From Division of Neurodegeneration
Center for Alzheimer Research
Department of Neurobiology, Care Sciences and Society
Karolinska Institutet, Stockholm, Sweden

Resolution of inflammation as a therapeutic target for Alzheimer's disease

Mingqin Zhu
朱明勤

Karolinska Institutet

Stockholm 2015

Published by Karolinska Institutet.

Printed by Åtta.45 Tryckeri AB.

ISBN 978-91-7549-922-2

Resolution of inflammation as a therapeutic target for Alzheimer's disease

THESIS FOR DOCTORAL DEGREE (Ph.D.)

The thesis will be defended at Hörsalen, Novum, 4th floor, Huddinge

on Friday May 29th, 2015, at 09:30

By

Mingqin Zhu

Principal Supervisor:
Marianne Schultzberg
Karolinska Institutet
Department of Neurobiology, Care Science and Society
Division of Neurodegeneration

Co-supervisor(s):
Erik Hjorth
Karolinska Institutet
Department of Neurobiology, Care Science and Society
Division of Neurodegeneration

Maria Eriksdotter
Karolinska Institutet
Department of Neurobiology, Care Science and Society
Division of Neurodegeneration

Opponent:
Michael T. Heneka
University of Bonn
Department of Physiology and Medicine

Examination Board:
Anna Fogdell Hahn
Karolinska Institutet
Department of Clinical Neuroscience

Anna Erlandsson
Uppsala University
Department of neuroscience, neurosurgery

Matti Viitanen
Karolinska Institutet
Department of Neurobiology, Care Science and Society
Division of Neurodegeneration

To my family

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, clinically manifested by memory impairment, loss of ability in conducting daily activity and increased need for care, thus causing immense suffering for the afflicted and relatives, as well as a huge economic burden to the society. Despite the existence of symptom-relieving drugs, there is no drug that can stop the progression or cure the disease. Therefore, new disease-modifying drugs are called for. Epidemiological studies suggest that among persons with long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) there is a lower prevalence of AD, and there is extensive evidence for the presence of a chronic inflammation in AD, that may promote pathology and pathogenesis, presenting inflammation as a therapeutic target. Resolution of inflammation belongs to the last phase of the inflammatory response, where inflammation is down-regulated and return to homeostasis is promoted. Chronic inflammation can thus be a result of a failure in resolution mechanisms. The levels of specialized pro-resolving lipid mediators (SPMs), mediators of resolution derived from polyunsaturated fatty acids (PUFA), are decreased in chronic inflammatory diseases including AD, opening a new field of therapeutic intervention for AD by stimulating resolution of inflammation.

In **Paper I**, studies on a human microglial cell line showed that both the omega-3 (n-3) FAs docosahexaenoic (DHA) and eicosapentaenoic acid (EPA), increased microglial phagocytosis of β-amyloid 1-42 ($A\beta_{42}$) peptide, and down-regulated the pro-inflammatory phenotype. In **Paper II**, we asked the question how $A\beta_{42}$ affects the resolution of inflammation. We showed that $A\beta_{42}$ is more suppressive on the resolution pathway in the human microglia compared to the inflammation induced by lipopolysaccharide (LPS), indicating that there is an impairment of resolution specific for this major molecular pathological hallmark of AD. **Paper III** describes lower levels of the SPMs maresin 1 (MaR1), protectin D1 (PD1) and resolvin (Rv) D5 in the entorhinal cortex in AD. Also, lipoxin A_4 (LXA_4), MaR1, RvD1 and protectin DX (PDX) exerted neuroprotective activity against staurosporine-induced apoptosis. MaR1 and RvD1 down-regulated $A\beta_{42}$-induced pro-inflammatory activation in human microglia. Microglial phagocytosis of $A\beta_{42}$ was increased by MaR1. Findings in **Paper IV** on the influence of apolipoprotein (Apo) E4 allele on the plasma SPM levels upon n-3 FA supplementation in AD patients, indicate that conversion of FAs to SPMs is not increased by supplementation with n-3 FAs, and showed that the proportion of patients with unchanged/improved cognition was higher in the n-3 FA-treated group compare to placebo, but only in ApoE4 non-carriers.

In conclusion, these studies show that resolution of inflammation is impaired in AD, and support the idea that stimulating the resolution of inflammation is a potential therapeutic strategy in AD.

LIST OF SCIENTIFIC PAPERS

I. Erik Hjorth*, **Mingqin Zhu***, Veronica Cortés-Toro, Inger Vedin, Jan Palmblad, Tommy Cederholm, Yvonne Freund-Levi, Gerd Faxen-Irving , Lars-Olof Wahlund, Hans Basun , Maria Eriksdotter & Marianne Schultzberg
Omega-3 fatty acids enhance phagocytosis of Alzheimer's disease-related amyloid-β_{42} by human microglia and decrease inflammatory markers
J Alzheimers Dis **35**, 697-713.

II. **Mingqin Zhu**, Xiuzhe Wang, Marianne Schultzberg & Erik Hjorth
Differential regulation of resolution in inflammation induced by Aβ_{42} and lipopolysaccharides in human microglia
J Alzheimers Dis **43**, 1237-1250.

III. **Mingqin Zhu**, Xiuzhe Wang, Erik Hjorth, Romain A. Colas, Lisa Schröder, Ann-Charlotte Granholm, Charles N. Serhan & Marianne Schultzberg
Pro-resolving lipid mediators improve neuronal survival and increase Aβ_{42} phagocytosis
Manuscript

IV. **Mingqin Zhu**, Erik Hjorth, Veronica Cortés-Toro, Yvonne Freund-Levi, Maria Eriksdotter, Jan Palmblad, Tommy Cederholm, Marianne Schultzberg and the OmegAD Study Group
Lipid mediators in plasma from AD patients receiving supplementation with n-3 fatty acids - relation to ApoE4. The OmegAD study
Manuscript

* indicates equal contribution

CONTENTS

LIST OF ABBREVIATIONS

AT-LXA_4	aspirin-triggered lipoxin A_4
α7nAChR	α7 nicotinic cholinergic receptor
Aβ	amyloid β
ACh	acetylcholine
AD	Alzheimer's disease
ALA	α-linoleic acid
ALX/FPR2	lipoxin A_4 (LXA_4)/formyl peptide receptor 2
ApoE	apolipoprotein E
APP	amyloid β (Aβ) precursor protein
BBB	blood-brain barrier
BLT1	leukotriene B_4 receptor 1
CD40L	CD40 ligand
ChEI	cholinesterase inhibitor
ChemR23	chemerin receptor 23
CNS	central nervous system
COX	cyclooxygenase
CR	complement receptor
CSF	cerebrospinal fluid
DAMP	damage-associated molecular pattern
DHA	docosahexaenoic acid
DP2	receptor for PGD_2
DPA	docosapentaenoic acid
DSM-IV	diagnostic and statistical manual, 4^{th} edition
ELISA	enzyme-linked immunosorbent assay
ENT	entorhinal cortex
EPA	eicosapentaenoic acid
ERK	extracellular signal-regulated kinase
FA	fatty acid
FPRL1	formyl peptide receptor-like 1
GFAP	glial fibrillary acidic protein
GPCR	G protein-coupled receptor

GPR32	G protein receptor 32
12, 15-HETE	12/15-hydroxyeicosatetraenoic acid
5-HETE	5-hydroxy-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid
ICD-10	international classification of disease, 10^{th} revision
IDE	insulin-degrading enzyme
IFN	interferon
IL	interleukin
LA	linoleic acid
LC-MS-MS	lipid chromatography - tandem-mass spectrometry
LDH	lactate dehydrogenase
LM	lipid mediator
LOX	lipoxygenase
LPS	lipopolysaccharide
LRP	low-density lipoprotein-related protein
LT	leukotriene
LTP	long-term potentiation
LXA_4	lipoxin A_4
MAPK	mitogen-activated protein kinase
MaR1	maresin 1
MCI	mild cognitive impairment
MCP-1	monocyte chemoattractant protein-1
MHC-II	major histocompatibility complex class II
MMP9	matrix metalloproteinase 9
MMSE	mini-mental state examination
MRI	magnetic resonance imaging
MS	multiple sclerosis
MVA	multivariate analysis
NF-κB	nuclear factor κ-light-chain-enhancer of activated B cells
NFT	neurofibrillary tangle
NSAID	non-steroidal anti-inflammatory drug
OPLS-DA	orthogonal projections to latent structures - discrimination analysis
PAMP	pathogen-associated molecular pattern
PD	Parkinson’s disease

PD1	protectin D1
PET	positron emission tomography
PG	prostaglandin
PHF	paired helical filament
PPAR	peroxisome proliferator-activated receptor
PRR	pattern recognition receptor
PUFA	polyunsaturated fatty acid
RA	retinoic acid
RAGE	receptor for advanced glycation end products
RNS	reactive nitrogen species
ROS	reactive oxygen species
RvD1	resolvin D1
RvE1	resolvin E1
SPM	specialized pro-resolving lipid mediator
SR	scavenger receptor
STS	staurosporine
ThT	thioflavin T
TNF	tumour necrosis factor
TREM2	triggering receptor expressed on myeloid cells

1 INTRODUCTION

1.1 ALZHEIMER'S DISEASE

In 1906, at the meeting of Psychiatrists of Southwest Germany, psychiatrist and neuropathologist Alois Alzheimer reported the neuropathological and clinical features of one of his cases, who died of dementia at the age of 55 [1]. The disease was named "Alzheimer's disease" in 1910 by his senior colleague Kraepelin [2]. Actually, the clinical features of Alzheimer's disease (AD) had been documented by the ancient Greeks, and Alois Alzheimer was not the first one to describe senile plaques, however, it was him who first reported the tangle pathology [2]. For a long period of time after the baptism of the disease, the term "Alzheimer's disease" was only used for describing the presenile dementia (less than 65 years old), and scientists did not at first separate between Alzheimer's disease and other types of dementia such as Pick's disease, Creuzfeldt-Jakob disease. Not until the 1970s, it became clear the late-onset "senile dementia" also have AD pathology, leading to altered definitions together with increased research funding and attention.

1.1.1 Epidemiology

The prevalence of dementia was estimated to be 35.6 million worldwide. The number was expected to be double every 20 years [3]. The total estimated costs of dementia were 604 billion dollars worldwide [4]. AD is the most common cause of dementia, accounting for 60-80% of all dementia cases. It is estimated that one in nine people above the age of 65 has AD, and about one-third of those above 85 [5]. It is noteworthy that about half of these cases display pathological changes related to other causes of dementia such as vascular pathology and Lewy body pathology, indicating the complexity and heterogeneity of the disease [6]. It has been suggested that AD is an under-diagnosed disease, since only those with onset of the clinical syndrome receives the diagnosis. However, AD pathology begins considerably earlier than the clinical manifestations. Therefore, a lot of people have a pre-clinical stage of AD unknowingly.

1.1.2 Risk factors

AD is a multifactorial disease, involving both genetic and environmental factors, among which aging is the strongest risk factor. However, AD is not a part of normal aging. Even though it was first described 100 years ago, the etiology is still unclear, and a tremendous amount of effort will be required before we fully understand the disease. Mutations in the genes encoding APP, presenilin 1 and presenilin 2, give rise to familial forms of AD, accounting for about 5% of AD cases [7]. However, the majority of AD cases belong to the

sporadic form, with unclear causality. The E4 allele of the apolipoprotein E (ApoE) gene is the highest risk factor for sporadic AD; individuals with a single ApoE4 allele have 2-5 fold increased risk of developing AD, while ApoE4 double carriers have 12-17 fold increased risk [8]. There are 3 ApoE gene types, E2, E3 and E4, which differ by single amino acid interchanges at residues 112 and 158. Other risk factors for sporadic AD include family history [9], and risk factors that are common with cardiovascular disease such as smoking, obesity, diabetes, high cholesterol and hypertension at mid-age [10-12]. Social and cognitive inactivity, low education and traumatic brain injury are also risk factors [13]. It appears that rather than having a single cause, AD is probably a consequence of multiple factors converging on a disruption of the homeostasis in the brain.

1.1.3 Clinical aspects – clinical manifestations, diagnosis and treatment strategy to date

The clinical symptoms are the reflection of the pathological changes in the brain. Neurons and synapses represent the fundamental basis for memories, thoughts and movements. Defects in the function of neuronal networks and synapses followed by the death of neurons (*i.e.* neurodegeneration) are the ultimate causes for the clinical symptoms of AD.

