Disruption of the retinoid signalling pathway causes a deposition of amyloid β in the adult rat brain," *European Journal of Neuroscience*, vol. 20, no. 4, pp. 896–902, 2004.

[36] Y. Ding, A. Qiao, Z. Wang, J. S. Goodwin, E.-S. Lee, M. L. Block, M. Allsbrook, M. P. McDonald, and G.-H. Fan, "Retinoic acid attenuates β-amyloid deposition and rescues memory deficits in an Alzheimer's disease transgenic mouse model," *Journal of Neuroscience*, vol. 28, no. 45, pp. 11622–11634, 2008.

[37] D. J. Mangelsdorf and D. A. Evans, "The RXR heterodimers and orphan receptors," *Cell*, vol. 83, no. 6, pp. 841–850, 1995.

[38] A. M. de Urquiza, S. Liu, M. Sjoberg, R. H. Zetterstrom, W. Griffiths, J. Sjovall, and T. Perlmann, "Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain," *Science*, vol. 290, no. 5499, pp. 2140–2144, 2000.

[39] P. F. Egea, A. Mitschler, and D. Moras, "Molecular recognition of agonist ligands by RXRs," *Molecular Endocrinology*, vol. 16, no. 5, pp. 987–997, 2002.

[40] J. Lengqvist, A. Mata de Urquiza, A.-C. Bergman, T. M. Willson, J. Sjövall, T. Perlmann, and W. J. Griffiths, "Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor α ligand-binding domain," *Molecular and Cellular Proteomics*, vol. 3, no. 7, pp. 692–703, 2004.

[41] S. C. Dyall, G. J. Michael, and A. T. Michael-Titus, "Omega-3 fatty acids reverse age-related decreases innuclear receptors and increase neurogenesis in old rats," *Journal of Neuroscience Research*, vol. 88, pp. 2091–2102, 2010.

[42] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[43] I. E. Camacho, L. Serneels, K. Spittaels, P. Merchiers, D. Dominguez, and B. De Strooper, "Peroxisome proliferator-activated receptor γ induces a clearance mechanism for the amyloid-β peptide," *Journal of Neuroscience*, vol. 24, no. 48, pp. 10908–10917, 2004.

[44] M. Sastre, I. Dewachter, G. E. Landreth, et al., "Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-gamma agonists modulate immunostimulated processing of amyloid precursor protein through regulation of beta-secretase," *Journal of Neuroscience*, vol. 23, pp. 9796–9804, 2003.

[45] M. Sastre, I. Dewachter, S. Rossner, et al., "Nonsteroidal anti-inflammatory drugs repress β-secretase gene promoter activity by the activation of PPARγ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 2, pp. 443–448, 2006.

[46] M. T. Heneka, M. Sastre, L. Dumitrescu-Ozimek, et al., "Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV717I transgenic mice," *Brain*, vol. 128, pp. 1442–1453, 2005.

[47] K. Fuenzalida, R. Quintanilla, P. Ramos, D. Piderit, R. A. Fuentealba, G. Martinez, N. C. Inestrosa, and M. Bronfman, "Peroxisome proliferator-activated receptor γ up-regulates the Bcl-2 anti-apoptotic protein in neurons and induces mitochondrial stabilization and protection against oxidative stress and apoptosis," *Journal of Biological Chemistry*, vol. 282, no. 51, pp. 37006–37015, 2007.

[48] G. S. Watson, B. A. Cholerton, M. A. Reger, L. D. Baker, S. R. Plymate, S. Asthana, M. A. Fishel, J. J. Kulstad, P. S. Green, D. G. Cook, S. E. Kahn, M. L. Keeling, and S. Craft, "Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study," *American Journal of Geriatric Psychiatry*, vol. 13, no. 11, pp. 950–958, 2005.

[49] C. Chambrier, J.-P. Bastard, J. Rieusset, E. Chevillotte, D. Bonnefont-Rousselot, P. Therond, B. Hainque, J.-P. Riou, M. Laville, and H. Vidal, "Eicosapentaenoic acid induces mRNA expression of peroxisome proliferator-activated receptor γ," *Obesity Research*, vol. 10, no. 6, pp. 518–525, 2002.

[50] T. Itoh, I. Murota, K. Yoshikai, S. Yamada, and K. Yamamoto, "Synthesis of docosahexaenoic acid derivatives designed as novel PPARγ agonists and antidiabetic agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 14, no. 1, pp. 98–108, 2006.

[51] K. Yamamoto, T. Itoh, D. Abe, M. Shimizu, T. Kanda, T. Koyama, M. Nishikawa, T. Tamai, H. Ooizumi, and S. Yamada, "Identification of putative metabolites of docosahexaenoic acid as potent PPARγ agonists and antidiabetic agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 15, no. 3, pp. 517–522, 2005.

[52] E. G. McGeer and P. L. McGeer, "Inflammatory processes in Alzheimer's disease," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 27, no. 5, pp. 741–749, 2003.

[53] W. S. T. Griffin, "Inflammation and neurodegenerative diseases," *American Journal of Clinical Nutrition*, vol. 83, no. 2, pp. 470S–474S, 2006.

[54] C. K. Combs, D. E. Johnson, S. B. Cannady, T. M. Lehman, and G. E. Landreth, "Identification of microglial signal transduction pathways mediating a neurotoxic response to amyloidogenic fragments of β-amyloid and prion proteins," *Journal of Neuroscience*, vol. 19, no. 3, pp. 928–939, 1999.

[55] M. D. Miranda, V. M. S. de Bruin, M. R. Vale, and G. S. B. Viana, "Lipid peroxidation and nitrite plus nitrate levels in brain tissue from patients with Alzheimer's disease," *Gerontology*, vol. 46, no. 4, pp. 179–184, 2000.

[56] T. I. Williams, B. C. Lynn, W. R. Markesbery, and M. A. Lovell, "Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease," *Neurobiology of Aging*, vol. 27, no. 8, pp. 1094–1099, 2006.

[57] D. Praticò, K. Uryu, S. Leight, J. Q. Trojanoswki, and V. M.-Y. Lee, "Increased lipid peroxidation precedes amyloid plaque formation in an animal model of alzheimer amyloidosis," *Journal of Neuroscience*, vol. 21, no. 12, pp. 4183–4187, 2001.

[58] J. D. Morrow, J. A. Awad, H. J. Boss, I. A. Blair, and L. J. Roberts II, "Non-cyclooxygenase-derived prostanoids (F_2-isoprostanes) are formed in situ on phospholipids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 22, pp. 10721–10725, 1992.

[59] J. Nourooz-Zadeh, B. Halliwell, and E. E. Änggård, "Evidence for the formation of F_3-isoprostanes during peroxidation of eicosapentaenoic acid," *Biochemical and Biophysical Research Communications*, vol. 236, no. 2, pp. 467–472, 1997.

[60] J. Nourooz-Zadeh, E. H. C. Liu, E. E. Änggård, and B. Halliwell, "F_4-isoprostanes: a novel class of prostanoids formed during peroxidation of docosahexaenoic acid (DHA)," *Biochemical and Biophysical Research Communications*, vol. 242, no. 2, pp. 338–344, 1998.

[61] S. S. Fam, L. J. Murphey, E. S. Terry, W. E. Zackert, Y. Chen, L. Gao, S. Pandalai, G. L. Milne, L. Jackson Roberts, N. A. Porter, T. J. Montine, and J. D. Morrow, "Formation of highly reactive A-ring and J-ring isoprostane-like compounds (A4/J4-neuroprostanes) in vivo from docosahexaenoic acid," *Journal of Biological Chemistry*, vol. 277, no. 39, pp. 36076–36084, 2002.

[62] J.-L. Cracowski, T. Durand, and G. Bessard, "Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications," *Trends in Pharmacological Sciences*, vol. 23, no. 8, pp. 360–366, 2002.

[63] L. J. Roberts II and J. F. Reckelhoff, "Measurement of F_2-isoprostanes unveils profound oxidative stress in aged rats," *Biochemical and Biophysical Research Communications*, vol. 287, no. 1, pp. 254–256, 2001.

[64] J. A. Youssef, L. S. Birnbaum, L. L. Swift, J. D. Morrow, and M. Z. Badr, "Age-independent, gray matter-localized, brain-enhanced oxidative stress in male fischer 344 rats: brain levels of F2-isoprostanes and F4-neuroprostanes," *Free Radical Biology and Medicine*, vol. 34, no. 12, pp. 1631–1635, 2003.

[65] J. Nourooz-Zadeh, E. H. C. Liu, B. Yhlen, E. E. Änggård, and B. Halliwell, "F_4-isoprostanes as specific marker of docosahexaenoic acid peroxidation in Alzheimer's disease," *Journal of Neurochemistry*, vol. 72, no. 2, pp. 734–740, 1999.

[66] E. E. Reich, W. R. Markesbery, L. J. Roberts II, L. L. Swift, J. D. Morrow, and T. J. Montine, "Brain regional quantification of F-ring and D-/E-ring isoprostanes and neuroprostanes in Alzheimer's disease," *American Journal of Pathology*, vol. 158, no. 1, pp. 293–297, 2001.

[67] J. P. Cosgrove, D. F. Church, and W. A. Pryor, "The kinetics of the autoxidation of polyunsaturated fatty acids," *Lipids*, vol. 22, no. 5, pp. 299–304, 1987.

[68] K. Kubo, M. Saito, T. Tadokoro, and A. Maekawa, "Changes in susceptibility of tissues to lipid peroxidation after ingestion of various levels of docosahexaenoic acid and vitamin E," *British Journal of Nutrition*, vol. 78, no. 4, pp. 655–669, 1997.

[69] M. Meydani, F. Natiello, B. Goldin, N. Free, M. Woods, E. Schaefer, J. B. Blumberg, and S. L. Gorbach, "Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women," *Journal of Nutrition*, vol. 121, no. 4, pp. 484–491, 1991.

[70] A. Jenkinson, M. F. Franklin, K. Wahle, and G. G. Duthie, "Dietary intakes of polyunsaturated fatty acids and indices of oxidative stress in human volunteers," *European Journal of Clinical Nutrition*, vol. 53, no. 7, pp. 523–528, 1999.

[71] J. P. Allard, R. Kurian, E. Aghdassi, R. Muggli, and D. Royall, "Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans," *Lipids*, vol. 32, no. 5, pp. 535–541, 1997.

[72] H. Grundt, D. W. T. Nilsen, M. A. Mansoor, and A. Nordøy, "Increased lipid peroxidation during long-term intervention with high doses of n-3 fatty acids (PUFAs) following an acute myocardial infarction," *European Journal of Clinical Nutrition*, vol. 57, no. 6, pp. 793–800, 2003.

[73] J. H. Song and T. Miyazawa, "Enhanced level of n-3 fatty acid in membrane phospholipids induces lipid peroxidation in rats fed dietary docosahexaenoic acid oil," *Atherosclerosis*, vol. 155, no. 1, pp. 9–18, 2001.

[74] K. Ando, K. Nagata, R. Yoshida, K. Kikugawa, and M. Suzuki, "Effect of n-3 polyunsaturated fatty acid supplementation on lipid peroxidation of rat organs," *Lipids*, vol. 35, no. 4, pp. 401–407, 2000.

[75] J. V. Higdon, J. Liu, S.-H. Du, J. D. Morrow, B. N. Ames, and R. C. Wander, "Supplementation of postmenopausal women with fish oil rich in eicosapentaenoic acid and docosahexaenoic acid is not associated with greater in vivo lipid peroxidation compared with oils rich in oleate and linoleate as assessed by plasma malondialdehyde and F_2-isoprostanes," *American Journal of Clinical Nutrition*, vol. 72, no. 3, pp. 714–722, 2000.

[76] P. Green, S. Glozman, L. Weiner, and E. Yavin, "Enhanced free radical scavenging and decreased lipid peroxidation in the rat fetal brain after treatment with ethyl docosahexaenoate," *Biochimica et Biophysica Acta*, vol. 1532, no. 3, pp. 203–212, 2001.