1.1.3.1 Clinical manifestation

The clinical symptoms of AD vary between individuals, the most common clinical manifestation being a gradually worsening in remembering new information. As the disease progresses, other areas of the brain are affected by the neurodegeneration and cause additional cognitive and behavioral symptoms, clinically manifested by a general difficulty in thinking, reasoning, planning, and the withdrawal from social activities, changes in mood and personality, and also associated with depression. Eventually, basic physiological functions such as swallowing are impaired. The life span after the diagnosis of AD is usually less than 10 years [14, 15].

1.1.3.2 Diagnosis

The ultimate criterion for diagnosing AD is the establishment of the presence of histopathological hallmarks by autopsy of the post-mortem brain. However, autopsy and post-mortem diagnosis is more helpful for research purpose. The most commonly used criteria for diagnosing AD in the clinic are the International Classification of Disease, 10th revision (ICD-10)(WHO 1992), the Diagnostic and Statistical Manual, 4th edit, (DSM-IV) (American Psychiatric Association 1994), the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS), and the Alzheimer's Disease and Related Disorders Association (ADRDA) workgroup in 1984 (NINCDS-ADRDA) criteria [16]. However, all of these criteria have limitations, since they require memory loss and

impairment of at least one non-memory domain function such as language, motor, perception and executive function. Early diagnosis is crucial for an early intervention, and with the development of positron emission tomography (PET), and magnetic resonance imaging (MRI) technologies, together with the development of assays for the analysis of biomarkers of AD in biological samples, new proposals have been published for diagnosing AD. In these, analysis of different aspects of brain imaging, and the levels of CSF biomarkers such as $A\beta_{42}$ and tau, were incorporated [17, 18].

1.1.3.3 Therapeutics and prevention

Drugs that are used clinically for treatment of AD patients target the cholinergic (Donepezil, Galantamine, Rivastigmine, Tacrine) or glutamate (Memantine) system. However, these drugs only provide limited short-term relief of symptoms, without affecting the underlying pathological mechanisms. There is currently no drug or treatment that can halt or reverse the progression of the disease.

Ongoing clinical trials include strategies that target Aβ, tau, neurotransmitters, inflammation, and other factors affected in AD. Several previous strategies have failed when tried in a larger scale [19-21], despite promising results in smaller clinical trials, shedding light on problems with the design of early clinical trials where patients may be "over-selected" so that they do not represent a general population. The lack of results after so much effort can produce a feeling of hopelessness. However, there are a plethora of pre-clinical studies, aiming to discover new treatment strategies with the ability to modify the pathological course of AD by affecting different pathways related to the disease. Since AD is a multifactorial disease, a combination of therapies, or drugs that have multiple targets, may have a higher chance for successful treatment. As AD pathology develops decades before the onset of clinical symptoms, prevention is also of great importance. Studies indicate that people with an active lifestyle, including physical and leisure activities, and an absence of vascular risk factors have a lower incidence of AD [13, 22].

1.1.4 Pathological aspects

The gross histopathology of AD is characterized by brain atrophy, deepening of the cerebral grooves and the enlargement of the cerebral ventricles. Histologically, AD is characterized by extracellular deposition of senile plaques, intracellular accumulation of neurofibrillary tangles (NFTs) [1], and chronic inflammation [23-26]. Genetic studies have shown that mutations in the amyloid β (Aβ) precursor protein (APP), or in the genes for APP-processing proteins, can cause early onset (familial) AD [27-29], while genes related to innate immunity and inflammation were found to be associated with late-onset (sporadic) AD [30, 31]. However, there is, so far, no evidence for mutations in the tau gene associated with AD. Even though *in*

vitro studies show that Aβ can induce tau hyperphosphorylation in neurons [32, 33], APP transgenic mice, which have an overabundance of Aβ, do not have tau pathology [34]. Interactions between the pathologies of Aβ and tau have not been clarified so far. There is evidence that inflammation can increase the production of Aβ and induce tau hyperphosphorylation in *in vitro* studies [35, 36]. It is highly likely that these three main pathological pathways interact with each other and drives the pathogenesis of AD.

1.1.4.1 Aβ

Aβ is a 37-43 amino acid long peptide, produced from sequential cleavage APP by the β- and γ-secretases [37]. Aβ has been considered as a main culprit of AD, and the Aβ hypothesis has been dominant for explaining the pathogenesis of AD [38]. There is a plethora of evidence that support the Aβ hypothesis: patients with an extra copy of chromosome 21, where the APP gene locates, develop dementia at an early age; APP transgenic mice exhibit significant cognitive impairment; the toxicity of Aβ has been documented extensively in *in vitro* studies [35, 39, 40].

Aβ is mainly produced by neurons, and in pathological conditions misfolded and abnormally aggregated. The 40 amino acid long Aβ species is abundant in the AD brain, but the majority of studies have indicated the $A\beta_{42}$ form as the main pathological species [41]. Aβ peptide is prone to aggregate to oligomers and large fibrils [42]. Histologically, different types of amyloid plaques are found in the AD brain, such as dense core and diffuse (cotton wool) plaques, indicating a differential aggregation status of Aβ [43]. Despite strong evidence supporting an etiological role of Aβ, there is no clear evidence for a correlation between the total Aβ burden and cognitive impairment [44]. Therefore, different aggregation forms of Aβ, the relative levels of which may differ between patients, may have different properties regarding neurotoxicity. Results from studies designed to address the neurotoxicity of different species of Aβ aggregation suggest that the oligomeric forms of Aβ are the most neurotoxic species [45, 46].

Aβ is produced in the normal healthy brain, and under physiological conditions, Aβ can be degraded or cleared from the brain by different pathways. Thus, Aβ can be degraded by enzymes such as neprilysin, insulin-degrading enzyme (IDE), and matrix metalloproteinase 9 (MMP9) [47-50]. Alternatively, Aβ can be transported from the brain and into the blood by transporters such as low-density lipoprotein-related protein (LRP) and the ATP-binding cassette (ABC) transporter permeability glycoprotein (P-GP) [51, 52]. In addition, Aβ can be cleared from the brain by non-specific flow of interstitial fluid to the blood [53]. Aβ can be transported into the CNS *via* the receptor for advanced glycation end products (RAGE) [54]. Therefore, a balance of the influx and efflux of Aβ to and from the brain is required to maintain homeostatic physiological conditions.

To cure a disease, the ideal way is to affect the disease etiology and target the molecule that is believed to drive the pathogenesis of the disease. In this regard, Aβ has received most of the attention during the past decade. Many hypotheses that involve Aβ production or clearance have been tested, both in animal models and in clinical trials, including immunization and inhibition of Aβ production. Great achievements have been made from the animal models. However, unfortunately, these findings could not be translated to humans in clinical trials due to insignificant beneficial effects or significant side effects (reviewed in [55]).

1.1.4.2 Tau

Tau is a microtubule-associated protein that binds to and stabilizes the microtubule, and thereby involved in the transport between the cell soma and the axonal terminals [56]. In the AD brain, tau protein is abnormally hyperphosphorylated, dissociated from the microtubules and accumulated in the soma and dendrites, there forming paired helical filaments (PHFs) and NFTs. The dissociation of tau from the microtubules can cause deficiency in axonal transport, resulting in the degeneration of the axon and causing death of the neuron [57]. In contrast to Aβ load, the NFT load correlates well with cognitive impairment [43]. In addition to a therapy based on reduction of Aβ, reducing the hyperphosphorylation of tau represents another major therapeutic target for AD. Strategies targeting tau pathology include vaccination, anti-tau aggregation [58], and the targeting of kinases that are involved in tau phosphorylation [59].

1.2 INFLAMMATION

The term “inflammation” was originally defined by Celsus, who described the four classical cardinal signs of inflammation; “tumor, rubor, calor and dolor” [60, 61]. As we grow to understand more about the molecular mechanisms in inflammation biology, inflammation has extended its original definition to a broad spectrum of situations, including diseases in the absence of the four cardinal signs.

1.2.1 Components of inflammation

Cellular and molecular mediators are fundamental units that mediate inflammation. Their levels can vary between different tissues, diseases, and even different stages of a disease. My thesis mainly focuses on inflammation in the brain in relation to AD except for paper IV in which inflammatory factors in the peripheral blood is studied.

1.2.1.1 Cellular components of inflammation in the brain

The inflammatory response is a synthesis of activities, and the interactions between the cell types present in the brain, primarily microglia, astrocytes and neurons, although oligodendrocytes and brain endothelial cells also play a role [62, 63].

1.2.1.1.1 Microglia

Microglial cells enter the brain during development from the yolk sac during hematopoiesis [64, 65], and remain in the brain as resident immune cells. Macrophages of peripheral origin can be found in the meninges, choroid plexus, and perivascular space. However, they cannot cross the blood brain barrier (BBB) under normal conditions. Therefore, the renewal of microglial cells in the adult brain depends on local progenitor cells [66, 67]. "Ramified" and "amoeboid" microglia can be found in the brain at different stages of diseases [68]. The morphology of microglia is adapted to their function. At the resting state, microglia exhibit a "ramified" morphology with long processes, thus increasing their ability to detect changes and threats in the microenvironment. For this function, microglial cells express various pattern recognition receptors (PRR) that can recognize pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs), so that they can detect and respond to the threat at the place of encounter.

Microglia are highly motile, which enables them to respond rapidly to a threat. Activated microglia exhibit an "amoeboid" shape, with an increased capacity for phagocytosis. As resident immune cells in the brain, apart from immune surveillance, microglial cells have critical physiological functions. During development, the microglia can remove excessive synapse growth, also known as synaptic pruning, which is crucial for neuronal development [69]. In addition, microglia can produce neuronal growth factors that support the survival and function of neurons [70].

Studies suggest that similarly to peripheral macrophages, microglial cells can be activated in different directions [71], although in the *in vivo* situation there is a continuum between the M1 – M2 phenotypes, which can also overlap. Therefore, this nomenclature should not be interpreted or used in a binary manner. However, the M1/M2 nomenclature can be helpful since the phenotype of microglia is a reflection of their function, and a dominance of either of them can be indicative of the situation for the tissue in which they reside. Pro-inflammatory M1 microglia are activated to "destroy" as they produce large amounts of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), while anti-inflammatory M2 microglia are activated for repair. The phenotype of the microglia is shaped by the surrounding microenvironment and can be viewed as a response to promote the homeostasis of the microenvironment. On the other hand, the products secreted by microglia contribute to the constitution of the microenvironment [72, 73], and can certainly perturb homeostasis by "over-reacting".

Microglial cells have been found in the vicinity of the senile plaques [23], and are positive for major histocompatibility complex class II (MHC-II) and complement receptors [24, 74], indicating a pro-inflammatory (M1) activation. The observation that microglia surround senile plaques indicates that they are attracted to deal with the overabundance of Aβ, and may therefore play a beneficial role in AD in clearance of Aβ. However, the phagocytosis and clearance of Aβ by microglia appears to be insufficient in AD. Furthermore, activated microglia produce molecules such as pro-inflammatory cytokines and ROS that are detrimental for the neurons, indicating that microglia may help drive the pathogenesis of AD. Therefore, modulation of microglia in the way that promotes phagocytosis without causing pro-inflammatory activation represents a possible treatment strategy for AD.

Misfolded Aβ can be recognized as DAMPs by microglial cells. Aβ interacts with various classes of PRRs, and distinct down-stream events may occur depending on which receptor Aβ binds to. CD36, which belongs to the scavenger receptor (SR) B family, has been reported to interact with fibrillar Aβ, and to induce the production of ROS, and therefore the activation of CD36 by Aβ is associated with a pro-inflammatory phenotype of microglia [75]. Complement receptor (CR) 3, also called CD11b/CD18 or macrophage antigen complex-1 (MAC-1), belongs to the leukocyte β2-integrin receptor family, and has been found to co-localize with senile plaques, indicating its role in clearance of Aβ [76]. CR3 has been reported to mediate microglial activation and ROS production in response to Aβ [77]. CD33, also known as siglec 3 can inhibit the phagocytosis of Aβ [78]. Notably, CD33 gene variance has been identified as a risk factor for AD, and increased levels of CD33 have been found in AD [79].

Aβ binds to the lipoxin A_4 (LXA_4)/formyl peptide receptor 2 (ALX/FPR2), also called formyl peptide receptor-like 1 (FPRL1), forming a ligand-receptor complex that leads to internalization [80]. Interestingly, ALX/FPR2 also transduces an anti-inflammatory signal, as it binds LXA_4, a mediator that plays an important role in resolution of inflammation (see below) [81].

The consequence of microglial interaction with Aβ is therefore a product of the form of Aβ and the expression profile of microglial Aβ-interacting proteins.

1.2.1.1.2 Astrocytes

Astrocytes play a supportive role for neurons and constitute up to 80% of the glial cells in the brain. Morphologically, astrocyte can exhibit large nuclei with thick irradiating processes (protoplasmic astrocytes), or smaller nuclei with thinner and longer processes (fibrous astrocytes). Glial fibrillary acidic protein (GFAP), a structural protein, and glutamine synthase, are molecular markers for astrocytes. Besides constituting a scaffold for structural support, protoplasmic astrocytes also play a role in removing excess neurotransmitters in the extracellular place, avoiding the over-excitation of postsynaptic neurons. Fibrous astrocytes

constitute a component of the BBB, thereby playing an important role in protecting the brain from the threats of peripheral origin, as well as in the transport of nutrients into the brain. Astrocytes also provide trophic support for neurons by producing neurotrophic factors.

Similarly to microglia, astrocytes are activated during pathological conditions, and produce harmful inflammatory mediators such as cytokines and ROS [82]. Reactive astrocytes, exhibiting higher immunoreactivity for GFAP are found around senile plaques in the AD brain [83], suggested to be an early event in AD pathology [84]. Astrocytes represent the major source of ApoE production in the brain, also co-localized with senile plaques [85]. Astrocytes can be activated by Aβ, upon which they produce cytokines and ROS that cause neuronal injury [86].

1.2.1.1.3 Neurons

In general, neurons are not considered to be inflammatory cells, even though they can produce inflammatory cytokines in cell culture conditions [87]. Neurons can also receive and respond to inflammatory signals by their expression of receptors for cytokines [88]. In the human brain, neuronal cytokines are believed to modulate neuronal signalling and plasticity [89], as well as being involved in sickness behaviour [90]. In AD, neurons express surface molecules that can interact with Aβ, such as RAGE, which contributes to intraneuronal Aβ accumulation and neuronal dysfunction [80].

Both neurons and astrocytes can regulate microglial activation and limit excessive inflammation by producing signals that interact with microglia. Depending on the situation, both activating and inhibitory signals can be produced. The CD200 ligand (CD200L), produced by both neurons and astrocytes, can interact with CD200R, a cell surface glycoprotein that is expressed by microglia. The interaction between CD200 and CD200R inhibits microglial activation [91], and there is evidence that both CD200 and CD200R are decreased in the AD brain [92], suggesting that a lack in inhibitory signals can be a factor that contributes to microglial activation in AD. However, neurons and astrocytes also send signals that can activate microglia, e.g. the CD40 ligand (CD40L). Increased levels of CD40 and CD40L have been found in the AD brain, and AD patients have higher serum levels of sCD40 [93]. Moreover, in the presence of low levels of Aβ, the ligation between CD40 and CD40L can activate microglia, which then produce pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, which may cause damage to the neurons [94].

1.2.1.2 Molecular components of inflammation in the brain

Cytokines, free radicals, lipid mediators (LMs), growth factors and neurotransmitters constitute molecular components in inflammation, and may play a role in the inflammatory response in the brain. In this thesis, I will focus on cytokines and LMs.

Cytokines are small bioactive proteins produced by immune cells as well as other cell types. Important cytokines include the interleukin (IL) family, the TNF family and the interferon (IFN) family. The synthesis of cytokines usually requires the activation of transcription factors such as nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and Janus kinase/signal transducer and activator of transcription (JAK-STAT) in response to threats detected by PRRs. The cytokines can act in an endocrine, autocrine or paracrine manner. Upon binding to their specific receptors [95], the pro- and anti- inflammatory cytokine signalling pathways are activated, leading to the activation of nuclear transcription factors, resulting in the synthesis of these proteins.

Cytokines play a dual role in the memory formation. It has been shown that IL-1β gene expression was increased during induction of long-term potentiation (LTP) both *in vitro* in hippocampal slides and i*n vivo* in rats, and LTP maintenance was impaired by blockage of IL-1 receptors [96], indicating normal physiological levels of cytokines are required for memory formation. In addition, studies on IL-1 receptor type I (IL-1RI) knockout mice showed an impairment of LTP [97], and transgenic expression of human soluble IL-1 receptor antagonist (hsIL-1ra) was found to impair long-term memory consolidation [98]. However, pathophysiological levels of IL-1β have been shown to directly inhibit LTP invoked by tetanic stimulation in rat hippocampus [99], and affect memory consolidation in aged rats following bacterial infection [100]. Increased levels of IL-1β were demonstrated in AD brain tissue [25], and therefore memory impairment mediated by pathological levels of cytokines may play a role in the pathogenesis of AD.

Increased levels of cytokines are usually interpreted as an indicator of inflammation, and have been proposed as potential peripheral biomarkers in the diagnosis of AD. However, reports on cytokine levels in mild cognitive impairment (MCI) or AD in plasma and cerebrospinal fluid (CSF) are inconclusive with contradicting results reviewed in [101]. The levels of cytokines appear to be differentially affected in AD at different stages of the disease pathogenesis. It has been suggested that cytokines can be classified into 3 groups, according to their changes with the progression of the disease [101]: *steadily increasing*, *changed only during conversion period*, and *unchanged*. Cytokines such as IL-1β, IL-6 and TNF-α increase steadily during the progression of the disease, whereas IL-18 and monocyte chemoattractant protein-1 **(**MCP-1) temporarily increase at the time of conversion from MCI to AD, and some cytokines, such as IL-1α and IL-2, remain unchanged during the disease progression. A standardization of cytokine measurements and patient recruitment characterization, together with longitudinal sampling over longer periods of time, is needed to better understand the role and interpretation of peripheral cytokine levels during the course of AD.

1.2.2 Involvement of inflammation in AD

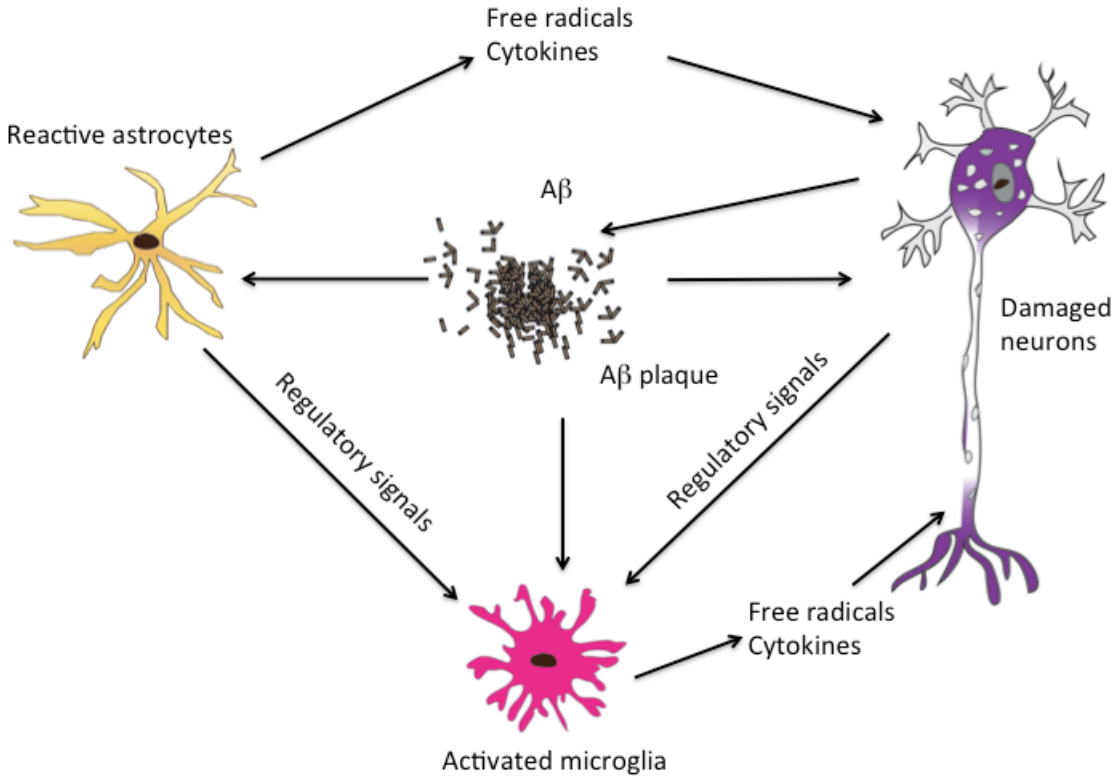

Fig. 1. Reciprocal interplay between Aβ and inflammation in Alzheimer's disease. Aβ peptide can by harmful for neurons by a direct action, or indirectly by activating microglia and astrocytes, which produce pro-inflammatory cytokines and free radicals that can damage the neurons. Inflammatory factors from microglia and astrocytes can stimulate the neuronal production and amyloidogenic processing of APP, resulting in increased levels of Aβ. Microglial cells receive regulatory signals from both neurons and astrocytes, and a loss of inhibitory signals contributes to microglial activation. Aβ = amyloid β.

Evidence accumulated during the last three decades have shown the existence of inflammation in AD, including activated microglia within and surrounding senile plaques [23, 24], and an increase in molecules involved in the innate immunity and inflammation such as CR1, human leukocyte antigen (HLA)-DR [74], and IL-1 [25]. Moreover, results from studies employing imaging techniques that detect microglial activation [102], and from genome-wide association studies (GWAS), showed that gene mutations involved in inflammation and innate immunity such as CR1 and CD33 are associated with increased incidence of AD [103]. Moreover, data from *in vitro* studies show that Aβ can activate microglia and astrocytes [104, 105], leading to increased production of pro-inflammatory cytokines, which can increase the production of APP [35, 106, 107], and amyloidogenic processing of APP, resulting in increased levels of Aβ. Furthermore, inflammation is further potentiated by cellular debris and the microenvironment of damaged tissue. Therefore, a self-reinforcing vicious circle has been proposed to exist in the pathogenesis of AD [35] (Fig. 1).

Other evidence supporting the involvement of inflammation in AD has been provided by epidemiological studies showing that among rheumatoid arthritis patients with long-term

medication of non-steroid anti-inflammatory drugs (NSAIDs) there was a lower incidence of AD [108]. However, clinical trials based on NSAIDs in patients with AD or MCI, have not been successful [109-111], or only beneficial in a subgroup of patients with higher baseline plasma levels of TNF-α and C-reactive protein (CRP) [112]. Several explanations for this have been suggested, such as treatment given when the pathology was already too extensive, too short duration of the intervention, or that the patient group was too heterogeneous. It may be that the beneficial effects of NSAIDs in AD only emerge in situations where a strong, chronic peripheral inflammation is present. This can hypothetically be associated with a profile of signalling and metabolism that is different from normal, and which therefore is receptive to the beneficial effects of NSAIDs.

1.3 RESOLUTION OF INFLAMMATION

Inflammation is a defensive response of the body to harmful stimuli such as infection and injury, for the purpose of eliminating the threat, after which restorative processes take place. In the end, the damaged tissue is meant to be repaired and return to homeostasis. Inflammation is a dynamic process under strict control of regulatory mechanisms that under normal conditions orchestrate the progression of the response from detection, activation, destructive defense, and lastly to down-regulation and restoration. In addition to inflammatory proteins such as cytokines, lipids represent groups of molecules that are involved in the regulation of inflammation. For instance, prostaglandins (PGs) can cause vasodilatation, leading to increased blood flow at the site of injury, and "redness" and "heat". PGs also mediate pain. Recent studies have shown that a group of LMs named specialized pro-resolving lipid mediators (SPMs) mediate the ending, or resolution, of inflammation. Therefore, understanding the regulation of lipid metabolism and lipid signalling in normal and pathological conditions is important.

1.3.1 General aspects of lipids

1.3.1.1 Lipids and the brain

Lipids are hydrophobic or amphiphilic molecules that can be classified into several categories, such as glycerides, free fatty acids (FAs), sterols, phospholipids, and fat-soluble vitamins [113]. The bilayer structure of cellular membranes is based on lipids, which supply energy when metabolized to ATP [114]. Moreover, lipids are also bioactive molecules that play important roles in cell signalling, immune regulation and neurotransmission [115, 116]. Lipids account for up to 50-60% of the brain dry weight, and within the brain gray matter contains 36-40% lipids, white matter 49-66%, and myelin 78–81% [117].