[77] M. S. Hossain, M. Hashimoto, and S. Masumura, "Influence of docosahexaenoic acid on cerebral lipid peroxide level in aged rats with and without hypercholesterolemia," *Neuroscience Letters*, vol. 244, no. 3, pp. 157–160, 1998.

[78] K. Kubo, M. Saito, T. Tadokoro, and A. Maekawa, "Dietary docosahexaenoic acid dose not promote lipid peroxidation in rat tissue to the extent expected from peroxidizability index of the lipids," *Bioscience, Biotechnology and Biochemistry*, vol. 62, no. 9, pp. 1698–1706, 1998.

[79] G. P. Lim, F. Calon, T. Morihara, F. Yang, B. Teter, O. Ubeda, N. Salem Jr., S. A. Frautschy, and G. M. Cole, "A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model," *Journal of Neuroscience*, vol. 25, no. 12, pp. 3032–3040, 2005.

[80] E. Yavin, A. Brand, and P. Green, "Docosahexaenoic acid abundance in the brain: a biodevice to combat oxidative stress," *Nutritional Neuroscience*, vol. 5, no. 3, pp. 149–157, 2002.

[81] M. S. Hossain, M. Hashimoto, S. Gamoh, and S. Masumura, "Antioxidative effects of docosahexaenoic acid in the cerebrum versus cerebellum and brainstem of aged hypercholesterolemic rats," *Journal of Neurochemistry*, vol. 72, no. 3, pp. 1133–1138, 1999.

[82] P. Green, S. Glozman, and E. Yavin, "Ethyl docosahexaenoate-associated decrease in fetal brain lipid peroxide production is mediated by activation of prostanoid and nitric oxide pathways," *Biochimica et Biophysica Acta*, vol. 1531, no. 1-2, pp. 156–164, 2001.

[83] M. Plourde, M. Fortier, M. Vandal, J. Tremblay-Mercier, E. Freemantle, M. Bégin, F. Pifferi, and S. C. Cunnane, "Unresolved issues in the link between docosahexaenoic acid and Alzheimer's disease," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 77, no. 5-6, pp. 301–308, 2007.

[84] S. C. Dyall, G. J. Michael, R. Whelpton, A. G. Scott, and A. T. Michael-Titus, "Dietary enrichment with omega-3 polyunsaturated fatty acids reverses age-related decreases in the GluR2 and NR2B glutamate receptor subunits in rat forebrain," *Neurobiology of Aging*, vol. 28, no. 3, pp. 424–439, 2007.

[85] T. Wyss-Coray, "Inflammation in Alzheimer disease: driving force, bystander or beneficial response?" *Nature Medicine*, vol. 12, no. 9, pp. 1005–1015, 2006.

[86] W. S. T. Griffin, L. C. Stanley, C. Ling, L. White, V. MacLeod, L. J. Perrot, C. L. White III, and C. Araoz, "Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 19, pp. 7611–7615, 1989.

[87] C. A. Murray and M. A. Lynch, "Evidence that increased hippocampal expression of the cytokine interleukin-1β is a common trigger for age- and stress-induced impairments in long-term potentiation," *Journal of Neuroscience*, vol. 18, no. 8, pp. 2974–2981, 1998.

[88] C. A. Murray, M. P. Clements, and M. A. Lynch, "Interleukin-1 induces lipid peroxidation and membrane changes in rat hippocampus: an age-related study," *Gerontology*, vol. 45, no. 3, pp. 136–142, 1999.

[89] M. C. P. Godoy, R. Tarelli, C. C. Ferrari, M. I. Sarchi, and F. J. Pitossi, "Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease," *Brain*, vol. 131, no. 7, pp. 1880–1894, 2008.

[90] W. S. T. Griffin, L. Liu, Y. Li, R. E. Mrak, and S. W. Barger, "Interleukin-1 mediates Alzheimer and Lewy body pathologies," *Journal of Neuroinflammation*, vol. 3, p. 5, 2006.

[91] A. M. Lynch and M. A. Lynch, "The age-related increase in IL-1 type I receptor in rat hippocampus is coupled with an increase in caspase-3 activation," *European Journal of Neuroscience*, vol. 15, no. 11, pp. 1779–1788, 2002.

[92] D. S. D. Martin, P. E. Lonergan, B. Boland, M. P. Fogarty, M. Brady, D. F. Horrobin, V. A. Campbell, and M. A. Lynch, "Apoptotic changes in the aged brain are triggered by interleukin-1β-induced activation of p38 and reversed by treatment with eicosapentaenoic acid," *Journal of Biological Chemistry*, vol. 277, no. 37, pp. 34239–34246, 2002.

[93] P. Taepavarapruk and C. Song, "Reductions of acetylcholine release and nerve growth factor expression are correlated with memory impairment induced by interleukin-1β administrations: effects of omega-3 fatty acid EPA treatment," *Journal of Neurochemistry*, vol. 112, no. 4, pp. 1054–1064, 2010.

[94] E. J. Coulson, L. M. May, A. M. Sykes, and A. S. Hamlin, "The role of the p75 neurotrophin receptor in cholinergic dysfunction in Alzheimer's disease," *Neuroscientist*, vol. 15, no. 4, pp. 317–323, 2009.

[95] S. Hong, K. Gronert, P. R. Devchand, R.-L. Moussignac, and C. N. Serhan, "Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells: autacoids in anti-inflammation," *Journal of Biological Chemistry*, vol. 278, no. 17, pp. 14677–14687, 2003.

[96] C. N. Serhan and N. Chiang, "Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus," *British Journal of Pharmacology*, vol. 153, no. 1, pp. S200–S215, 2008.

[97] M. Arita, F. Bianchini, J. Aliberti, A. Sher, N. Chiang, S. Hong, R. Yang, N. A. Petasis, and C. N. Serhan, "Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1," *Journal of Experimental Medicine*, vol. 201, no. 5, pp. 713–722, 2005.

[98] A. Ariel, P.-L. Li, W. Wang, W.-X. Tang, G. Fredman, S. Hong, K. H. Gotlinger, and C. N. Serhan, "The docosatriene protectin D1 is produced by TH2 skewing promotes human T cell via lipid raft clustering," *Journal of Biological Chemistry*, vol. 280, no. 52, pp. 43079–43086, 2005.

[99] T. R. Billiar, P. E. Bankey, B. A. Svingen, R. D. Curran, M. A. West, R. T. Holman, R. L. Simmons, and F. B. Cerra, "Fatty acid intake and Kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation," *Surgery*, vol. 104, no. 2, pp. 343–349, 1988.

[100] A. L. Cooper, L. Gibbons, M. A. Horan, R. A. Little, and N. J. Rothwell, "Effect of dietary fish oil supplementation on fever and cytokine production in human volunteers," *Clinical Nutrition*, vol. 12, no. 6, pp. 321–328, 1993.

[101] S. Endres, R. Ghorbani, V. E. Kelley, K. Georgilis, G. Lonnemann, J. W. M. Van Der Meer, J. G. Cannon, T. S. Rogers, M. S. Klempner, P. C. Weber, E. J. Schaefer, S. M. Wolff, and C. A. Dinarello, "The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells," *New England Journal of Medicine*, vol. 320, no. 5, pp. 265–271, 1989.

[102] K. Fritsche, "Fatty acids as modulators of the immune response," *Annual Review of Nutrition*, vol. 26, pp. 45–73, 2006.

[103] S. N. Meydani, S. Endres, M. M. Woods, B. R. Goldin, C. Soo, A. Morrill-Labrode, C. A. Dinarello, and S. L. Gorbach, "Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women," *Journal of Nutrition*, vol. 121, no. 4, pp. 547–555, 1991.

[104] S. N. Meydani, A. H. Lichtenstein, S. Cornwall, M. Meydani, B. R. Goldin, H. Rasmussen, C. A. Dinarello, and E. J. Schaefer, "Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived N-3 fatty acid enrichment," *Journal of Clinical Investigation*, vol. 92, no. 1, pp. 105–113, 1993.

[105] G. Renier, E. Skamene, J. DeSanctis, and D. Radzioch, "Dietary n-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice. Modulation of macrophage secretory activities," *Arteriosclerosis and Thrombosis*, vol. 13, no. 10, pp. 1515–1524, 1993.

[106] S. Kew, M. D. Mesa, S. Tricon, R. Bitckley, A. M. Minihane, and P. Yaqoob, "Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans," *American Journal of Clinical Nutrition*, vol. 79, no. 4, pp. 674–681, 2004.

[107] O. Obajimi, K. D. Black, D. J. MacDonald, R. M. Boyle, I. Glen, and B. M. Ross, "Differential effects of eicosapentaenoic and docosahexaenoic acids upon oxidant-stimulated release and uptake of arachidonic acid in human lymphoma U937 cells," *Pharmacological Research*, vol. 52, no. 2, pp. 183–191, 2005.

[108] I. Vedin, T. Cederholm, Y. F. Levi, et al., "Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study," *American Journal of Clinical Nutrition*, vol. 87, no. 6, pp. 1616–1622, 2008.

[109] R. Malek, K. K. Borowicz, M. Jargiello, and S. J. Czuczwar, "Role of nuclear factor kappaB in the central nervous system," *Pharmacological Reports*, vol. 59, no. 1, pp. 25–33, 2007.

[110] B. Kaltschmidt and C. Kaltschmidt, "NF-kappaB in the nervous system," *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 3, Article ID a001271, 2009.

[111] M. P. Mattson and S. Camandola, "NF-κB in neuronal plasticity and neurodegenerative disorders," *Journal of Clinical Investigation*, vol. 107, no. 3, pp. 247–254, 2001.

[112] T. E. Novak, T. A. Babcock, D. H. Jho, W. S. Helton, and N. J. Espat, "NF-κB inhibition by ω-3 fatty acids modulates LPS-stimulated macrophage TNF-α-transcription," *American Journal of Physiology*, vol. 284, no. 1, pp. L84–L89, 2003.

[113] C.-J. Lo, K. C. Chiu, M. Fu, R. Lo, and S. Helton, "Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NFκB activity," *Journal of Surgical Research*, vol. 82, no. 2, pp. 216–221, 1999.

[114] Y. Zhao, S. Joshi-Barve, S. Barve, and L. H. Chen, "Eicosapentaenoic acid Prevents LPS-induced TNF-α expression by preventing NF-κB activation," *Journal of the American College of Nutrition*, vol. 23, no. 1, pp. 71–78, 2004.

[115] H.-C. Pan, T.-K. Kao, Y.-C. Ou, D.-Y. Yang, Y.-J. Yen, C.-C. Wang, Y.-H. Chuang, S.-L. Liao, S.-L. Raung, C.-W. Wu, A.-N. Chiang, and C.-J. Chen, "Protective effect of docosahexaenoic acid against brain injury in ischemic rats," *Journal of Nutritional Biochemistry*, vol. 20, no. 9, pp. 715–725, 2009.

[116] P. Barberger-Gateau, L. Letenneur, V. Deschamps, K. Pérès, J.-F. Dartigues, and S. Renaud, "Fish, meat, and risk of dementia: cohort study," *British Medical Journal*, vol. 325, no. 7370, pp. 932–933, 2002.

[117] M. C. Morris, "Docosahexaenoic acid and Alzheimer disease," *Archives of Neurology*, vol. 63, no. 11, pp. 1527–1528, 2006.

[118] S. Yehuda, S. Rabinovtz, R. L. Carasso, and D. I. Mostofsky, "Essential fatty acids preparation (SR-3) improves Alzheimer's patients quality of life," *International Journal of Neuroscience*, vol. 87, no. 3-4, pp. 141–149, 1996.

[119] P. F. Boston, A. Bennett, D. F. Horrobin, and C. N. Bennett, "Ethyl-EPA in Alzheimer's disease—a pilot study," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 71, no. 5, pp. 341–346, 2004.

[120] Y. Freund-Levi, M. Eriksdotter-Jönhagen, T. Cederholm, H. Basun, G. Faxén-Irving, A. Garlind, I. Vedin, B. Vessby, L.-O. Wahlund, and J. Palmblad, "ω-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: omegAD study: a randomized double-blind trial," *Archives of Neurology*, vol. 63, no. 10, pp. 1402–1408, 2006.