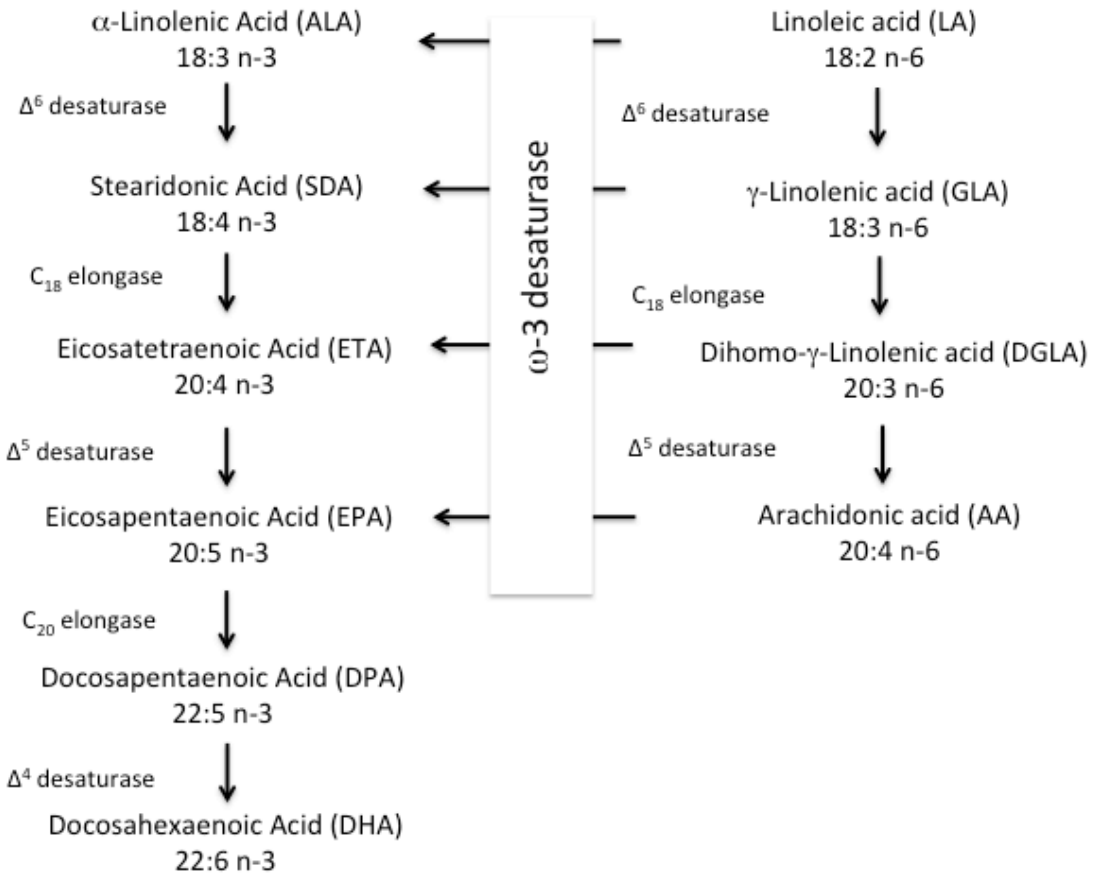

Fig. 2. Synthesis of DHA, EPA and AA from essential fatty acids.

1.3.1.2 Polyunsaturated fatty acids (PUFAs)

Polyunsaturated fatty acids (PUFAs) are FAs with more than one carbon-carbon double bond, classified into omega-3 (n-3) and omega-6 (n-6) FAs based on the position of this, counting from the first double-carbon bond from the methyl end towards the carbonyl end [118]. The n-3 FA family is composed of α–linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA, C20:5), docosapentaenoic acid (DPA, C22:5) and docosahexaenoic acid (DHA, C22:6). The n-6 FAs include linoleic acid (LA, C18:2), arachidonic acid (AA, C20:4), gamma-linoleic acid (GLA, 18:3) and dihomo-gamma-linoleic acid (DGLA, 20:3) [119]. ALA and LA are essential for humans, since they are required for normal physiological functions, but cannot be produced endogenously. Other n-3 and n-6 FAs can be converted from ALA or LA, and are therefore considered as "conditioned essential" [120, 121] (Fig. 2).

1.3.1.3 Metabolism of PUFAs

Some species of fish are enriched in PUFAs, and other dietary sources include nuts, sunflower oil, etc [122]. Dietary sources of lipids are absorbed and synthesized in the liver. Lipids can reach the brain *via* the blood as it has been proved that PUFAs can cross the BBB [123]. DHA is an important constituent of the bilayer of biological membranes, enriched in

neurons, retinoid membranes, therefore being important in neuronal function and vision. Lipids undergo dehydration and esterification, and can be processed to small LMs, shown to be important for mediating inflammation, pain, thrombosis and vascular function, etc. [117]. Enzymes involved in the processing of lipids include cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 (CYP450) [124].

1.3.2 Lipid dysregulation in AD

Lipid dysregulation is a feature of several conditions in which chronic inflammation is present, including obesity, vascular disease and diabetes. Interestingly, these diseases share many risk factors and features with AD, suggesting a complex picture of overlapping and interacting etiologies. A dysregulation of lipids belonging to the PUFA class is suggested by studies showing decreased levels of DHA in the human AD hippocampus compared to control subjects [125], and by dysfunctional conversion from dietary FAs to omega-3 FAs in the liver in AD [126]. Evidence that strongly support a dysregulation of lipids in AD is provided by studies focused on the polymorphisms in the ApoE gene. ApoE is a protein involved in cholesterol transportation and lipid metabolism [127]. It has been demonstrated that the E4 allele is the largest risk factor for sporadic AD [8]. In AD, ApoE is associated with senile plaques [128], however, the total levels of ApoE were found to be decreased in AD compared to controls [129, 130]. Interestingly, in AD patients given a single dose of uniformly ^{13}C-labelled DHA supplementation, the clearance of DHA in plasma was slower in ApoE4 carriers than in non-carriers [131]. Furthermore, the ApoE4 genotype is associated with higher lipid peroxidation [132], which can cause oxidative stress.

Lipid rafts are microdomains rich in sphingolipids, cholesterol and saturated FAs, and play an important role in cell signalling, and are crucial for neuronal functions. Lipid dysregulation in AD is indicated by an altered lipid profile in lipid rafts in the brain [133]. Lipid rafts are also involved in APP-processing, and it has been suggested that APP inside lipid rafts is processed by β-secretase, while APP outside lipid rafts is processed by α-secretase [134]. The alterations in lipid rafts may therefore hypothetically promote amyloidogenic processing of APP, and thus increased levels of Aβ. Moreover, there is evidence for alterations in enzymes involved in FA-processing in the brain, including COXs, 5-LOX and 15-LOX [135, 136].

Furthermore, the mRNA levels for both PGD synthase and the receptor for PGD_2 (DP) were increased in glial cells associated with senile plaques in brains from AD patients, as well as from a mouse model of AD [137]. PGD_2 has been shown to contribute to neurotoxicity mediated by microglia exposed to $A\beta_{42}$ and prion proteins [138]. In addition, altered levels of receptors for LMs, such as peroxisome proliferator-activated receptor (PPAR)-γ that receives and conduct lipid signalling, have been showed in AD [136, 137]. Rebalancing and restoring this altered lipid metabolism seen in AD may have beneficial effects.

1.3.3 Specialized pro-resolving lipid mediators (SPMs)

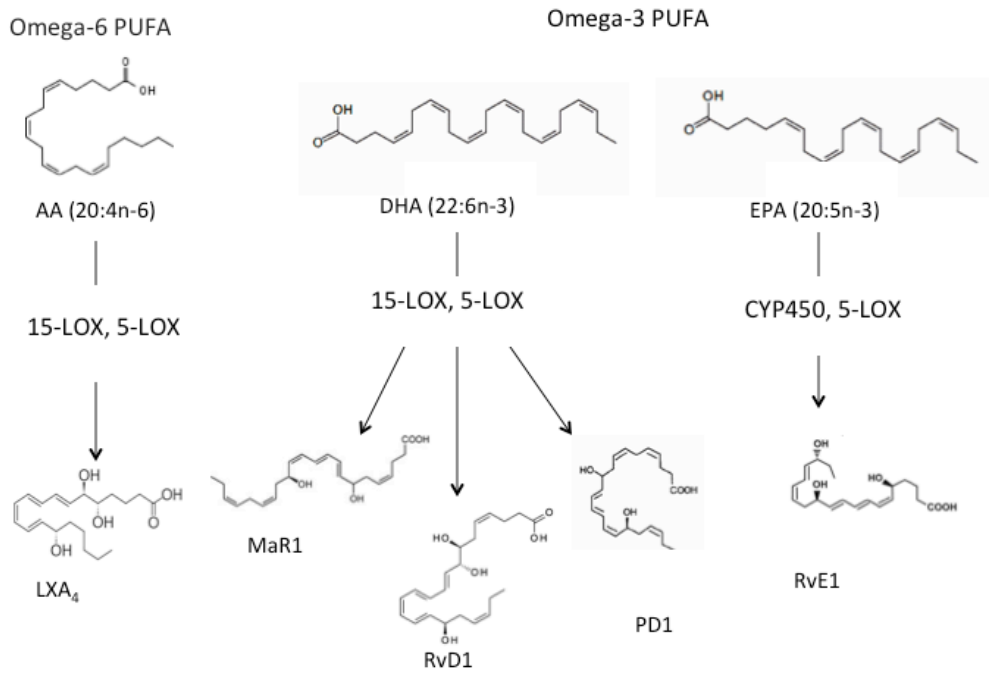

Fig. 3. Synthesis of SPMs from PUFAs. AA = arachidonic acid, CYP450 = cytochrome P450, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, LOX= lipoxygenase, LXA_4 = lipoxin A_4, MaR1 = maresin 1, PD1 = protectin D1, PUFA = polyunsaturated fatty acid， RvD1 = resolvin D1, RvE1 = resolvin E1, SPMs = specialized pro-resolving lipid mediators.

Since inflammation is a protective but potentially self-destructive biological response, it should be ended by resolution after the pathogen is eliminated. Inflammation has commonly been viewed to end by a passive dissipation of the inflammatory mediators. However, accumulating evidence support that the resolution of inflammation is a highly regulated process [124]. Using a liquid chromatography tandem mass spectrometry (LC-MS-MS) technique, a new group of LMs were discovered, the levels of which were found to be increased at the later phase of inflammation [139-141]. The chemical structures of these molecules have been elucidated and their biological functions are under investigation, the work in this thesis is a part of that effort. The molecules were named SPMs, and 4 classes have been identified so far. They include LXA_4, which is derived from AA, the D-series resolvins, protectins and maresins derived from DHA, and the E-series resolvins derived from EPA [124, 142, 143] (see Fig. 3).

The ideal outcome of inflammation is a complete resolution, meaning that the pathogenic threat has to be cleared, and that the damaged tissue returns to normal, *i.e.* a return to homeostasis. However, under pathological conditions, inflammation may persist and lead to chronic inflammation. Chronic inflammation can be a consequence of deficiency in the resolution mechanism. A self-limiting inflammatory response normally occurs, where pro-resolving activities are sufficient to counteract the pro-inflammatory response. However, in

the situation of a chronic inflammatory disease, pro-resolving activities are not sufficient to counteract the pro-inflammatory signalling, leading to persistent inflammation which may lead to chronic inflammatory disease.

A deficiency in resolution has been described in chronic inflammatory diseases including AD. In patients with severe asthma, a chronic inflammatory airway allergic disease, the levels of LXA_4 and neuroprotectin (NP) D1were found to be reduced [144, 145], and lower plasma levels were described in patients with localized aggressive periodontitis (LAP) compared to healthy individuals [146]. Reduced levels of DHA and its derivative NPD1 were demonstrated in the hippocampus of AD patients [125], and the LXA_4 levels were lower in the hippocampus as well as the CSF from AD patients [136]. Furthermore, the levels of LXA_4 and resolvin (Rv) D1 in CSF correlates with cognitive function as determined by MMSE [136], indicating that these SPMs may play a role in preserving memory functions.

In light of the described deficiency in resolution in AD, it is important to understand the biological function of the SPMs in the brain. The biological function of SPMs in the periphery has been studied, but little is known so far with regard to their role and activities in the central nervous system (CNS). Activities of SPMs in relation to the cell type are summarized in Fig. 4.

Given the beneficial functions of SPMs described above, utilizing SPMs as means to treat inflammatory diseases has been investigated in several models of inflammatory diseases. Thus, treatments with SPMs have demonstrated protective effects in models for asthma [147-149], colitis [150, 151], and peritonitis [152, 153]. Furthermore, there is evidence of beneficial effects of SPMs in animal models involving the nervous system, *i.e.* for cerebral ischemia [154-158] and pain [159-163] (see Table 1). In studies on transgenic animal models for AD, treatment with aspirin-triggered (AT)-LXA_4 was shown to ameliorate Aβ- and tau-pathology, and to improve memory function [164, 165]. Reduced severity of eczema has been demonstrated in a clinical trial with 15(R/S)-methyl-LXA_4 [166]. The synthetic Rv analogue RX-10001 has been shown to provide protection against goblet cell loss in a murine model of dry eye [167, 168], and RX-10001 and another Rv analogue RX-100045, have completed phase II clinical trials for treating dry eye syndrome.

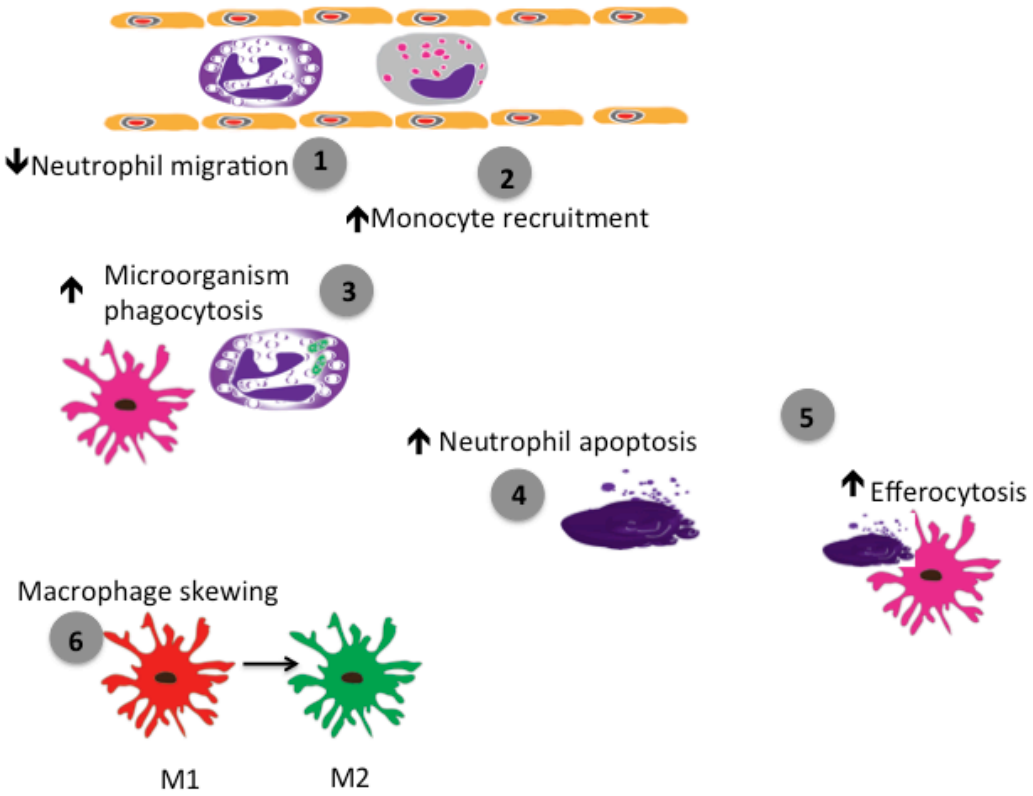

Fig. 4. Activities of SPMs in relation to the cell type: 1) inhibition of leukocyte recruitment, 2) stimulation of monocyte recruitment and of their differentiation into macrophages, 3) promoting macrophage phagocytosis of pathogens, 4) stimulation of neutrophil apoptosis, 5) stimulation of phagocytosis of apoptotic neutrophils (efferocytosis), and 6) modification of the macrophage phenotype from pro-inflammatory M1 to anti-inflammatory M2 phenotype.

Table.1. Beneficial effects of specialized pro-resolving lipid mediators (SPMs) in animal models.

Treatment	SPM analogue	Disease model	Effects in disease model	Reference
LXA_4	LXA_4	Corneal neovascularization	Reduced inflammatory angiogenesis	Leedom et al., 2010
		Stroke	Neuroprotection, anti-inflammation	Sobrado et al., 2009
		Pain	Attenuated mechanical hypersensitivity	Abdelmoaty et al., 2013
			Protection in development of nociceptive behaviours	Sun et al., 2012
	LXA_4ME	Stroke	Ameliorated BBB dysfunction, neurological dysfunctions and reduced infarction volume	Wu et al., 2012; Ye et al., 2010
	ATL	Alzheimer's disease (AD)	Reduced β-amyloid pathology, improved cognition	Medeiros et al., 2013; Dunn et al., 2015
	ATL, LXA_4	Cancer induced bone pain	Attenuated bone caner pain, supressed inflammation	Hu et al., 2012
	ATL, 3-oxa-15-epi-LXA_4 analogue	Allergy	Reduced allergic airway responses	Levy et al., 2007
	An -oxidation-resistant 3-oxa-AT-LXA_4 analogue	Colitis	Attenuated colitis in rodents	Fiorucci et al., 2004
	ATL synthetic mimetic	Transplantation	Protection in graft-versus-host disease	Devchand et al., 2005

Treatment	SPM analogue	Disease model	Effects in disease model	Reference
Maresin1	Maresin1	Colitis	Reduced cellular infiltration and colonic tissue damage	Marcon et al., 2013
		Surgry in planaria	Tissue regeneration and pain control	Serhan et al., 2012
		Peritonitis	Enhanced phagocytosis	Serhan et al., 2009
NPD	AT-NPD1	Stroke	Attenuated cerebral ischemic injury	Bazan et al., 2012; Marcheselli et al., 2003
	NPD1	Peritonitis	Promoting resolution of acute inflammation	Yamada et al., 2011
		Choroidal neovascularization	Attenuated leakage and neovascularization	Sheets et al., 2013
		Pain induced by nerve trauma	Prevention of mechanical allodynia	Rajasagi et al., 2013; Xu et al., 2013
		HSV-induced corneal stromal keratitis	Reduced severity of stromal keratitis	Bazan et al., 2012
Resolvin	RvD1	Lateral paw incision surgery	Reduced postoperative pain	Huang et al., 2011
		Wound healing	Promoted would healing	Tang et al., 2013
		Peritonitis	Attenuated neutrophil recruiment	Norling et al., 2012
		Pancreatitis	Attenuated pain	Quan-Xin et al., 2012
	AT-RvD1	Stabilized tibia fracture	Reversed signs of systemic injury and inflammation	Terrando et al., 2013
	RvD1, AT-RvD1	Allergy	Decreased airway eosinophilia and mucus metaplasia	Rogerio et al., 2012
	RvD2	Pain	Prevented formalin-induced spontaneous pain	Park et al., 2011
	RvD3, AT-RvD3	Peritonitis	Proresolving actions	Dalli et al., 2013
	RvE1, RvD1	Fibrosis caused by ureteric obstruction	Inhibitition of interstitial fibrosis in obstructed kidney	Qu et al., 2012
	RvE1	Asthma	Decreased airway eosinophil and lymphocyte recruitment, down-regulated Th2 cytokines, and decreased airway hyperresponsiveness	Aoki et al., 2008
		Acute lung injury	Promoted neutrophil apoptosis, enhanced resolution in lung	El Kebir et al., 2012
		Peritonitis	Reduced neutrophil infiltration	Arita et al., 2007
		Asthma	Inhibition of allergic pulmonary inflammation	Flesher et al., 2014

ATL = aspirin-triggered lipoxin A_4, AT-RvD = aspirin-triggered resolvin D, AT- NpD = aspirin-triggered neuroprotein D, LXA_4 = lipoxin A_4, LXA_4ME = lipoxin A_4 methyl ester, NpD = neuroprotein D, 3-oxa-15-epi-LXA_4 = 5*S*,6*R*,7*E*,9*E*,13*E*,15*S*)-16-(4-fluorophenoxy)-3-oxa-5,6,15-trihydroxy-7,9,13-hexadecatrien-11-ynoic acid, RvD = resolvin D, RvE = resolvin E.

1.3.4 Receptors for SPMs

Although there is evidence for beneficial effects of SPMs, the underlying molecular mechanisms are largely unknown. Receptors for LXA_4, RvD1 and RvE1 have been identified, while the receptors for other SPMs remain unknown. All the receptors identified so far belong to 7-transmembrane (7-TM) G protein-coupled receptors (GPCRs). LXA_4 and RvD1 have been found to bind to ALX/FPR2 and G protein receptor (GPR) 32 [169, 170], and RvE1 binds to chemerin receptor 23 (ChemR23) and the leukotriene B4 receptor 1 (BLT1) [153, 171]. RvE1 binds to BLT1 as a partial agonist, and counteracts pro-inflammatory signals transduced by BLT1 to mediate the resolution of inflammation [153]. These receptors bind other ligands upon which they can transduce a pro-inflammatory signal. ALX/FPR2 has been identified as a receptor for Aβ [172], which activates microglia, and thereby transducing pro-inflammatory signals. ChemR23 binds chemerin [173], a chemotactic peptide [173],

associated with increased inflammation [174]. Furthermore, the nuclear receptor PPAR-γ has been reported to mediate protective effects of SPMs [175]. GPR120 binds long chain FAs including DHA and EPA [176], and may also be a candidate receptor for SPMs.

In a recent study, alterations in the expression of receptors for SPMs have been demonstrated in the AD brain [136]. In the human brain, both neurons and glial cells express ChemR23, with higher levels found in AD compared to control subjects [136]. Similar to ChemR23, ALX/FPR2 was described to occur in both neurons and glia in the human brain, with higher levels in AD [136]. Furthermore, the levels of PPAR-γ were higher in the hippocampus of AD patients [136]. The increased levels of SPM receptors in the AD brain may be interpreted as a compensatory mechanism for the reduced levels of SPMs [136]. Interestingly, at least some of the anti-inflammatory effects of SPMs seem to be due to competition of SPMs with pro-inflammatory ligands such as LTB_4, acting as partial agonists or antagonists [153]. The plurality of ligands with pro-inflammatory effects, and the increased levels of SPM receptors in AD brains can therefore also be interpreted as a feature of the pronounced chronic inflammatory state in the AD brain.

1.3.5 Enzymes involved in the resolution of inflammation

The SPMs are synthesized from their PUFA precursors via the activities of LOXs and COXs. LOXs add oxygen to the carbon chain of PUFAs, and depending on the site of incorporation the enzyme was classified as 5- or 15-LOX [177]. Notably, besides the role in synthesis of SPMs, 5-LOX is also involved in the synthesis of pro-inflammatory leukotrienes (LTs). COXs are crucial enzymes involved in the synthesis of PGs, which mediate inflammation and pain (Fig. 5). Interestingly, COXs together with LOXs can give rise to aspirin-triggered (AT) forms of SPMs in response to aspirin. Thus, the production profile of LMs by this enzymatic machinery can change from pro-inflammatory classes of LMs to pro-resolving classes of

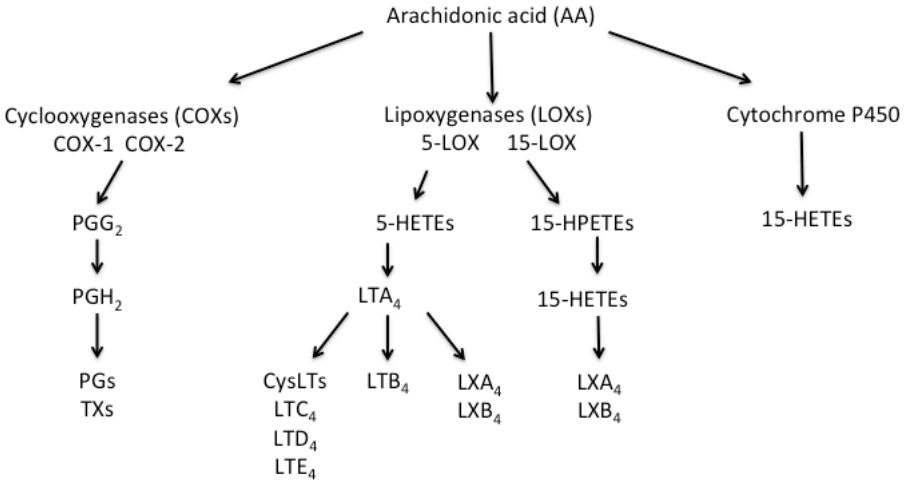

Fig. 5. Synthesis of lipid mediators from AA. AA = arachidonic acid, CysLTs = cysteinyl leukotrienes, 5-HETE = 5-hydroxy-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid, 15-HETE = 15-hydroxy-5Z, 8Z, 11Z, 13E-eicosatetraenoic acid, 15-HPETE = 15-hydroperoxy-5Z, 8Z, 11Z, 13E-eicosatetraenoic acid, LT = leukotriene, LX = lipoxin, PG = prostaglandin, TX = thromboxane.