[121] N. G. Bazan, "Neuroprotectin D1 (NPD1): a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress," *Brain Pathology*, vol. 15, no. 2, pp. 159–166, 2005.

[122] T. L. Huang, P. P. Zandi, K. L. Tucker, et al., "Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4," *Neurology*, vol. 65, no. 9, pp. 1409–1414, 2005.

[123] M. Plourde, M. C. Vohl, M. Vandal, P. Couture, S. Lemieux, and S. C. Cunnane, "Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men," *British Journal of Nutrition*, vol. 102, pp. 1121–1124, 2009.

[124] L. J. Whalley, I. J. Deary, J. M. Starr, K. W. Wahle, K. A. Rance, V. J. Bourne, and H. C. Fox, "n-3 Fatty acid erythrocyte membrane content, APOE ε4, and cognitive variation: an observational follow-up study in late adulthood," *American Journal of Clinical Nutrition*, vol. 87, no. 2, pp. 449–454, 2008.

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Research Article

Intereleukin-10 Promoter Polymorphism in Mild Cognitive Impairment and in Its Clinical Evolution

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Specific proinflammatory alleles are associated with higher risk of Alzheimer disease (AD) in different onset age. The homozygosis for the A allele of −1082 polymorphism (G/A) of interleukin-10 (IL-10) promotes a higher risk of AD and reduced IL-10 generation in peripheral cells after amyloid stimulation. In this paper we analysed genotype and allele frequencies of this polymorphism in 138 subjects with mild cognitive impairment (MCI) diagnosed, respectively, as amnestic (a-MCI) and multiple impaired cognitive domains (mcd-MCI). The genotype frequencies were similar in a-MCI and AD subjects, whereas in mcd-MCI comparable to controls (AA genotype: 50% in a-MCI, 49.2% in AD, 28.7% in mcd-MCI and 31.8% in controls). Consequently, both allele and genotype distributions were significantly different between a-MCI and mcd-MCI (allele: $P = .02$, genotype: $P < .05$). These results support the theory that polymorphisms of cytokine genes can affect neurodegeneration and its clinical progression. IL-10 may partly explain the conversion of a-MCI to AD or be a genetic marker of susceptibility.

1. Introduction

The pathogenic process of Alzheimer's disease (AD) starts decades before the clinical onset of the disease [1]. During this preclinical phase, there is a gradual loss of axons and neurons, and at a certain threshold the first symptoms, most often impaired episodic memory, appear. At this stage, patients do not fulfil the criteria for dementia and may be diagnosed with mild cognitive impairment (MCI). There is considerable clinical heterogeneity of this pathology since different clinical patterns can be recognized: amnestic MCI (a-MCI), MCI with multiple impaired cognitive domains (mcd-MCI), and single nonmemory domain MCI [2]. Although a-MCI may be the preclinical stage of AD, there is no established method to predict progression to AD in individuals with MCI.

Inflammation is accepted to be a feature of AD [3, 4] and the pathogeneses of neurodegeneration have been at least in part attributed to the release of proinflammatory cytokines from brain resident cells [5, 6] and, although less consistently, from peripheral cell [7, 8]. Furthermore, an increased intrathecal production of the proinflammatory cytokine TNF-α and a decreased production of the anti-inflammatory cytokine TGF-β have been demonstrated in the brain of patients with MCI, suggesting there is a proinflammatory state in such patients at high risk for AD [9].

Moreover, circulating acute phase reactant levels in middle age predict AD risk in old age and in particular certain functional promoter polymorphisms in cognate genes that modulate inflammation are often found at elevated frequency among AD cases.

Recently specific risk sets of proinflammatory alleles were identified that characterize AD in different onset age (before age 65, at ages 65–74, and at older ages) [10].

These alleles comprise also the −1082 promoter gene polymorphisms of IL-10 (G/A substitution) [11].

IL-10 maps to chromosome 1 between 1q31 and 1q32 is highly polymorphic, and its production is correlated to biallelic polymorphisms at positions −1082 (G to A),

−819 (T to C), and −592 (A to C). The polymorphism at position −1082 lies within an Ets (E-twenty-six specific)-like recognition site and may affect the binding of this transcriptional factor and, therefore, alter transcription activation; the −1082 A allele correlates with low IL-10 generation after stimulation of T cells in vitro [12], while polymorphisms at positions −819 and −592 do not seem to be involved.

In a previous study, we found that the homozygosis for the A allele of the IL-10 −1082 G/A single nucleotide polymorphism (SNP) was associated with six-fold higher risk of AD. In the same study, we also analysed the production of IL-10 in Peripheral Blood Mononuclear Cells (PBMCs) of AD patients and age-matched controls after specific stimulation with amyloid peptide, LPS, and Flu. Since the generation of IL-10 was reduced in patients after amyloid stimulation, we concluded that these specific immune responses may be selectively impaired in AD [13].

The aim of this study was to analyse the genotype and allele frequencies of these IL-10 SNPs in 138 subjects with MCI and to compare them with those previously shown in AD and healthy controls (HCs) [13].

2. Materials and Methods

2.1. Study Protocol. This study comprised 138 subjects with MCI age 80.37 ± 5.93 years (mean ± standard deviation (SD)). All patients were Caucasian, living in Northern Italy, and selected from a larger ambulatory population sample followed at the Geriatric Unit of the Ospedale Maggiore Policlinico IRCCS, University of Milan, Italy and the Geriatric Clinic of the University of Milan-Bicocca, Italy.

At enrolment, MCI subjects were divided into two groups based on cognitive features and diagnosed, respectively, as a-MCI (30 patients) and mcd-MCI (108 patients).

In particular, a-MCI met the criteria described by Petersen [14]: subjects with memory impairment only (>1.5 SD above the age- and education-specific norms) and no difficulties in any other area of cognitive functions. mcd-MCIs were subjects diagnosed with impairment in at least two cognitive domains of more than 1 SD below the mean of the respective age- and education-matched population, and with cognitive decline confirmed by the individuals themselves or reliable informants, but in whom no diagnosis of dementia could be achieved. A cut-off score of 1 SD was applied, which is less severe than that used for a-MCI, in order to obtain higher diagnostic sensitivity even though diagnostic specificity was reduced. Because the presence of more than one cognitive deficit and frequently initial impairment in Lawton's instrumental activities of daily living also characterized mcd-MCI, it may be mistaken for dementia; thus, a less severe criterion (>1 SD) allows better differentiation between mcd-MCI and dementia [15].

At this time, 74 patients out of the 138 completed a four-year follow-up and 24 were diagnosed with AD, 22 with vascular dementia (MCI → VD), and 28 with stable MCI [16]. Subjects who developed AD during follow-up were required to meet the DSM IV (Diagnostic and Statistical Manual of mental Disorders—4th ed.) and NINCDS-ADRDA (National Institute of Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association Work Group) criteria [17].

Within MCI who progressed to AD (MCI → AD), only two were diagnosed as mcd-MCI at enrolment; all the others were diagnosed as a-MCI.

In order to minimize the risk of possible inflammatory processes, all subjects were selected in the absence of clinical signs of inflammation (e.g., normal body temperature, no concomitant inflammatory condition) and with normal blood chemistry (red blood cell sedimentation rate, albumin, transferring, and C reactive protein plasma levels).

Informed consent was obtained from all subjects and the Ethics Committee of both universities approved the study, which was conducted according to the Helsinki II declaration. This population was matched with AD patients ($n = 63$) and nonsdemented sex- and age-matched healthy controls ($n = 63$) enrolled for our previous study [13].

2.2. Gene Polymorphism Analysis. Whole blood was collected by venipuncture in Vacutainer tubes containing EDTA (Becton Dickinson Co., Rutherford, NJ).

Genomic DNA was extracted by the salting-out method as described in [18]. The concentration and purity of DNA were determined by spectrophotometric analysis. In order to establish IL-10 genotypes we employed a polymerase chain reaction using sequence-specific primers (PCR-SSPs). The sequence in the promoter region of the IL-10 gene (polymorphic positions −1082, −819, and −592) was amplified using the cytokine genotyping tray method (One Lambda, Canoga Park, CA, USA). The human β-globin gene was amplified as an internal control for the genomic DNA preparation. PCR conditions were indicated by the One Lambda PCR program (OLI-1) and the PCR products were visualised by electrophoresis in 2.5% agarose gel.

ApoE genotypes were determined by means of PCR amplification of a 234 base-pair fragment of exon 4 of the ApoE gene, followed by digestion with Cfo1. The restriction patterns were revealed by means of 4% agarose gel electrophoresis [13].

2.3. Statistical Analysis. Statistical analysis was performed with the SPSS statistical package (SPSS version 17, Chicago, IL). Genotype and allele frequencies in the study groups were compared using the χ^2-test. $P < .05$ was taken as the cut-off for statistical significance.

3. Results

3.1. Distribution of IL-10 Genotypes in MCI Subjects. The genotype and allele frequencies of the biallelic polymorphism at position −1082 are reported in Table 1. This SNP alters transcriptional activation with a gene dosage-related effect, so GG genotype correlates with high, GA with intermediate, and AA with low IL-10 production after stimulation of T cells in vitro [12].

Table 1: Distribution of genotype and allele frequencies of −1082 (G/A) SNP in Alzheimer's disease patients (AD), control subjects (CT), and mild cognitive impairment patients (MCI).

	GG (H)	GA (M)	AA (L)	G	A
AD	4 (6.4%)	28 (44.4%)	31 (49.2%)	36 (28.6%)	90 (71.4%)
CT	14 (22.2%)	29 (46%)	20 (31.8%)	57 (45.2%)	69 (54.8%)
MCI	21 (15.2%)	71 (51.4%)	46 (33.3%)	113 (40.9%)	163 (59.1%)

Genotype: χ^2 9.480, d.f. 4; $P = .05$.
Allele: χ^2 8.257, d.f. 2; $P = .02$.

Table 2: Distribution of genotype and allele frequencies of −1082 (G/A) SNP in amnestic MCI (a-MCI) and multiple cognitive domains MCI patients (mcd-MCI).

	GG (H)	GA (M)	AA (L)	G	A
a-MCI	1 (3.3%)	14 (46.7%)	15 (50%)	16 (26.7%)	44 (73.3%)
mcd-MCI	20 (18.5%)	57 (52.8%)	31 (28.7%)	97 (44.9%)	119 (55.1%)

Genotype: χ^2 6.927, d.f. 2; $P < .05$.
Allele: χ^2 5.729, d.f. 1; $P = .02$.

Table 3: Distribution of genotype and allele frequencies of −1082 (G/A) SNP in MCIs that remain stable, progressed to AD (MCI → AD), and progressed to VD (MCI → VD).

	GG (H)	GA (M)	AA (L)	G	A
MCI stable	8 (28.6%)	12 (42.8%)	8 (28.6%)	28 (50%)	28 (50%)
MCI → AD	2 (8.3%)	12 (50%)	10 (41.7%)	16 (33.3%)	32 (66.7%)
MCI → VD	5 (22.7%)	11 (50%)	6 (27.3%)	21 (47.7%)	23 (52.3%)

Genotype distribution compared percentages: χ^2 15.604, d.f. 4; $P = .004$.
Allele distributions compared percentages: χ^2 6.661, d.f. 2; $P < .05$.

As previously described [13], AD patients show a significant higher frequency of the −1082A low producer allele, which skews the genotype distribution in AD compared to HC, with a significant decrease of −1082 GG high producer genotype.

MCI subjects as a whole had an intermediate pattern between AD and HC subjects, the percentages of G allele and GG genotype being 40.9% and 15.2%, while the percentage of A allele and AA genotype being 59.1% and 33.3%, respectively, (allele: $P = .02$, genotype: $P = .05$) (Table 1).