LMs, a process termed “class-switching”. Increased knowledge on the activation and regulatory mechanisms of these enzymes, and how this can prevent or result in class-switching, is necessary to increase the understanding of chronic inflammatory diseases, and how tissue regeneration may be stimulated.

1.3.5.1 5-LOX

5-LOX is encoded by the gene ALOX, which is located in chromosome 6. In the brain, 5-LOX is expressed by all types of cells [178], and higher levels have been shown in AD brains as well as in an AD mouse model [135]. Crossing 5-LOX knockout mice with a mouse model for AD, based on transgenic expression of mutated human APP, resulted in reduced Aβ pathology and improved cognitive performance [179]. The tetracycline antibiotic minocycline inhibits 5-LOX, and has been used for the treatment of asthma. Interestingly, minocycline has been shown to inhibit BACE1, and reduce Aβ pathology and inflammation in APP transgenic mice [180]. 5-LOX is activated by phosphorylation at three currently known sites, Ser663, Ser271 and Ser523. Ser271 is phosphorylated by P38 mitogen-activated protein kinase (MAPK), and extracellular-signal-regulated kinases (ERK) phosphorylate 5-LOX at Ser663 [181]. Interestingly, phosphorylation of 5-LOX at Ser523 has been reported to shift the production of LMs toward LXA_4, while the production of LTB_4 is decreased, and Ser523 is therefore considered to be an anti-inflammatory phosphorylation site, that is involved in class-switching [182, 183]. 5-LOX is irreversibly inactivated by its end products, such as LTB_4.

1.3.5.2 15-LOX-1 and 15-LOX-2

Two isoforms of 15-LOXs can be found in the brain: 15-LOX-1 and 15-LOX-2 [136, 184]. 15-LOX-1 is also been referred to as 12/15 LOX due to they can oxygenate AA at both C12 and C15 position [185]. 15-LOXs are implicated in many diseases including AD [186]. It has been reported that in the AD brain, in pathologically affected areas such as frontal and temporal region, the levels of 15-LOX-1 were higher, and its metabolic products 15-hydroxyeicosatetraenoic acids (15-HETE), were also markedly elevated in the CSF of AD patients compared to controls [184, 187]. Increased levels of 15-LOX-2 were reported in the hippocampus as well [136]. Interestingly, decreased levels of both 15-LOX-1 and15-LOX-2 were observed in cancer [188, 189], and *in vitro* overexpression of 15-LOX-1 in colorectal cells inhibits cell growth and induces apoptosis [190, 191]. It has been suggested that the balance between 15-LOX-1 and 15-LOX-2 is important. For instance, the shift in expression from 15-LOX-2 to 15-LOX-1 is associated with neoplastic progression [192]. The role of 15-LOXs in AD is unclear, since 15-LOXs can directly oxidize lipids in the cell membrane [193], generating oxidative stress that can be detrimental for the neurons. However, 15-LOXs are also involved in the synthesis SPMs, and thus related to anti-inflammatory and pro-resolving activities. In addition, the anti-inflammatory cytokines IL-4 and IL-13 induce the expression of 15-LOX-1 [194]. Inhibition of 15-LOX-1 in APP-overexpressing

neuroblastoma cells significantly reduced the levels of soluble APPβ and β-secretase (BACE) [195]. Moreover, crossing APP transgenic mice with 15-LOX knockout mice resulted in a progeny with reduced Aβ pathology [196]. To summarize, 15-LOX may play a dual role in AD, but further studies are needed to elucidate the biology of this enzyme and how it is regulated.

1.3.5.3 COX-1 and COX-2

The genes encoding COX-1 and COX-2 are located on chromosome 9 and 1, respectively. In a study where the authors stained frontal and temporal brain tissue from post-mortem AD and control subjects by immunohistochemistry, COX-1 immunoreactivity was found both in neurons and microglial cells, while COX-2 immunoreactivity was found only in neurons, and the number of COX-2 positive neurons were increased in AD brains compared to control brains [197]. Later studies have shown increased levels of COX-2 at early stages of AD, whereas decreased levels were observed at a late stage [198, 199]. Aspirin, which is a well-known NSAID, widely used for treatment of inflammation. Low doses of aspirin are prescribed for the prevention of cardiovascular events. The mechanism of action for aspirin is through irreversible acetylation of COX-1 and COX-2 and thereby affecting its catalytic activity and inhibiting the synthesis of PGs and thromboxanes (TXs) [200]. Interestingly, aspirin was found to trigger transcellular formation of a novel series of aspirin-triggered lipoxins (ATLs) during co-incubation of human umbilical vein endothelial cells with neutrophils [201]. Later studies showed that ATLs inhibited the neutrophil infiltration in a mouse ear inflammation model, showing anti-inflammatory and pro-resolving properties [81]. Further studies on the mechanisms of ATL synthesis showed that acetylated COX-2 is still active and can convert AA to 15-RHETE and give rise to ATL [202].

1.3.6 Class-switching

Signals that can promote class-switching represent another pathway for stimulating resolution of inflammation, but need further investigation. Acetylcholine (ACh) has been suggested to be involved in the class-switching mechanisms. ACh is a neurotransmitter that is involved in memory and learning [203], and the degeneration of cholinergic neurons in the AD brain is a major part of the neuropathology, and the basis for symptomatic treatment with cholinesterase inhibitors (ChEIs). Furthermore, there is a loss of cholinergic receptors in the cerebral cortex of AD patients [204-206]. Aβ can bind with high affinity to the α7 nicotinic cholinergic receptor (α7nAChR) in neurons [207], thus impairing the ability to be activated by ACh [208]. α7nAChRs are also expressed by microglia, and ACh inhibits microglial activation [209]. It has been reported that activation of α7nAChR in microglia by nicotine reduced lipopolysaccharide (LPS)-induced production of the pro-inflammatory cytokines TNF-α and IL-18 [210], indicating an anti-inflammatory effect of ACh. However, ACh also

increases COX-2 and the classical pro-inflammatory LM PGE_2 [211]. Interestingly, exposure of human blood neutrophils to PGE_2 increased the production of the pro-resolving LXA_4, while reducing the anti-inflammatory LTB_4, indicating a role of PGE_2 in class-switching between LMs [212]. Thus, the anti-inflammatory effect of activation of a7nAChR may be due to class-switching through PGE_2. However, the direct effect of a7nAChR activation on LOX enzymes and SPM production has not yet been investigated, but is of great interest.

2 AIMS

The main aim of this thesis was to investigate the role of resolution of inflammation in AD, and whether stimulation of resolution of inflammation can be a novel therapy for AD.

The specific aims were to investigate:

Paper I: the effects of DHA and EPA on microglial phagocytosis of Aβ on secreted and cellular markers of immune activity.

Paper II: whether Aβ, the major component of senile plaques in the AD brain, may have a negative influence on components of the resolution cascade.

Paper III: possible alterations in SPMs in the entorhinal cortex (ENT) using LC-MS-MS, and the actions of SPMs in AD-related *in vitro* models.

Paper IV: the effects of n-3 FA supplementation on the release of SPMs in relation to ApoE4 genotype in AD patients.

3 MATERIALS AND METHODS

3.1 CLINICAL SAMPLES

3.1.1 Post-mortem human brain samples

Post-mortem analysis of brain pathology is the golden standard for AD diagnosis. Furthermore, post-mortem brain samples provide valuable research material for understanding the underlying pathology and mechanisms.

3.1.2 Plasma samples

The collection of CSF is an invasive procedure. Therefore, blood-based biomarkers are of great interest for the diagnosis of AD. So far, no single reliable blood-based biomarker has been identified for AD. Probably, a combination of several biomarkers will be required to predict AD with sufficient sensitivity and specificity.

3.2 CELLULAR MODELS

3.2.1 Human microglial cell line

3.2.1.1 General characteristics

The human microglial cell line CHME-3 was derived from 8-10 weeks old embryonic human microglia transfected by plasmid-containing cDNA encoding SV40 T antigen [213]. The CHME-3 cells maintain certain properties of primary microglial cells, such as phagocytosis, and the expression and secretion of immune molecules in response to inflammation induced by LPS and Aβ that have been demonstrated by the authors in the work in this thesis. However, compared to primary microglia, the CHME-3 cells spontaneously secrete IL-6, providing stimulatory signals at basic culture conditions. Furthermore, the phagocytic capacity of the CHME3 cells appears to be lower than in primary microglia, possibly a result of the chronic IL-6 stimulation. The CHME-3 cells are heterogeneous with regard to size and morphology. During control conditions, the CHME-3 cells express both pro-inflammatory M1 markers such as CD40 and CD86, and anti-inflammatory M2 markers such as CD163 and CD206. We have shown that activation of CHME-3 cells with $A\beta_{42}$ leads to increased levels of M1 markers and increased secretion of TNF-α (**Paper III**).

3.2.1.2 Resolution pathway in CHME-3 cells

CHME-3 cells express receptors to SPMs, including ALX/FPR2, GPR32 and ChemR23 (Fig. 6A-C). CHME-3 cells also possess the enzymatic machinery (COX, 5-LOX, 15-LOX) that is needed for the synthesis of SPMs. We have shown that CHME-3 cells can secrete the SPMs LXA_4 and RvD1 in culture. Therefore, in human brain, microglial cells can be a source of SPMs. However, this does not exclude other cell types as sources of SPMs in the brain.

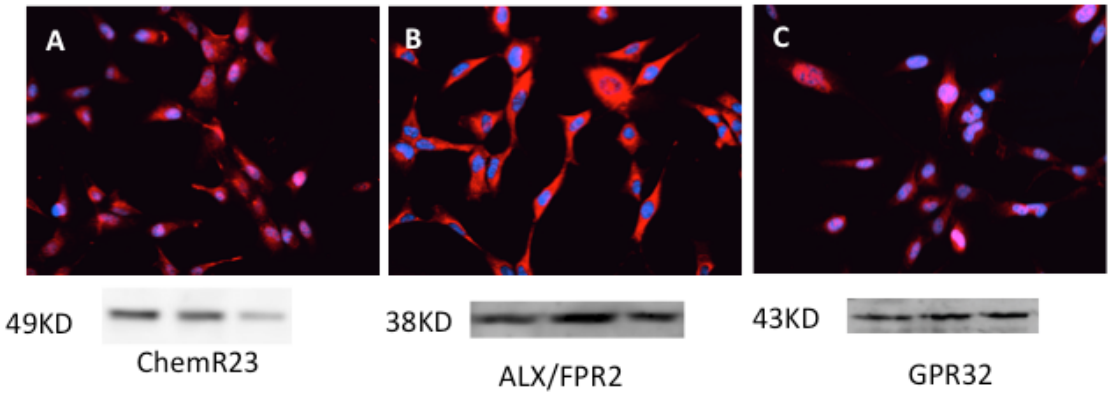

Fig. 6. SPM receptors in human microglia. A) ChemR23: receptor for RvE1. B) ALX/FPR2: receptor for LXA_4 and RvD1. C) GPR32: receptor for LXA_4 and RvD1. ALX/FPR2 = lipoxin A_4 (LXA_4)/formyl peptide receptor 2, ChemR23 = chemerin receptor 23, GPR32 = G protein-coupled receptor 32, LXA_4 = lipoxin A_4, RvD1 = resolvin D1, RvE1 = resolvin E1. SPMs = specialized pro-resolving lipid mediators.