It is interesting to note that the genotype frequencies of the −1082 SNP in a-MCI subjects were similar to those of AD subjects, whereas those of mcd-MCI were comparable to HC (AA genotype 50% in a-MCI and 49.2% in AD; 28.7% and 31.8% in mcd-MCI and HC, resp.) (Table 2). Consequently, the allele and genotype distributions were significantly different between a-MCI and mcd-MCI (allele: $P = .02$, genotype: $P < .05$).

The same SNP is linked with two other SNPs at positions −819 and −592. They combine with microsatellite alleles to form haplotypes where the difference in IL-10 production is mainly accounted by the −1082 SNP [19, 20]. The genotype and allele frequencies of −819 and −592 SNPs were distributed similarly in our samples (data not shown).

3.2. Distribution of Apolipoprotein E Genotype in MCI Subjects. The frequency of ApoE ε4 in our sample was in line with the data already published [21–24]. In particular genotyping of our MCI patients globally considered revealed the presence of ε4 allele in 40% of cases and, during follow-up, in 54% of MCI → AD and 39% in stable MCI. The ApoE4 status is an independent risk factor for AD [13].

3.3. Follow-Up. After a 4-year follow-up 24 MCI progressed to AD (MCI → AD) [16] and 22 progressed to vascular dementia (MCI → VD). Table 3 shows −1082 SNP distributions in MCI progressing and not progressing to AD (stable MCI).

In MCI → AD both A allele and AA genotype were higher than in stable MCI and in MCI → VD.

Due to the limited number of patients that completed the follow-up period, the data reached the statistical significances only comparing genotype and allele percentage (allele: $P < .05$, genotype $P = .004$).

4. Discussion

A "cytokine cycle" has been proposed where [25] the anti-inflammatory cytokines (IL-4, IL-10, and IL-13) regulate β-amyloid-induced microglial/macrophage inflammatory responses and modify the microglial activity surrounding amyloid neuritic plaques [26]. These cytokines can inhibit the induction of IL-1, TNF-α, and MCP-1 in differentiated human monocytes and, above all, IL-10 causes dose-dependent inhibition of the IL-6 secretion induced by β-amyloid in these cells and in murine microglia [25].

In a previous paper, we described not only a significantly higher percentage of IL-10 −1082 AA low-producing genotype among AD cases, but also a reduced IL-10 generation in peripheral blood mononuclear cells from these patients after β-amyloid stimulation [13].

Interestingly a report on Italian centenarians, who are clearly less prone than younger persons to age-related diseases, showed that extreme longevity is significantly associated with the high IL-10-producing genotypes [27].

In the present study, the allele frequencies of −1082 SNP in a-MCI subjects were similar to those of AD patients, whereas those of mcd-MCI were comparable to HC (the frequencies of the low-producer AA genotype were 50% and 28.7%, in a-MCI and mcd-MCI, resp.).

It is to note that, after an adequate period of follow-up, the twenty-four a-MCI subjects that progressed to AD showed a higher percentage of AA carriers (41.7%) compared to those of MCI that remain stable (28.6%) and compared to those progressed in vascular dementia (27.3%). The similar genotype distribution of this IL-10 SNP in AD and a-MCI but not in mcd-MCI and the data retrospectively obtained after the follow-up suggest that it is potentially involved in the conversion of a-MCI to AD.

However, our results support the theory that the overall risk of developing AD may be governed by a multifactorial "susceptibility profile" and that polymorphisms of cytokine genes can affect neurodegeneration and its clinical progression.

In addiction, IL-10 may partly explain the conversion of a-MCI to AD or, at least, be a genetic marker of susceptibility [28].

Therefore, it is extremely relevant to closely define intrinsic (i.e., genetic) individual risk profiles in prevention and treatment trials. The finding that the set of gene variants in innate immunity associated with earlier onset predicted rapid clinical progression suggests that interventions to control inflammation might be useful especially for relatively younger cases to delay disease progression.

References

[1] J. L. Price and J. C. Morris, "Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease," *Annals of Neurology*, vol. 45, no. 3, pp. 358–368, 1999.

[2] R. C. Petersen, R. Doody, A. Kurz et al., "Current concepts in mild cognitive impairment," *Archives of Neurology*, vol. 58, no. 12, pp. 1985–1992, 2001.

[3] H. W. Querfurth and F. M. LaFerla, "Alzheimer's disease," *The New England Journal of Medicine*, vol. 362, no. 4, pp. 329–344, 2010.

[4] T. Wyss-Coray, "Inflammation in Alzheimer disease: driving force, bystander or beneficial response?" *Nature Medicine*, vol. 12, no. 9, pp. 1005–1015, 2006.

[5] E. Tarkowski, K. Blennow, A. Wallin, and A. Tarkowski, "Intracerebral production of tumor necrosis factor-α, a local neuroprotective agent, in Alzheimer disease and vascular dementia," *Journal of Clinical Immunology*, vol. 19, no. 4, pp. 223–230, 1999.

[6] E. Tarkowski, A.-M. Liljeroth, Å. Nilsson, L. Minthon, and K. Blennow, "Decreased levels of intrathecal interleukin 1 receptor antagonist in Alzheimer's disease," *Dementia and Geriatric Cognitive Disorders*, vol. 12, no. 5, pp. 314–317, 2001.

[7] P. Bermejo, S. Martín-Aragón, J. Benedí et al., "Differences of peripheral inflammatory markers between mild cognitive impairment and Alzheimer's disease," *Immunology Letters*, vol. 117, no. 2, pp. 198–202, 2008.

[8] K. Bonotis, E. Krikki, V. Holeva, C. Aggouridaki, V. Costa, and S. Baloyannis, "Systemic immune aberrations in Alzheimer's disease patients," *Journal of Neuroimmunology*, vol. 193, no. 1-2, pp. 183–187, 2008.

[9] S. Ray, M. Britschgi, C. Herbert et al., "Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins," *Nature Medicine*, vol. 13, no. 11, pp. 1359–1362, 2007.

[10] F. Licastro, E. Porcellini, C. Caruso, D. Lio, and E. H. Corder, "Genetic risk profiles for Alzheimer's disease: integration of APOE genotype and variants that up-regulate inflammation," *Neurobiology of Aging*, vol. 28, no. 11, pp. 1637–1643, 2007.

[11] D. Lio, F. Licastro, L. Scola et al., "Interleukin-10 promoter polymorphism in sporadic Alzheimer's disease," *Genes and Immunity*, vol. 4, no. 3, pp. 234–238, 2003.

[12] J. M. Kim, C. I. Brannan, N. G. Copeland, N. A. Jenkins, T. A. Khan, and K. W. Moore, "Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes," *Journal of Immunology*, vol. 148, no. 11, pp. 3618–3623, 1992.

[13] B. Arosio, D. Trabattoni, L. Galimberti et al., "Interleukin-10 and interleukin-6 gene polymorphisms as risk factors for Alzheimer's disease," *Neurobiology of Aging*, vol. 25, no. 8, pp. 1009–1015, 2004.

[14] R. C. Petersen, "Mild cognitive impairment as a diagnostic entity," *Journal of Internal Medicine*, vol. 256, no. 3, pp. 183–194, 2004.

[15] M. Zanetti, C. Ballabio, C. Abbate, C. Cutaia, C. Vergani, and L. Bergamaschini, "Mild cognitive impairment subtypes and vascular dementia in community-dwelling elderly people: a 3-year follow-up study," *Journal of the American Geriatrics Society*, vol. 54, no. 4, pp. 580–586, 2006.

[16] O. Hansson, H. Zetterberg, P. Buchhave, E. Londos, K. Blennow, and L. Minthon, "Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study," *Lancet Neurology*, vol. 5, no. 3, pp. 228–234, 2006.

[17] G. McKhann, D. Drachman, and M. Folstein, "Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease," *Neurology*, vol. 34, no. 7, pp. 939–944, 1984.

[18] S. A. Miller, D. D. Dykes, and H. F. Polesky, "A simple salting out procedure for extracting DNA from human nucleated cells," *Nucleic Acids Research*, vol. 16, no. 3, p. 1215, 1988.

[19] J. Eskdale, P. Wordsworth, S. Bowman, M. Field, and G. Gallagher, "Association between polymorphisms at the human IL-10 locus and systemic lupus erythematosus," *Tissue Antigens*, vol. 49, no. 6, pp. 635–639, 1997.

[20] J. Eskdale, G. Gallagher, C. L. Verweij, V. Keijsers, R. G. J. Westendorp, and T. W. J. Huizinga, "Interleukin 10 secretion in relation to human IL-10 locus haplotypes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 16, pp. 9465–9470, 1998.

[21] D. J. Berlau, M. M. Corrada, E. Head, and C. H. Kawas, "ApoE ε2 is associated with intact cognition but increased Alzheimer pathology in the oldest old," *Neurology*, vol. 72, no. 9, pp. 829–834, 2009.

[22] G. Bu, "Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy," *Nature Reviews Neuroscience*, vol. 10, no. 5, pp. 333–344, 2009.

[23] P. P. Singh, M. Singh, and S. S. Mastana, "APOE distribution in world populations with new data from India and the UK," *Annals of Human Biology*, vol. 33, no. 3, pp. 279–308, 2006.

[24] J. Kim, J. M. Basak, and D. M. Holtzman, "The role of apolipoprotein E in Alzheimer's disease," *Neuron*, vol. 63, no. 3, pp. 287–303, 2009.

[25] A. M. Szczepanik, S. Funes, W. Petko, and G. E. Ringheim, "IL-4, IL-10 and IL-13 modulate Aβ(1-42)-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line," *Journal of Neuroimmunology*, vol. 113, no. 1, pp. 49–62, 2001.

[26] C. C. Chao, T. W. Molitor, and S. Hu, "Neuroprotective role of IL-4 against activated microglia," *Journal of Immunology*, vol. 151, no. 3, pp. 1473–1481, 1993.

[27] D. Lio, L. Scola, A. Crivello et al., "Gender-specific association between—1082 IL-10 promoter polymorphism and longevity," *Genes and Immunity*, vol. 3, no. 1, pp. 30–33, 2002.

[28] O. Combarros, P. Sánchez-Juan, J. A. Riancho et al., "Aromatase and interleukin-10 genetic variants interactively modulate Alzheimer's disease risk," *Journal of Neural Transmission*, vol. 115, no. 6, pp. 863–867, 2008.

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Review Article

Neuroinflammation, Microglia and Implications for Anti-Inflammatory Treatment in Alzheimer's Disease

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Neuroinflammation has been implicated in the pathology of Alzheimer's disease (AD) for decades. Still it has not been fully understood when and how inflammation arises in the course of AD. Whether inflammation is an underling cause or a resulting condition in AD remains unresolved. Mounting evidence indicates that microglial activation contributes to neuronal damage in neurodegenerative diseases. However, also beneficial aspects of microglial activation have been identified. The purpose of this review is to highlight new insights into the detrimental and beneficial role of neuroinflammation in AD. It is our intention to focus on newer controversies in the field of microglia activation. Precisely, we want to shed light on whether neuroinflammation is associated to brain tissue damage and functional impairment or is there also a damage limiting activity. In regard to this, we discuss the limitations and the advantages of anti-inflammatory treatment options and identify what future implications might result from this underling neuroinflammation for AD therapy.

1. Introduction

The pathology of Alzheimer's disease (AD) is characterized by the deposition of amyloid-β (Aβ) plaques in the brain parenchyma and neurofibrillary tangles within neurons [1].

Apart from the disease's distinct pathological markers, its neurodegenerative conditions are characterized by chronic neuroinflammatory processes. Yet, those inflammatory markers are not exclusively associated with AD. Also brains of "healthy aged" individuals show concentrations of serum markers related to inflammation, homocysteine and cholesterol homeostasis are associated with cognitive functioning in the nondemented healthy aging population [2]. In the AD pathology, these aging-related inflammatory processes are increased. The suggestion that inflammation may participate in AD first came up more than two decades ago. As several clinical trials have shown a beneficial effect for nonsteroidal anti-inflammatory drugs for the occurrence and course of AD, the inflammatory hypothesis in AD gained a lot of attention. In regard to treatment and prevention of AD, several classes of medications have emerged to the market, which improve the cognitive symptoms of this disorder (e.g. the cholinesterase inhibitors). But the relief that these drugs provide remains symptomatic—so it is a major goal for the future to develop effective disease-modifying therapy. Different substantial efforts have been made to identify potential strategies to ameliorate or prevent AD pathology, with data stemming from basic research as well as from animal and epidemiological studies. Because many investigators have concluded that neuroinflammation contributes to neuronal damage in the brain during AD [3, 4], the use of anti-inflammatory drugs as a possible treatment option has been widely investigated [5–7]. Anti-inflammatory therapy has therefore been credited as a strategy for reducing the risk or slowing the progression of AD. However, the results of these studies remain inconsistent [8].