3.2.2 Human neuroblastoma cell line

3.2.2.1 General characteristics

The human neuroblastoma SH-SY5Y cell line is a sub-line of SK-N-SH cells, which were derived from the biopsy from a metastatic neuroblastoma patient in the 1970s [214]. The SK-N-SH cells have three subtypes: neuronal ("N" type), Schwanninan ("S" type) and intermediate ("I" type) [215]. The SH-SY5Y cell line mainly contains cells of the N type. The cells have been shown to be dopaminergic, adrenergic, cholinergic and glutamatergic, and are therefore widely used as a neuronal model for Parkinson's disease (PD) and AD. SH-SY5Y cells are neoplastic and possess stem cell properties, and can be differentiated by various agents such as retinoic acid (RA), 12-tetradecanoyl-13-acetyl-beta-phorbol (TPA), growth factors and vitamin D3, to become more similar to mature neurons. Morphologically, undifferentiated SH-SY5Y cells have multiple short spiny processes (Fig. 7A).

In this thesis, differentiated SH-SY5Y cells were used, obtained by sequential treatment with RA for 5 days and brain-derived neurotrophic factor (BDNF) for another 5 days. Upon treatment with RA, the cells become elongated and begin to extend processes to some degree, while continuing to divide and proliferate. After switching to serum-free medium containing BDNF, the proliferation stops and the cell bodies decrease in size and gradually extend long, extensively branched neurites (Fig. 7B-D), showing a morphology similar to that of primary neurons. Differentiated SH-SY5Y cells also have more mature neuronal molecular characteristics compared with undifferentiated cells, exemplified by an increase in cholinergic markers, and mature isoforms of tau protein [216], which makes them suitable for experiments related to AD, as compared to undifferentiated cells. Another important advantage of the differentiated cells is that they stop dividing upon switching to serum-free medium, thereby avoiding confounding effects of cell division, which is major drawback of continuously dividing cell lines.

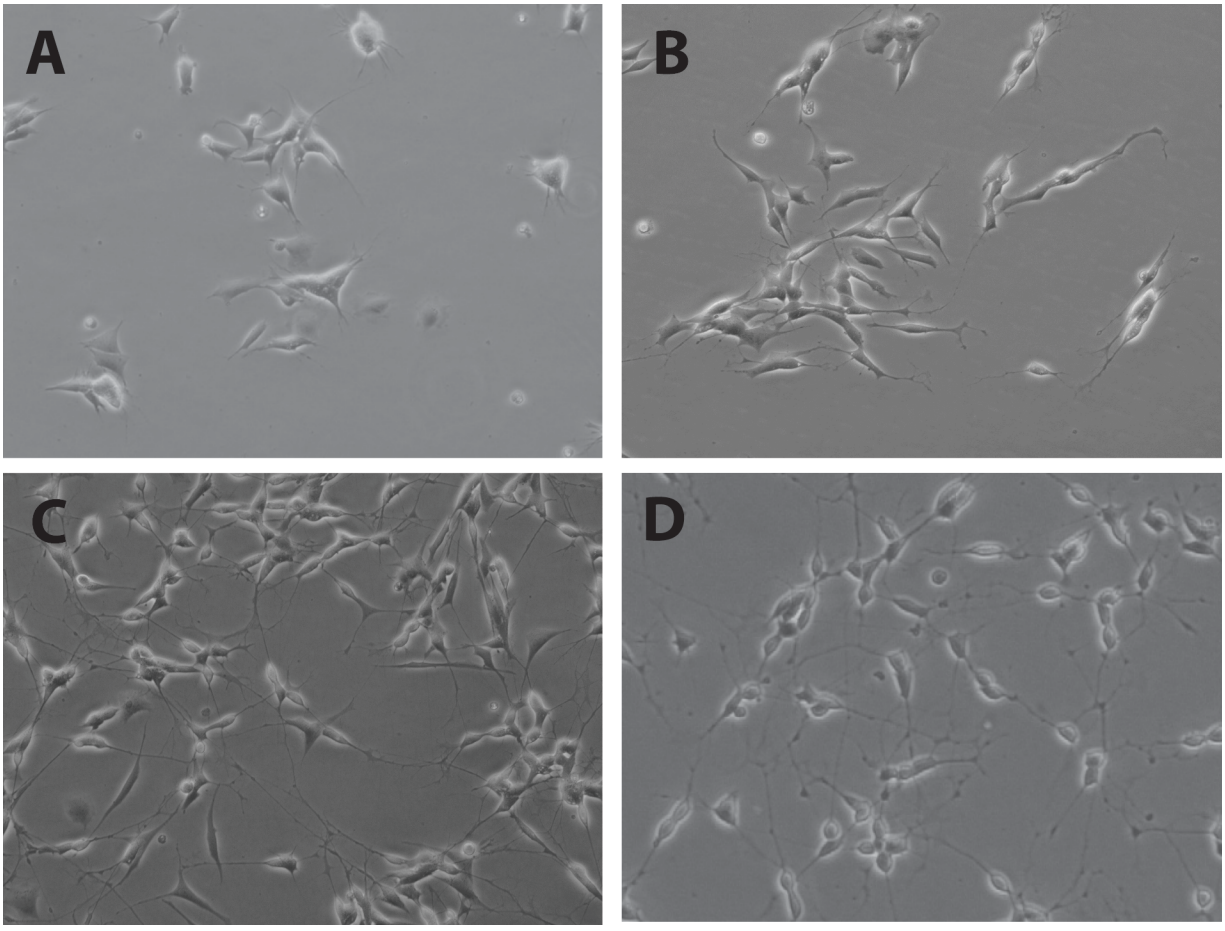

Fig. 7. Differentiation of SH-SY5Y neuroblastoma cells. A) Undifferentiated cells. B) Cells incubated with RA for 24 h. C) Cells incubated with RA for 5 days. D) Cells incubated with BDNF for 2 days, following the 5 days of incubation with RA. BDNF = brain-derived neurotrophic factor, RA = retinoic acid.

3.2.2.2 Resolution pathway in SH-SY5Y cells

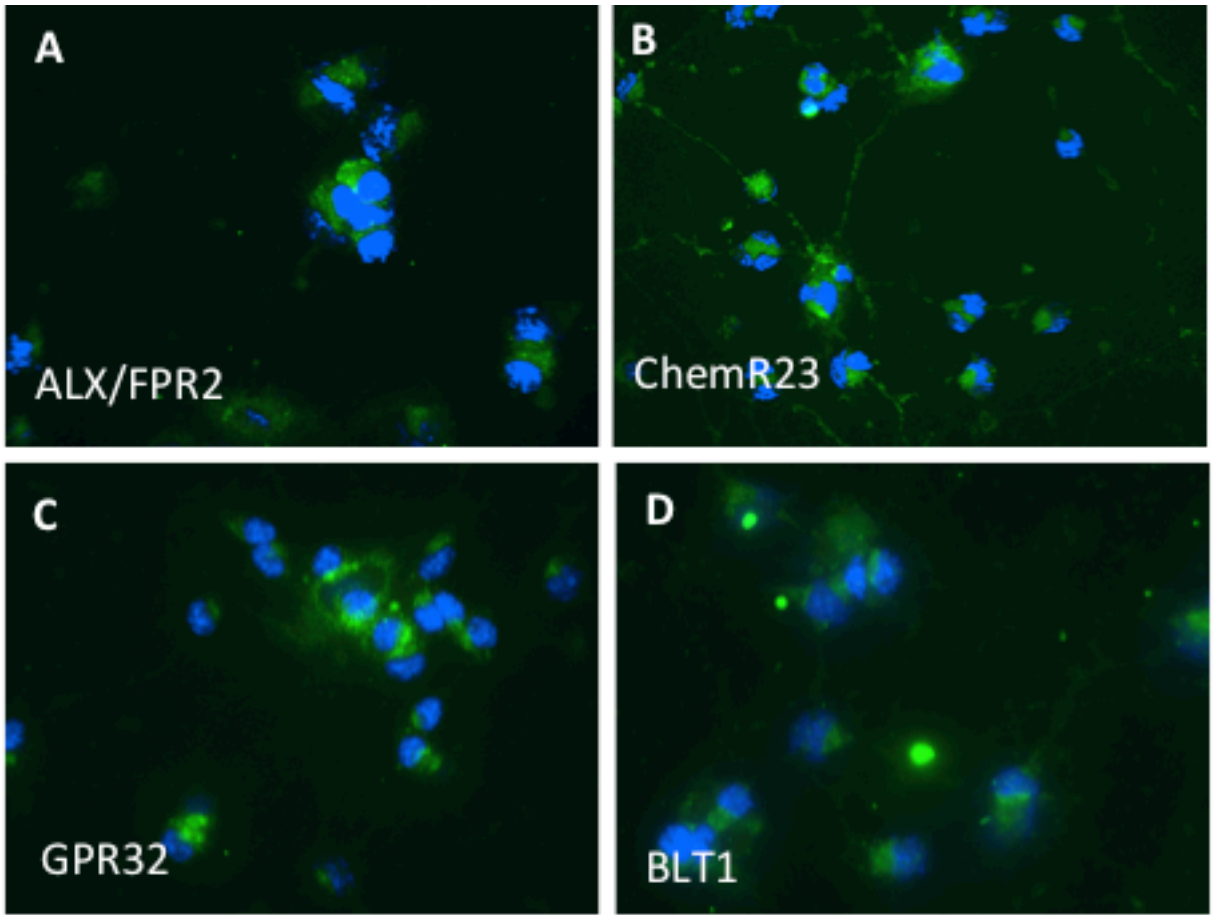

Fig. 8. SPM receptors in human differentiated neuroblastoma cells. Micrographs show SH-SY5Y neuroblastoma cells differentiated with RA and BDNF. A) ALX/FPR2: receptor for LXA_4 and RvD1. B) ChemR23: receptor for RvE1. C) GPR32: receptor for LXA_4 and RvD1. D) BLT1 receptor for RvE1 and LTB4. ALX/FPR2 = lipoxin A_4 (LXA_4)/formyl peptide receptor 2, BDNF = brain-derived neurotrophic factor, BLT1 = leukotriene B4 receptor type I, ChemR23 = chemerin receptor 23, GPR32 = G protein receptor 32, LXA_4 = lipoxin A_4, RA = retinoic acid, RvD1 = resolvin D1, RvE1 = resolvin E1. SPMs = specialized pro-resolving lipid mediators.

SH-SY5Y cells differentiated with RA and BDNF express receptors to SPMs, *i.e.* ALX/FPR2 and GPR32, which are receptors for LXA_4 and RvD1 (Fig. 8A and C), as well as ChemR23 and BLT1, which are receptors for RvE1 (Fig. 8B and D). Therefore, differentiated SH-SY5Y cells are able to receive signals of the resolution pathway, supporting its use as a neuronal model in studies on resolution of inflammation.

3.2.3 Advantages and disadvantages of using cell lines *vs.* primary cultures

3.2.3.1 Advantages

Cell lines are continuously dividing cells that can be passaged many times, and can therefore provide an almost unlimited source of cells for experiments. Compared to primary cultures,

cell lines are also easier to culture - primary cultures usually require more supplements to the medium, are less resilient, and have a higher risk of contamination. Many cell lines are commercially available and have been well characterized, enabling interpretation and comparison of results from different laboratories using the same cell line. Primary cells derived from patients can behave differently depending on the genetics, age and origin, and most of them are less well characterized, making it difficult to compare results from different laboratories.

3.2.3.2 Disadvantages

Cell lines are artificially or naturally or artificially immortalized cells that escape the normal cellular senescence, and are therefore continuously dividing. Compared to primary cells, the responses of a cell line can be less similar to the *in viv*o situation. In particular, the rapid proliferation often seen in cell lines can be a confounding factor, especially when studying toxicity and the protection against this. Because of the genetic modification and the loss of normal genome integrity, the cells of a cell line have a higher rate of gene mutations and may therefore change their original phenotype, resulting in the loss of genes relevant to the research question, or result in different and even contradicting responses to the same stimuli between passages. Therefore, it has been suggested to use a limited number of passages for cell biological research. Or if not possible, perform continuous characterization of the cell line upon growing passage number.