Until now, many questions regarding the inflammatory response are still unresolved. Discussion continues whether neuroinflammation is an underling cause or a resulting condition in AD.

There are several studies showing that an intact immune response including intact T cell immunity is a prerequisite for cognitive function. T cell deficient mice show impaired

learning abilities, which can be reversed with T cell substitution [9, 10].

Inflammation in the brain is characterized by activation of glial cells (mainly microglia and astrocytes) and expression of key inflammatory mediators as well as neurotoxic free radicals. It has been suggested that neuroinflammation is associated with neurodegenerative disorders—both acute (e.g. stroke, injury) and chronic (e.g. multiple sclerosis, AD). In this context, microglia cells play a crucial role and therefore microglia and cytokines have been extensively studied in these conditions. In the central nervous system, microglia are the resident phagocytes of the innate immune system. Microglia are found in a highly activated state in close anatomical proximity to senile plaques within the AD brain. In this activated state, microglia produce various proinflammatory cytokines and other immune mediators that create a neurotoxic milieu leading to disease progression [4, 11].

The purpose of this review is to highlight our new insights into the role of neuroinflammation in the pathophysiology of AD. It is our intention to focus on newer controversies in the field of microglia activation and its function in AD pathology. For this, we asked ourselves some questions: are neuroinflammatory alterations neuroprotective—or are they rather an underlying cause of AD? And what strategies result from this underling neuroinflammation for future treatment options?

2. Characteristics of Neuroinflammation in AD

The relevance of neuroinammation to AD pathology has been established by multiple lines of direct and indirect evidence. One argument is that increased microglial activation has been shown in regions associated with Aβ deposition [12]. Upregulated inammatory mechanisms colocalize in the AD brain with those regions that exhibit high levels of AD pathology (e.g. frontal and limbic cortex) and are minimal in brain regions with low AD pathologic susceptibility (e.g. cerebellum) [13].

As a second point, many of the inammatory mechanisms that have been uncovered in the AD brain are established to be cytotoxic in the periphery. Therefore it seems likely that they are also cytotoxic in the brain, an organ that is sensitive to inammation (e.g. meningitis, edema). However, inflammation in the brain is different from inflammation in the periphery. AD brains lack the classical hallmarks of inflammation such as neutrophil infiltration and perivascular mononuclear cuffing. As for other neurodegenerative diseases, a local inflammatory reaction is sustained by activated microglia and reactive astrocytes. This is indicated by the presence of antigens associated with microglia activation and inflammatory mediators, such as factors of the complement system, cytokines, and free radicals [14].

For AD a huge variety of proinflammatory markers have been identified, whereas this was not the case for other forms of dementia. A relevant reduction of monocyte chemotactic protein-1 levels in the grey matter in dementia patients has been shown. For interleukin-6 (IL) and related markers of this proinflammatory cytokine system, decreases were observed in the demented population [15, 16]. It is discussed, however, whether this decrease is related to further psychopathological symptoms such as depression [16]. On the other hand, IL-6 has also neuroprotective properties and decreased IL-6 might be associated with decreased neuroprotection [17],

Only modest elevations of inammatory markers are found in the autopsy of patients lacking a clinical presentation of dementia but who exhibit sufficient Aβ and neurobrillary tangles to otherwise qualify for the diagnosis of AD. Their level of inflammatory markers is signicantly greater than levels of nondemented patients, but dramatically less than AD patients [18]. These findings further strengthen that an inflammation is a necessity for clinical symptoms of AD.

There also is direct evidence of inammatory toxicity in the AD brain. For instance, complement xation and lysis of neurites could be demonstrated ultrastructurally in Alzheimer's disease cortex, but in contrast it was only very weakly detected in nondemented elderly cortex under the same conditions [19].

Finally, many clinical and animal studies have strongly suggested that especially nonsteroidal anti-inflammatory drugs (NSAIDs) could be used as preventive or treatment strategies in AD. This aspect is further discussed in a later section of this paper, where we focus on anti-inflammatory treatment.

Even though there are many indicators that neuroinflammation plays a key role in AD pathology, this does not answer which of these inflammatory activities are causing disease progression. The question remains: do some of these processes help to fight against the disease? In order to address this, the role of microglia seems important, because these cells are known for neuroprotective and degenerative functions.

3. Controversy: Do Activated Microglia Cause Neuroprotection or -Degeneration in the AD Brain?

Microglia are one of three glial cell types in the central nervous system (CNS). They play an important role as resident immunocompetent and phagocytic cells in the event of injury and disease. Del Rio Hortega determined in 1927 that microglia belong to a distinct glial cell type apart from astrocytes and oligodendrocytes [20]. Since the 1970s there has been wide recognition that microglia are immune effectors in the CNS that respond to pathological conditions and participate in initiation and progression of neurological disorders (including AD) by releasing potentially cytotoxic molecules such as proinflammatory cytokines, reactive oxygen intermediates, proteinases, and complement proteins [21]. This means that their phagocytic function can be beneficial while their inflammation related functions might be detrimental.

Several studies give evidence for an increased number of morphologically reactive microglia in AD brains compared to nondemented individuals [22, 23]. The location of these

reactive microglia has been indentified directly around plaques [24]. This finding has been verified in a recent imaging study, which showed increased microglial activation in regions associated with amyloid deposition [12]. Up to now, the exact timing of this association could not be identified. Microgliosis might be an early component of the disease process and not necessarily dependent upon Aβ plaque interaction as a stimulus. What is known so far is that activation of microglia by Aβ fibrils is associated with a chemotactic response and extensive clustering of microglia around Aβ plaques in the AD brain [25]. These findings indicate the prominent role of microglial cells in AD. Nonetheless it remains unclear, whether their functions are beneficial or detrimental.

The following section explains the checkered role of activated microglia in AD pathology.

4. Neuroprotective Properties of Microglia in AD

Is there a possibility that activated microglia cells are beneficial in neurodegenerative diseases? It is known that the microglia population can be neuroprotective by degrading Aβ plaques in AD. Mouse models found that microglia mainly recruit macrophages from the periphery that then transform into microglia in the brain. Therefore most of the microglia that were associated with plaques in the mouse brain came from the bone marrow [26]. Furthermore it has been suggested that newly recruited microglia have different phagocytotic properties than intrinsic microglia, which is important for Aβ elimination. Lysosmes from the macrophage cell line are more acidic than those of microglial lysosomes [27]. This indicates that microglia derived from the periphery might be more efficient in eliminating Aβ than brain microglia. Furthermore, phagocytic activity of microglia is dampened by proinflammatory cytokines like tumor necrosis factor α (TNF) [28]. These findings show that microglia that are committed to an inflammatory response may have a lower phagocytotic capacity, than newly recruited microglia. In mouse models of AD it could be demonstrated that anti-inflammatory drugs like minocycline improve cognitive functions and reduce the activation of microglial cells but do not alter the Aβ plaques deposition and distribution [29]. Seabrook et al. showed in amyloid precursor protein transgenic mice an age dependent effect of minocycline: in young animals the drug increased the amyloid load indicating a beneficial effect of microglia in clearing amyloid [30]. Not only for AD minocycline was investigated as a potential treatment, also in schizophrenia an add-on therapy with minocycline appeared to be effective on the cognitive performance by reducing a broad range of psychotic symptoms [31]. On the other hand an additional mechanism might help microglia cells with the elimination process. Transforming growth factor-β 1 has been demonstrated to promote microglial Aβ clearance and reduce plaque burden [32]. This could support the idea that microglial activation is useful in the clearance of Aβ.

A further suggestion for the beneficial role of microglia is that neuroprotection results from the microglial glutamate removal. Glutamate has been indentified as a relevant neurotoxic substance that acts through N-methyl-D-aspartic acid (NMDA) receptors on neurons and can lead to increased neuronal cell death. Microglial cells can increase their capacity to take up glutamate upon stimulation with lipopolysaccaride (LPS) over a mechanism that is TNFα dependent [33]. For AD this microglial function could be relevant because memantine (the NMDA receptor antagonist) has been shown to improve cognition, function (activities of daily living), agitation, and delusions in AD patients [34]. Taken this together, microglial cells are important for the control of glutamate levels and might therefore contribute to neuronal survival. There is also evidence that microglia are capable of secreting neurotrophic or neuron survival factors (e.g. nerve growth factor and neurotrophin 3) upon activation via inflammation or injury [35].

A recent review explains that microglia—when they are challenged—may adapt to different stimulatory contexts and pass through a sequence of reactive profiles. This is in line with the finding that microglia are not just "resting" but have active sensor and versatile functions [36].

Are most microglial cells functions beneficial in AD? Several studies suggest an overbalance of the detrimental microglial properties. This issue is discussed in the next section.

5. Microglia—Are They Responsible for Neurodestruction and -Degeneration?

In order to address this question, it is important to focus on timing. One must investigate when microglial activity begins during the time course of the disease. An increase in microglial activation has been observed in very early stages of AD. This increase surprisingly disappeared over time [37]. The suggestion of Vehmas et al. strengthens the assumption that microglial activation begins early in disease progression [37]. This could be a hint that microglia initially try to eliminate Aβ, but over time of the disease fail and therefore decrease their activity. Alternatively, the microglial role in AD could be detrimental and they initiate the underlying AD pathology. In order to further evaluate this issue, a closer look needs to be taken on what causes the microglial activation in AD and it seems important to distinguish between acute and chronic stimulation of microglial cells. While an acute insult may trigger oxidative and nitrosative stress, it is typically short-lived and unlikely to be harmful to long-term neuronal survival. Therefore it is believed that an acute neuroinflammatory response is generally beneficial to the CNS, since it tends to minimize further injury and contributes to repair of damaged tissue. The opposite is the case for a chronic stimulation: chronic neuroinflammation is most often detrimental and damaging to nervous tissue. Thus, whether neuroinflammation has beneficial or harmful outcomes in the brain may depend critically on the duration of the inflammatory response. The progressive deposition of Aβ in AD disease might provide a chronic stimulus to microglial cells. Also the chemotactic functions of Aβ to attract microglia contribute further to the ongoing

inflammatory process [25]. The ratio of the proinflammatory cytokine IL-1β to the anti-inflammatory cytokine IL-10 is drastically elevated in the serum of AD patients, giving these patients a definite long-term proinflammatory profile [38], indicating a chronic neuroinflammatory state of the CNS. In addition, the accumulating loss of neurons that characterizes AD further contributes to generation of debris and keeps microglia activated indefinitely maintaining microglia in an activated state long term. This data indicates that in AD the inflammation might be rather chronic and therefore contributing to disease progression.

There is also the emerging idea that an inflamed CNS environment may influence the ability of microglia to contribute to plaque deposition rather than plaque removal [28]. This strongly suggests that the microenvironment of the brain can influence whether microglia perform beneficial or deleterious functions in pathophysiological states. This means that microglia cells functionally adapt to their environment [36]. Recent studies show that in response to certain environmental toxins and endogenous proteins, microglia can enter an overactivated state and release reactive oxygen species (ROS) that cause neurotoxicity [39]. Overactivated microglia can be detected using imaging techniques and therefore this knowledge offers an opportunity not only for early diagnosis, but eventually also for the development of targeted anti-inflammatory therapies that might diminish the progression of the disease [21].

In addition, activated microglia release the excitotoxin quinolinic acid [40], and microglia activated by AD plaques produce an apparently novel amine that evokes fulminant excitotoxicity [41]. One interesting implication of an excitotoxic contribution to inammatory mechanisms is the potential for limited damage to functional cellular compartments. Because excitatory amino acid receptors are restricted to synapses and dendrites, these subcellular compartments are preferentially vulnerable.

As a result, microglia-produced excitotoxins may lead to cognitive impairment that is not necessarily correlated with neuronal cell loss [3]. However, activated microglia do not only produce neurotoxic metabolites. Some of their products like 3-hydroxyanthralinic acid (which is—like quinolinic acid—one of the downstream products of the tryptophan metabolism) exert antioxidant and anti-inflammatory functions [42, 43]. Therefore the balance of these products that result from activated microglia is important for the inflammation process.

To sum up the results from microglial studies: clear indications for the important role of neuroinflammation contributing to disease progression in AD were found. However, some parts of microglial activation might also be beneficial during the course of AD. These issues are shown in Figure 1.

6. The Role of COX Inhibitors in Neurodegeneration

As explained above, neuroinflammation is a critical event in AD. It has been suggested that anti-inammatory therapy could be benecial in delaying the onset or slowing the progression of AD.

Cyclooxygenase (COX) is a unique enzyme. First, it exhibits two catalytic activities, a bis-oxygenase activity, which catalyses prostaglandin G_2 (PG) formation from arachidonic acid and a peroxidase activity, which reduces PG G_2 to PG H_2. The peroxidase activity also results in the production of free radicals, which are in part utilized by COX itself [44]. Although NSAIDs may have other effects as well, it is generally assumed that their primary mechanism of action is by competitive inhibition of COX activity, thereby reducing the production of inammatory prostaglandins from membrane-derived arachidonate. COX not only helps mediate production of prostaglandins and other inammatory factors, it is itself upregulated by pro-inammatory mediators [44].

In AD, Aβ neurotoxicity may result from several mechanisms, most likely in combination. These mechanisms include oxidative damage, direct cytotoxicity, and induction of destructive inflammatory mechanisms; efforts have been directed at the control of each of these processes [45]. See Figure 1 for the involvment of COX in the AD pathology.

The treatment of AD with NSAIDs is one of the most promising approaches.

7. Possible Mechanisms of Action of NSAID in AD

If NSAIDs are beneficial in AD, the presumed mechanism would be inhibition of COX expressed in the brain. Both COX-1 and COX-2 are expressed there and COX-2 plays a unique role in the brain compared to the periphery: only in the brain COX-2 is expressed constitutively whereas elsewhere the expression is activation-dependent. Although in vivo the majority of COX-2 appears to be made in neurons, COX-2 was also seen in rat astrocytes and microglia [46]. It has been demonstrated that COX-inhibiting NSAIDs reduce microglial activation following infusion of Aβ in rats [47]. Neuronal stress, such as ischaemia and excitotoxicity, is associated with strong upregulation of neuronal COX-2 expression. This suggests that COX-2 is involved in neurotoxic mechanisms and may therefore represent a target for drug therapy in the treatment of AD [48, 49].

Several studies provide the background for possible mechanisms of action of NSAIDs in AD. Neuronal COX-2 is upregulated in response to exposure to Aβ [50], and focal increases in COX-2 have been shown in the region of amyloid plaques in double transgenic mice carrying genes that encode both mutant APP and mutant presenilin 1 [51]. Many studies seem to show that COX-2 inhibition confers neuroprotection [52–55]. Some studies have revealed an upregulation of neuronal COX-2 in the brains of patients with AD [56, 57], though this has not been a universal finding [58, 59]. One explanation for the variation of COX expression is the short half-life of COX-2 transcripts or individual variability of inflammatory-related processes.

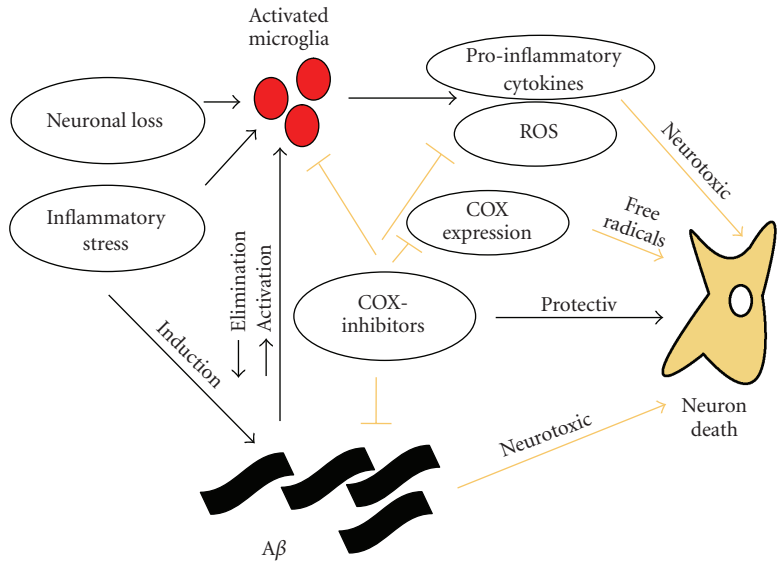

Figure 1: possible interactions of COX-inhibitors and Alzheimer's disease pathology. The fair arrows show neurotoxic properties of Aβ, COX-expression cytokines. In addition it is indicated that COX-inhibitors block COX-expression, activated microglia, ROS, and Aβ. ROS: radical oxygen species; COX: cyclooxygenase; Aβ: amyloid β.

Another principle of how NSAIDs could act, comes from the finding that prostaglandin E2 levels are elevated in patients with AD, especially in early stages of the disease [60]. Therefore NSAIDs blocking prostaglandin E2 synthesis might be beneficial. This issue is further strengthened by glial culture studies indicating that prostaglandins, particularly prostaglandin E, alter the production of several inammation-related molecules, including IL-6, chemokines, and APP [61–63].

In addition to the more traditional inammatory mechanisms associated with COX, unique functions of COX-mediated damage may also occur in the AD brain. For example, several of the prostanoid products of arachidonate metabolism potentiate glutamate excitotoxicity, and COX-2 overexpressing transgenic mice exhibit increased neuronal susceptibility to excitotoxic insult [64].

Some of the previously mentioned studies of COX in ischemia also suggest that intraneuronal COX-2 levels may contribute to neuronal death by production of free radicals [65]. In addition, increased COX-2 levels in AD neurons may directly damage neurons or increase their vulnerability to other detrimental processes occurring in AD brain [65]. Thus, NSAIDs actions to inhibit COX-mediated production of apoptotic factors by neurons could be one of the mechanisms by which these drugs seem to exert benecial effects in AD.

Another non-COX-dependent mechanism of NSAIDs is to attenuate inammatory processes in a manner by directly activating the peroxisome proliferator-activated receptor gamma (PPARγ), a receptor and nuclear transcription factor [66–68]. PPARγ is a member of the orphan nuclear receptor family and in cells of monocytic lineage, including microglia, acts to suppress the expression of a broad range of proinammatory genes [66, 68]. Some NSAIDs act as PPARγ agonists, directly binding to it and initiating its transcriptional activity. Activation of PPARγ inhibits the Aβ-stimulated activation of microglia and monocytes and their secretion of proinammatory and neurotoxic products. For example, PPARγ agonists act to inhibit the Aβ-stimulated expression of IL-6 and TNF-alpha [69], by microglia and monocytes, and to prevent Aβ-mediated conversion of microglia into an activated phenotype [70].

A further underlying mechanism of AD pathology is oxidative stress [71, 72]. Activated microglial cells are known to release ROS, which might possibly cause this oxidative stress. Though glia cells can also exhibit antioxidative functions by releasing hemeoxygenase-1 (HO-1) triggered by accumulation of 3-hydroxyanthralinic acid (3-HAA), a down-stream product of the tryptophan metabolism. The association of neuronal injury in AD and oxidative stress has been demonstrated by overexpression of immunoreactive HO-1 protein in neurons and astrocytes of the cerebral cortex and hippocampus. HO-1 was found to be colocalized to senile plaques, neurofibrillary tangles, and corpora amylacea [73]. It is widely accepted that a moderate activation of heme catabolism is neuroprotective and contributes to degradation of neurotoxic protein aggregates. Regulatory interactions between HO-1 and COX pathways have also been reported [74]. However, experimental observations indicate that the extent of HO-1 induction may be critical because excessive heme degradation may result in toxic levels of carbon monoxide, bilirubin and iron. Pharmacological modulation of HO-1 levels in the brain shows promising results in models of AD and Parkinson's disease [75].

Referring to the oxidative stress underlying AD pathology, one further aspect of these reactive oxygen species includes activation of COX-1/2, which are blocked by NSAIDs. It has been shown that daily doses of NSAIDs

increase circulating levels of antioxidants [76]. In a rat model of AD it was suggested that treatment with a COX-2 inhibitor reduces oxidative stress and might therefore be beneficial for the course of AD [77].

As another mechanism it has been suggested that NSAIDs directly affect amyloid pathology in the brain by reducing Aβ-42 peptide levels over the gamma-secretase activity independently of COX activity [78]. Weggen et al. reported that the NSAIDs ibuprofen, indomethacin, and sulindac sulphide preferentially decrease the highly amyloidogenic Aβ-42 peptide produced from a variety of cultured cells by as much as 80% [79]. However, for some NSAIDs the lowering effect of Aβ-42 could not be shown; instead, an increase in Aβ-42 levels was observed [80]. The underlying mechanism of how NSAIDs decrease Aβ-42 was clarified by Lleo et al., who demonstrated that Aβ-42 lowering NSAIDs specifically affect the proximity between APP and presenilin 1 and alter a novel allosteric mechanism of action [81].

8. Anti-Inflammatory Treatment Studies in AD

In recent years it has become widely accepted that inflammatory processes are an underlying condition of AD. Therefore a number of clinical trials investigating different anti-inflammatory treatment regimens have been performed. In the following paragraph, we summarize the most import findings in regard to first mainly COX-2 dominant and second COX-1 inhibitors.

A prospective cohort study with 6989 subjects showed that long-term use of NSAIDs protects against AD but not against vascular dementia [5]. More recently, Szekely et al. provided very similar findings: they concluded that NSAIDs use reduced the risk of preferentially AD versus vascular dementia but mainly in those individuals having an apolipoprotein E (APO) epsilon 4 allele. This study was done with over 3,000 subjects aged 65 years and older [6]. Not only selective COX-2 inhibitors were shown to be associated with decreased risk of AD; a reduced occurrence of AD could also be demonstrated for the use of the COX-1 inhibitor aspirin [7]. A meta-analysis of 17 epidemiological studies yielded strong, generally consistent, statistical evidence that NSAID and steroid use is associated with reduced risk of AD [82]. Vlad et al. investigated 49,349 patients with AD and 196,850 controls: long-term (>5 years) nonsteroidal anti-inflammatory drug use was shown to be protective against Alzheimer disease. These findings were clearest for ibuprofen, but did not appear for other NSAIDs [83].

Not all studies showed a positive outcome for COX inhibitors in AD patients: the failure of selective COX-2 inhibition (rofecoxib) over placebo was stated in a one-year randomized controlled study. The authors argued that their results could indicate that the disease process was too advanced to be modified, as the goal of the study was slowing the progression of dementia in patients with already established AD [8]. For another COX-2 inhibitor, celecoxib, no beneficial effect on the occurrence of AD could be demonstrated in an age group over 70 years [84]. Also Wolfson et al. looked retrospectively at a case control population and found no support for a beneficial effect for NSAIDs in the AD subjects [85]. However, this negative result may have been caused by an insufficient period of data collection before disease onset.

9. Conclusion

It is indisputable that neuroinammation plays a key role in AD pathology. Mechanisms that parallel those encountered in localized peripheral inammatory responses are readily identied, along with detailed pathways for how the mechanisms interact. On balance, it is likely that AD neuroinammation exacerbates AD pathogenesis.

A general treatment principle in psychiatry, that an intervention as early as possible leads to the best outcome, seems to be especially true for AD. Many lines of evidence show that Aβ-induced neuroinflammation is an early event in neurodegeneration of AD [86], as increases in microglial activation has been observed in very early stages of AD and disappeared over time [37]. The fact that neuroinflammation occurs very early in AD could explain why anti-inflammatory treatment seems to be most efficient as preventive or early treatment. There are several reasons why an early use of NSAIDs is superior to a late one: Cox-expression in the brain decreases over time in AD brains [87]. And the CSF PG E_2 levels in patients with Alzheimer's disease were high when their short-term memory scores were just below those of controls, but were low in later stages of the disease. These findings further support that inflammatory processes predominate early in Alzheimer's disease [88] and therefore require early intervention with anti-inflammatory treatment.

This could explain the failure of some prospective clinical trials of selective COX-2 inhibitors: it may be related to a delayed onset of treatment, but eventually also to drug selection (regarding different effects of COX-1 and COX-2) and dose and duration of treatment. Especially the drug selection seems essential as some NSAIDs have recently been shown to increase Aβ-42 levels [77]. It also has to be noted that the protective effects of NSAIDs may be via non-COX-inhibitory mechanisms, such as lowering of Aβ levels and activation of the peroxisome proliferator-activated receptor-[gamma] [89] and these non-COX-dependent mechanisms might be differentially distributed among COX-inhibitors.

However, two major aspects should be kept in mind when considering the significance of COX-2 activity in brain diseases. The first thing: COX-2 is expressed under normal conditions and contributes to fundamental brain functions such as synaptic activity, memory consolidation, and functional hyperemia. The second thing: the term neuroinflammation is a much more controlled reaction than inflammation in peripheral tissues. In degenerative diseases, it mainly occurs in the absence of blood-borne infiltrating cells and is sustained by activated glial cells, particularly microglia.

In summary, the harmful inflammatory processes seem to dominate AD pathology, but there are also some beneficial functions for inflammatory subsets. If AD neuroinammation is approached with realistic expectations and rational drug

design, AD patients could significantly benefit from anti-inammatory treatment, especially with NSAIDs.

A future goal could be to utilize not only the efficient treatment properties of NSAIDs in early AD, but also makes use of the neuroprotective aspects of neuroinflammation with a combination therapy that maximizes the potential of glial activation. This would include treatment with NSAIDs and drugs that enforce anti-inflammatory and antioxidative properties (e.g. with 3-HAA and HO-1 enhancement).

References

[1] J. Götz and N. N. Götz, "Animal models for Alzheimer's disease and frontotemporal dementia: a perspective," *ASN Neuro*, vol. 1, no. 4, article e00019, 2009.

[2] C. E. Teunissen, M. P. J. van Boxtel, H. Bosma, et al., "Serum markers in relation to cognitive functioning in an aging population: results of the Maastricht Aging Study (MAAS)," *Tijdschrift voor Gerontologie en Geriatrie*, vol. 34, no. 1, pp. 6–12, 2003.

[3] E. G. McGeer and P. L. McGeer, "Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy," *Journal of Alzheimer's Disease*, vol. 19, no. 1, pp. 355–361, 2010.

[4] H. Akiyama, S. Barger, S. Barnum, et al., "Inflammation and Alzheimer's disease," *Neurobiology of Aging*, vol. 21, no. 3, pp. 383–421, 2000.

[5] B. A. in 't Veld, A. Ruitenberg, A. Hofman, et al., "Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease," *The New England Journal of Medicine*, vol. 345, no. 21, pp. 1515–1521, 2001.

[6] C. A. Szekely, J. C. S. Breitner, A. L. Fitzpatrick, et al., "NSAID use and dementia risk in the Cardiovascular Health Study: role of APOE and NSAID type," *Neurology*, vol. 70, no. 1, pp. 17–24, 2008.

[7] J. C. Anthony, J. C. S. Breitner, P. P. Zandi, et al., "Reduced prevalence of AD in users of NSAIDS and H2 receptor antagonists: the Cache County study," *Neurology*, vol. 54, no. 11, pp. 2066–2071, 2000.

[8] S. A. Reines, G. A. Block, J. C. Morris, et al., "No effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study," *Neurology*, vol. 62, no. 1, pp. 66–71, 2004.

[9] J. Kipnis, H. Cohen, M. Cardon, Y. Z IV, and M. Schwartz, "T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 21, pp. 8180–8185, 2004.

[10] Y. Ziv, N. Ron, O. Butovsky, et al., "Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood," *Nature Neuroscience*, vol. 9, no. 2, pp. 268–275, 2006.

[11] T. Wyss-Coray, "Inflammation in Alzheimer disease: driving force, bystander or beneficial response?" *Nature Medicine*, vol. 12, no. 9, pp. 1005–1015, 2006.

[12] P. Edison, H. A. Archer, A. Gerhard, et al., "Microglia, amyloid, and cognition in Alzheimer's disease: an [11C](R)PK11195-PET and [11C]PIB-PET study," *Neurobiology of Disease*, vol. 32, no. 3, pp. 412–419, 2008.

[13] J. Rogers and Y. Shen, "A perspective on inflammation in Alzheimer's disease," *Annals of the New York Academy of Sciences*, vol. 924, pp. 132–135, 2000.

[14] V. H. Perry, T. A. Newman, and C. Cunningham, "The impact of systemic infection on the progression of neurodegenerative disease," *Nature Reviews Neuroscience*, vol. 4, no. 2, pp. 103–112, 2003.

[15] E. Mulugeta, F. Molina-Holgado, M. S. Elliott, et al., "Inflammatory mediators in the frontal lobe of patients with mixed and vascular dementia," *Dementia and Geriatric Cognitive Disorders*, vol. 25, no. 3, pp. 278–286, 2008.

[16] S. Stübner, T. Schön, F. Padberg, et al., "Interleukin-6 and the soluble IL-6 receptor are decreased in cerebrospinal fluid of geriatric patients with major depression: no alteration of soluble gp130," *Neuroscience Letters*, vol. 259, no. 3, pp. 145–148, 1999.

[17] X.-Q. Wang, Y.-P. Peng, J.-H. Lu, B.-B. Cao, and Y.-H. Qiu, "Neuroprotection of interleukin-6 against NMDA attack and its signal transduction by JAK and MAPK," *Neuroscience Letters*, vol. 450, no. 2, pp. 122–126, 2009.

[18] L.-F. Lue, L. Brachova, W. H. Civin, and J. Rogers, "Inflammation, Aβ deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration," *Journal of Neuropathology and Experimental Neurology*, vol. 55, no. 10, pp. 1083–1088, 1996.

[19] S. Webster, L.-F. Lue, L. Brachova, et al., "Molecular and cellular characterization of the membrane attack complex, C5b-9, in Alzheimer's disease," *Neurobiology of Aging*, vol. 18, no. 4, pp. 415–421, 1997.

[20] P. del Rio-Hortega, "Art and artifice in the science of histology," *Histopathology*, vol. 22, no. 6, pp. 515–525, 1993.

[21] M. L. Block, L. Zecca, and J.-S. Hong, "Microglia-mediated neurotoxicity: uncovering the molecular mechanisms," *Nature Reviews Neuroscience*, vol. 8, no. 1, pp. 57–69, 2007.

[22] P. Cras, M. Kawai, S. Siedlak, et al., "Neuronal and microglial involvement in β-amyloid protein deposition in Alzheimer's disease," *American Journal of Pathology*, vol. 137, no. 2, pp. 241–246, 1990.

[23] S. D. Styren, W. H. Civin, and J. Rogers, "Molecular, cellular, and pathologic characterization of HLA-DR immunoreactivity in normal elderly and Alzheimer's disease brain," *Experimental Neurology*, vol. 110, no. 1, pp. 93–104, 1990.

[24] L. S. Perlmutter, E. Barron, and H. C. Chui, "Morphologic association between microglia and senile plaque amyloid in Alzheimer's disease," *Neuroscience Letters*, vol. 119, no. 1, pp. 32–36, 1990.

[25] L.-F. Lue, R. Rydel, E. F. Brigham, et al., "Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro," *GLIA*, vol. 35, no. 1, pp. 72–79, 2001.

[26] A. R. Simard and S. Rivest, "Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia," *FASEB Journal*, vol. 18, no. 9, pp. 998–1000, 2004.

[27] A. Majumdar, D. Cruz, N. Asamoah, et al., "Activation of microglia acidifies lysosomes and leads to degradation of Alzheimer amyloid fibrils," *Molecular Biology of the Cell*, vol. 18, no. 4, pp. 1490–1496, 2007.

[28] J. Koenigsknecht-Talboo and G. E. Landreth, "Microglial phagocytosis induced by fibrillar β-amyloid and IgGs are differentially regulated by proinflammatory cytokines," *Journal of Neuroscience*, vol. 25, no. 36, pp. 8240–8249, 2005.

[29] R. Fan, F. Xu, M. L. Previti, et al., "Minocycline reduces microglial activation and improves behavioral deficits in a transgenic model of cerebral microvascular amyloid," *Journal of Neuroscience*, vol. 27, no. 12, pp. 3057–3063, 2007.

[30] T. J. Seabrook, L. Jiang, M. Maier, and C. A. Lemere, "Minocycline affects microglia activation, Aβ deposition, and behavior in APP-tg mice," *GLIA*, vol. 53, no. 7, pp. 776–782, 2006.

[31] C. Chaves, C. R. Marque, C. Trzesniak, et al., "Glutamate-N-methyl-D-aspartate receptor modulation and minocycline for the treatment of patients with schizophrenia: an update," *Brazilian Journal of Medical and Biological Research*, vol. 42, no. 11, pp. 1002–1014, 2009.

[32] T. Wyss-Coray, C. Lin, F. Yan, et al., "TGF-β1 promotes microglial amyloid-β clearance and reduces plaque burden in transgenic mice," *Nature Medicine*, vol. 7, no. 5, pp. 612–618, 2001.

[33] M. Persson, M. Brantefjord, E. Hansson, and L. Rönnbäck, "Lipopolysaccharide increases microglial GLT-1 expression and glutamate uptake capacity in vitro by a mechanism dependent on TNF-α," *GLIA*, vol. 51, no. 2, pp. 111–120, 2005.

[34] P. T. Francis, "Altered glutamate neurotransmission and behaviour in dementia: evidence from studies of memantine," *Current Molecular Pharmacology*, vol. 2, no. 1, pp. 77–82, 2009.

[35] S. U. Kim and J. de Vellis, "Microglia in health and disease," *Journal of Neuroscience Research*, vol. 81, no. 3, pp. 302–313, 2005.

[36] U.-K. Hanisch and H. Kettenmann, "Microglia: active sensor and versatile effector cells in the normal and pathologic brain," *Nature Neuroscience*, vol. 10, no. 11, pp. 1387–1394, 2007.

[37] A. K. Vehmas, C. H. Kawas, W. F. Stewart, and J. C. Troncoso, "Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease," *Neurobiology of Aging*, vol. 24, no. 2, pp. 321–331, 2003.

[38] E. J. Remarque, E. L. E. M. Bollen, A. W. E. Weverling-Rijnsburger, J. C. Laterveer, G. J. Blauw, and R. G. J. Westendorp, "Patients with Alzheimer's disease display a pro-inflammatory phenotype," *Experimental Gerontology*, vol. 36, no. 1, pp. 171–176, 2001.

[39] N. G. Innamorato, I. Lastres-Becker, and A. Cuadrado, "Role of microglial redox balance in modulation of neuroinflammation," *Current Opinion in Neurology*, vol. 22, no. 3, pp. 308–314, 2009.

[40] M. G. Espey, O. N. Chernyshev, J. F. Reinhard Jr., M. A. A. Namboodiri, and C. A. Colton, "Activated human microglia produce the excitotoxin quinolinic acid," *NeuroReport*, vol. 8, no. 2, pp. 431–434, 1997.

[41] D. Giulian, "Senile plaques stimulate microglia to release a neurotoxin found in Alzheimer brain," *Neurochemistry International*, vol. 27, no. 1, pp. 119–137, 1995.

[42] G. Leipnitz, C. Schumacher, K. B. Dalcin, et al., "In vitro evidence for an antioxidant role of 3-hydroxykynurenine and 3-hydroxyanthranilic acid in the brain," *Neurochemistry International*, vol. 50, no. 1, pp. 83–94, 2007.

[43] S. R. Thomas, P. K. Witting, and R. Stocker, "3-hydroxyanthranilic acid is an efficient, cell-derived co-antioxidant for α-tocopherol, inhibiting human low density lipoprotein and plasma lipid peroxidation," *Journal of Biological Chemistry*, vol. 271, no. 51, pp. 32714–32721, 1996.

[44] W. L. Smith, R. Michael Garavito, and D. L. DeWitt, "Prostaglandin endoperoxide H syntheses (cyclooxygenases)-1 and -2," *Journal of Biological Chemistry*, vol. 271, no. 52, pp. 33157–33160, 1996.

[45] P. S. Aisen and K. L. Davis, "The search for disease-modifying treatment for Alzheimer's disease," *Neurology*, vol. 48, no. 5, pp. S35–S41, 1997.

[46] W. D. Hirst, K. A. Young, R. Newton, V. C. Allport, D. R. Marriott, and G. P. Wilkin, "Expression of COX-2 by normal and reactive astrocytes in the adult rat central nervous system," *Molecular and Cellular Neurosciences*, vol. 13, no. 1, pp. 57–68, 1999.

[47] B. Hauss-Wegrzyniak, P. Vraniak, and G. L. Wenk, "The effects of a novel NSAID on chronic neuroinflammation are age dependent," *Neurobiology of Aging*, vol. 20, no. 3, pp. 305–313, 1999.

[48] A. M. Planas, M. A. Soriano, E. Rodríquez-Farré, and I. Ferrer, "Induction of cyclooxygenase-2 mRNA and protein following transient focal ischemia in the rat brain," *Neuroscience Letters*, vol. 200, no. 3, pp. 187–190, 1995.

[49] G. Tocco, J. Freire-Moar, S. S. Schreiber, S. H. Sakhi, P. S. Aisen, and G. M. Pasinetti, "Maturational regulation and regional induction of cyclooxygenase-2 in rat brain: implications for Alzheimer's disease," *Experimental Neurology*, vol. 144, no. 2, pp. 339–349, 1997.

[50] G. M. Pasinetti and P. S. Aisen, "Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain," *Neuroscience*, vol. 87, no. 2, pp. 319–324, 1998.

[51] Y. Matsuoka, M. Picciano, B. Maleste, et al., "Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer's disease," *American Journal of Pathology*, vol. 158, no. 4, pp. 1345–1354, 2001.

[52] S. J. Hewett, T. F. Uliasz, A. S. Vidwans, and J. A. Hewett, "Cyclooxygenase-2 contributes to N-methyl-D-aspartate-mediated neuronal cell death in primary cortical cell culture," *Journal of Pharmacology and Experimental Therapeutics*, vol. 293, no. 2, pp. 417–425, 2000.

[53] L. B. Willard, B. Hauss-Wegrzyniak, W. Danysz, and G. L. Wenk, "The cytotoxicity of chronic neuroinflammation upon basal forebrain cholinergic neurons of rats can be attenuated by glutamatergic antagonism or cyclooxygenase-2 inhibition," *Experimental Brain Research*, vol. 134, no. 1, pp. 58–65, 2000.

[54] T. Kunz and E. H. Oliw, "The selective cyclooxygenase-2 inhibitor rofecoxib reduces kainate-induced cell death in the rat hippocampus," *European Journal of Neuroscience*, vol. 13, no. 3, pp. 569–575, 2001.

[55] E. Araki, C. Forster, J. M. Dubinsky, M. E. Ross, and C. Iadecola, "Cyclooxygenase-2 inhibitor NS-398 protects neuronal cultures from lipopolysaccharide-induced neurotoxicity," *Stroke*, vol. 32, no. 10, pp. 2370–2375, 2001.

[56] K. Yasojima, C. Schwab, E. G. McGeer, and P. L. McGeer, "Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs," *Brain Research*, vol. 830, no. 2, pp. 226–236, 1999.

[57] L. Ho, D. Purohit, V. Haroutunian, et al., "Neuronal cyclooxygenase 2 expression in the hippocampal formation as a function of the clinical progression of Alzheimer disease," *Archives of Neurology*, vol. 58, no. 3, pp. 487–492, 2001.

[58] W. J. Lukiw and N. G. Bazan, "Cyclooxygenase 2 RNA message abundance, stability, and hypervariability in sporadic Alzheimer neocortex," *Journal of Neuroscience Research*, vol. 50, no. 6, pp. 937–945, 1997.

[59] J. W. Chang, P. D. Coleman, and M. K. O'Banion, "Prostaglandin G/H synthase-2 (cyclooxygenase-2) mRNA expression is decreased in Alzheimer's disease," *Neurobiology of Aging*, vol. 17, no. 5, pp. 801–808, 1996.

[60] T. J. Montine, K. R. Sidell, B. C. Crews, et al., "Elevated CSF prostaglandin E2 levels in patients with probable AD," *Neurology*, vol. 53, no. 7, pp. 1495–1498, 1999.

[61] R. K. K. Lee, S. Knapp, and R. J. Wurtman, "Prostaglandin E2 stimulates amyloid precursor protein gene expression: inhibition by immunosuppressants," *Journal of Neuroscience*, vol. 19, no. 3, pp. 940–947, 1999.

[62] M. A. A. Blom, M. G. H. Van Twillert, S. C. de Vries, et al., "NSAIDS inhibit the IL-1 β-induced IL-6 release from human post-mortem astrocytes: the involvement of prostaglandin E2," *Brain Research*, vol. 777, no. 1-2, pp. 210–218, 1997.

[63] B. L. Fiebich, M. Hüll, K. Lieb, K. Gyufko, M. Berger, and J. Bauer, "Prostaglandin E2 induces interleukin-6 synthesis in human astrocytoma cells," *Journal of Neurochemistry*, vol. 68, no. 2, pp. 704–709, 1997.

[64] K. A. Kelley, L. Ho, D. Winger, et al., "Potentiation of excitotoxicity in transgenic mice overexpressing neuronal cyclooxygenase-2," *American Journal of Pathology*, vol. 155, no. 3, pp. 995–1004, 1999.

[65] G. M. Pasinetti, "Cyclooxygenase and inflammation in Alzheimer's disease: experimental approaches and clinical interventions," *Journal of Neuroscience Research*, vol. 54, no. 1, pp. 1–6, 1998.

[66] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[67] J. M. Lehmann, J. M. Lenhard, B. B. Oliver, G. M. Ringold, and S. A. Kliewer, "Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs," *Journal of Biological Chemistry*, vol. 272, no. 6, pp. 3406–3410, 1997.

[68] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[69] C. K. Combs, D. E. Johnson, S. B. Cannady, T. M. Lehman, and G. E. Landreth, "Identification of microglial signal transduction pathways mediating a neurotoxic response to amyloidogenic fragments of β-amyloid and prion proteins," *Journal of Neuroscience*, vol. 19, no. 3, pp. 928–939, 1999.

[70] C. K. Combs, D. E. Johnson, J. C. Karlo, S. B. Cannady, and G. E. Landreth, "Inflammatory mechanisms in Alzheimer's disease: inhibition of β-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARγ agonists," *Journal of Neuroscience*, vol. 20, no. 2, pp. 558–567, 2000.

[71] M. A. Ansari and S. W. Scheff, "Oxidative stress in the progression of alzheimer disease in the frontal cortex," *Journal of Neuropathology and Experimental Neurology*, vol. 69, no. 2, pp. 155–167, 2010.

[72] M. A. Smith, X. Zhu, M. Tabaton, et al., "Increased iron and free radical generation in preclinical Alzheimer disease and mild cognitive impairment," *Journal of Alzheimer's Disease*, vol. 19, no. 1, pp. 353–372, 2010.

[73] H. M. Schipper, W. Song, H. Zukor, J. R. Hascalovici, and D. Zeligman, "Heme oxygenase-1 and neurodegeneration: expanding frontiers of engagement," *Journal of Neurochemistry*, vol. 110, no. 2, pp. 469–485, 2009.

[74] M. J. Alcaraz, P. Fernandez, and M. I. Guillén, "Anti-inflammatory actions of the heme oxygenase-1 pathway," *Current Pharmaceutical Design*, vol. 9, no. 30, pp. 2541–2551, 2003.

[75] A. Cuadrado and A. I. Rojo, "Heme oxygenase-1 as a therapeutic target in neurodegenerative diseases and brain infections," *Current Pharmaceutical Design*, vol. 14, no. 5, pp. 429–442, 2008.

[76] K. Kimura, "Mechanisms of active oxygen species reduction by non-steroidal anti-inflammatory drugs," *International Journal of Biochemistry and Cell Biology*, vol. 29, no. 3, pp. 437–446, 1997.

[77] M. Nivsarkar, A. Banerjee, and H. Padh, "Cyclooxygenase inhibitors: a novel direction for Alzheimer's management," *Pharmacological Reports*, vol. 60, no. 5, pp. 692–698, 2008.

[78] C. Guardia-Laguarta, M. Pera, and Lleó A, "γ-secretase as a therapeutic target in Alzheimer's disease," *Current Drug Targets*, vol. 11, no. 4, pp. 506–517, 2010.

[79] S. Weggen, J. L. Eriksen, P. Das, et al., "A subset of NSAIDs lower amyloidogenic Aβ42 independently of cyclooxygenase activity," *Nature*, vol. 414, no. 6860, pp. 212–216, 2001.

[80] T. Kukar, M. P. Murphy, J. L. Eriksen, et al., "Diverse compounds mimic Alzheimer disease-causing mutations by augmenting Aβ42 production," *Nature Medicine*, vol. 11, no. 5, pp. 545–550, 2005.

[81] A. Lleó, O. Berezovska, L. Herl, et al., "Nonsteroidal anti-inflammatory drugs lower Aβ42 and change presenilin 1 conformation," *Nature Medicine*, vol. 10, no. 10, pp. 1065–1066, 2004.

[82] P. L. McGeer, M. Schulzer, and E. G. McGeer, "Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies," *Neurology*, vol. 47, no. 2, pp. 425–432, 1996.

[83] S. C. Vlad, D. R. Miller, N. W. Kowall, and D. T. Felson, "Protective effects of NSAIDs on the development of Alzheimer disease," *Neurology*, vol. 70, no. 19, pp. 1672–1677, 2008.

[84] B. K. Martin, C. Szekely, J. Brandt, et al., "Cognitive function over time in the Alzheimer's disease anti-inflammatory prevention trial (ADAPT): results of a randomized, controlled trial of naproxen and celecoxib," *Archives of Neurology*, vol. 65, no. 7, pp. 896–905, 2008.

[85] C. Wolfson, A. Perrault, Y. Moride, J. M. Esdaile, L. Abenhaim, and F. Momoli, "A case-control analysis of nonsteroidal anti-inflammatory drugs and Alzheimer's disease: are they protective?" *Neuroepidemiology*, vol. 21, no. 2, pp. 81–86, 2002.

[86] J. M. Craft, D. M. Watterson, and L. J. Van Eldik, "Human amyloid β-induced neuroinflammation is an early event in neurodegeneration," *GLIA*, vol. 53, no. 5, pp. 484–490, 2006.

[87] A. V. Yermakova and M. Kerry O'Banion, "Downregulation of neuronal cyclooxygenase-2 expression in end stage Alzheimer's disease," *Neurobiology of Aging*, vol. 22, no. 6, pp. 823–836, 2001.

[88] M. Combrinck, J. Williams, M. A. De Berardinis, et al., "Levels of CSF prostaglandin E2, cognitive decline, and survival in Alzheimer's disease," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 77, no. 1, pp. 85–88, 2006.

[89] P. S. Aisen, "The potential of anti-inflammatory drugs for the treatment of Alzheimer's disease," *Lancet Neurology*, vol. 1, no. 5, pp. 279–284, 2002.