3.3 TECHNIQUES

3.3.1 Flow-cytometry

Flow-cytometry is a technique that is widely used in research and clinical practice for immune phenotype analysis. It is a powerful technique that simultaneously analyses multiple physical characteristics of cells as they flow in a fluid stream through a beam of laser light, while at the same time allowing immunocytochemical detection by several fluorophore-conjugated antibodies directed at the respective antigen, the number being limited by the laser and filter characteristics of the flow-cytometer. The characteristics including size, internal complexity and relative fluorescence intensity, can be determined by an optical-to-electronic coupling system. Flow-cytometry is widely used for the diagnosis of blood cancers. It is also used for cell cycle analysis, and cell sorting for selection and purification of cells.

3.3.2 Enzyme-linked immunosorbent assay (ELISA)

3.3.2.1 Sandwich ELISA

Also named indirect ELISA, which is the most common type of ELISA being used in research. A capture antibody is bound to the plastic surface in the wells of a multi-well plate. Before the antigen is applied the surface is blocked with an inert protein such as bovine serum albumin (BSA), to decrease unspecific binding of the antigen to the surface of the well. The sample containing the antigen of interest is incubated in the well, allowing it to bind to the antibody. Next, an enzyme-conjugated secondary antibody specific to another epitope of the antigen is added, thereby 'locking' the antigen between the capture and secondary antibody, and hence the name "sandwich ELISA". A substrate for the conjugated enzyme is added, and the resulting product is detected by absorbance, fluorescence or chemiluminescence. It is a method with high sensitivity; however the specificity of the ELISA is dependent on the quality of the antibodies.

3.3.2.2 Competitive enzyme immunoassay (EIA) – for analysis of lipid mediators

The LMs LXA_4, RvD1 and LTB_4 were analysed in the cell culture medium and human samples using EIA, which is a technique based on competitive binding between the antigen in the sample and an enzyme-conjugated antigen that is added to the sample. Conjugated antigen competes with the antigen in the sample for the primary antibody binding sites. Therefore, the signal derived from the enzymatic reaction is inversely proportional to the amount of free antigen in the well during incubation.

3.3.3 Immunohistochemistry

Immunohistochemistry, or immunocytochemistry in the case of cell cultures, is a classical and useful technique to investigate the presence and location of a specific antigen in tissue or cells by using a specific antibody that binds to the antigen, thereby allowing visualization under a microscope. The information obtained is both qualitative morphological and quantitative. By using image analysis software, it is possible to determine the approximate levels of an antigen. Similarly to ELISA and to all methods employing antibodies, the quality and specificity of the antibody is determinant of the quality of the output data.

3.3.4 Western blot

Western blot is a technique involving gel electrophoresis and immunoblotting. Electrophoresis separates the denatured proteins by the molecular weight of the protein. The addition of sodium dodecyl sulphate (SDS), an anionic surfactant that disrupts non-covalent

bonds and denatures proteins, results in a charge that is proportional to the size of the protein, independent of the charge of the amino acids. The protein thereby travels in the electrical field through the gel, which offers physical resistance, with a speed that is proportional to its size, resulting in separation in the gel according to size. Subsequently, the proteins are transferred to a nitrocellulose membrane and stained with antibodies specific for the protein of interest. Compared to immunohistochemistry, it is more specific due to additional information offered by size separation. It is also easier to quantify. In addition, in some situations, it can give some information regarding post-translational modification (phosphorylation, glycosylation), or aggregation form (dimerization, etc.), according to the molecular weight of the protein. A disadvantage is the lack of information on morphological cellular localization.

3.3.5 Lipid chromatography – tandem-mass spectrometry (LC-MS-MS)

Lipids are of crucial importance for their involvement in biological and pathophysiological process, hence knowing the composition and concentration of lipid metabolites in biological samples will expand our knowledge and understanding of a disease. However, due to the diversity of the structural and physical properties of lipids, analysis of wide-scale lipid profiles has been considered difficult. With the development of new purification systems and advanced mass spectrometry (MS) techniques, it is now possible to analyze and quantify lipids with accuracy in a large scale. Lipid chromatography (LC) enables separation of lipids in a biological sample, allowing a subsequent advanced MS analysis. Briefly, samples are prepared in a pH-adjusted water-organic mixture (mobile phase), and then forced through a column (stationary phase) in order to accomplish a particular type of separation. MS is based on the analysis of mass-to-charge ratio of the charged particles. It is a powerful technique to determine the mass, composition and chemical structure of a sample or molecule. In the MS instrument the sample undergoes vaporization for ionization to generate charged molecules, and then the ions are separated according to their mass-to-charge ratio by electromagnetic fields. The ions are detected and the signals are presented in mass spectra. MS technique is both qualitative and quantitative, and can be used to identify an unknown molecule or quantify the concentration of a known molecule in a sample.

3.3.6 Measurement of cell viability and death

3.3.6.1 Resazurin assay

The resazurin assay (also known as Alamar Blue assay) is a widely used method for analysis of viability of mammalian cells and bacteria. Living cells are metabolically active, and the non-fluorescence resazurin dye is reduced in the mitochondria of living cells by a reducing

enzyme to resorufin, a fluorescent compound. The fluorescence signal is proportional to the number of viable cells. The resazurin assay correlates well with another commonly used assay based on formazan, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, but it is safer and easier to use, and we have also found it to be more sensitive. In addition, it is not toxic for the cells, allowing long-term incubation and other subsequent applications.

3.3.6.2 Lactate dehydrogenase (LDH) assay

Lactate dehydrogenase (LDH) is an enzyme present in all types of cells, and it leaks out through the broken plasma membrane in damaged cells. The LDH assay is a sensitive, convenient and precise assay based on the oxidization of lactate to pyruvate by LDH released to the culture medium. The resulting pyruvate reacts with a tetrazolium salt (INT, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium) to form formazan, which has an orange colour. The strength of the signal from the colorimetric reaction in the culture medium is proportional to the number of lysed (dead) cells in the culture.

3.4 STATISTICS

3.4.1 Multivariate analysis (MVA)

MVA analysis was used in **Paper II** and **IV** in the thesis. MVA is a powerful statistical method that analyses all variables together. It gives information about the overall pattern of the data such as clustering and class separation. It has been widely used for analysis of databases that include many variables, such as in the "omics" fields. It is also useful for experimental data with fewer variables, since it may provide a better interpretation of the data.

The principle of MVA is to reduce the dimensionality (equals to number of variables) of the data and focus on the information of interest into several latent variables (equals to the number of components of the model), similar to how shadows of a multi-dimensional cloud of variable spots can be projected onto a plane [217]. Compared to univariate analysis, MVA also avoids type I and type II errors by avoiding doing multiple comparison analysis. The MVA in this thesis were performed using the SIMCA P+ software package (UMETRICS AB, Umeå, Sweden).

Principle component analysis (PCA) is a statistical method that used to examine the interrelations among a set of variables in order to identify the underlying structure of those variables, so that similar samples clustered together and distinct samples far apart. PCA is the basis of all MVA analysis. It is an unsupervised non-parametric analysis method that is independent of any hypothesis about data distribution, and gives an overview about the

structure (patterns of similarities in the observations) of the data. PCA is based on projection of the high dimensional data to principle components (PCs), and each principle component can be viewed as one latent variable that describes the information extracted from the dataset: the first principle component (PC1) finds the largest variance of the data set comes to lie on the first axis, and the PC2 find the second largest variance comes to line on the second axis, which is orthogonal to the first axis. Subsequently, next PC will find the next largest variation and comes to lie on the next axis that is orthogonal to the previous one.

Orthogonal projections to latent structures (OPLS) is a prediction and regression method that finds the association between the quantitative X data that is related to known information, the Y data. Therefore, it is a supervised analysis method. In OPLS, the X data are separated into two components based on their association with the Y data: predictive component describes information that are correlated to the Y data and orthogonal component that describes the information that are not related to the Y data. If the Y data are composed of discrete variables, which may indicate for example treatment or diagnostic groups, the analysis is called OPLS- DA (discriminant analysis). The ease of interpretation is an advantage of OPLS-DA, since it shows which variables are responsible for class discrimination. The degree of contribution of each variable for the class separation can be obtained by analysing correlations between predictive X data and Y data, and visualized in the loading plot.

The quality of MVA can be determined using the following parameters: R^2 estimates the goodness of fit and indicates how well the model explains the dataset. In OPLS-DA, the R^2X value for the predictive components represents the fraction of variation in X related to variation not related to Y. A low R^2X value for the predictive component indicates that X contains much variation not related to Y. The R^2Y value is the correlation between the measured and predicted Y values and gives an indication how well the model describes the response. Q^2 values give information about predictive ability based on cross validation, which is a model validation technique for assessing how the results of a statistical accurately a predictive model will perform in practice.

3.4.2 Univariate analysis

The univariate analyses were performed by IBM SPSS statistics 20.0 software (IBM Corporation, NY, USA) or Statistica 12 (Dell Software, Aliso Viejo, USA).

For *in vitro* studies, the data were normalized to the average of that particular experiment (**Paper I, II and III)**. The data were logarithmized to allow for parametric statistical analysis (**Paper I**). Normally distributed data were analysed by one-way ANOVA followed by Fisher's *post hoc* test (**Paper I**), whereas non-normally distributed data were analysed by the Mann-Whitney or the Kruskal-Wallis test (**Paper II – IV**). For dependent variables, non-parametric Wilcoxon matched-pairs test were used (**Paper I, IV**). Correlation analysis was

performed with the non-parametric Spearman's rho test (**Paper IV**). For all the studies, 95% confidence interval and p-values < 0.05 was used to indicate statistical significance.

4 ETHICAL CONSIDERATIONS

The use of human CSF and post-mortem brain samples was approved by the ethical committee at Karolinska Institutet, the regional human ethics committee of the Stockholm County, and the Swedish Ministry of Health and Social Affairs. The ethical permit numbers are: 2011/680-31/1 for the human CSF samples, and Dnr 024/01 and 2011/962-31/1 for human post-mortem brain tissues.

5 RESULTS AND DISCUSSION

5.1 FORMS OF Aβ, AGGREGATION AND PHAGOCYTOSIS

5.1.1 Aβ species

In **Paper I**, **II** and **III**, we treated human microglia cells with different concentrations of $A\beta_{42}$. For the studies on phagocytosis of $A\beta_{42}$ we used 1μg/ml (0.22 μM), and for investigating the effects on microglial activation and inflammation 1-100 μg/ml (0.22 – 22 μM) of $A\beta_{42}$ was used. The choice to use $A\beta_{42}$ over other species of lengths of Aβ for our studies was motivated by the evidence indicating that $A\beta_{42}$ is the species most associated with the pathology in AD.

5.1.2 Concentration of $A\beta_{42}$

It is difficult to determine which concentration of $A\beta_{42}$ to be used in *in vitro s*tudies where the culture conditions is supposed to be as similar as possible to the situation in the human AD brain. The concentrations of $A\beta_{42}$ in the AD brain may depend on the region, and it is plausible that the concentration around the senile plaques is higher than in other areas. The post-mortem time can also influence the levels. Most *in vitro* studies have used a μM range for studies on the toxicity of $A\beta_{42}$ in neuronal models, and this has been criticised as being higher than physiological concentrations, suggested to be in the pM range in the human brain [27, 218, 219]. Although concentrations in the μM range are commonly used to induce toxicity in neuronal *in vitro* models, the concentration required is dependent on the parameter chosen for indicating toxicity. Lower concentrations of Aβ that do not reduce cell viability may still cause impairment in synaptic function. Furthermore, the effects of Aβ in cellular experiments can only mimic acute toxicity of Aβ, while Aβ accumulation in *in vivo* conditions in AD patients may have occurred decades before the onset of clinical symptoms [220, 221]. Therefore, results from studies on neurotoxicity of $A\beta_{42}$ and treatments offering protection against this are difficult to translate to the situation in the human brain.

5.1.3 Aggregation of $A\beta_{42}$

In **Papers I** and **III**, we characterized the aggregation form of $A\beta_{42}$ at different times of incubation in cell culture medium (Paper I: incubation with 1 and 5 μg/ml $A\beta_{42}$ for 0, 6 and 24 hours; Paper III: incubation with 0, 10 and 100 μg/ml $A\beta_{42}$ for 24 hours), using western blot and thioflavin T (ThT) assay. The results showed that in our experimental conditions, at 1 μg/ml, there is no detectable band as measured by western blot. The total amount of protein loaded at 1 μg/ml was 75 ng, which may be too low for detection by western blot. However,

the concentrations of 5, 10 and 100 μg/ml resulted in detectable levels after 6 and 24 hours incubation, showing bands in the western blots for of a mixture of monomers, dimers, and oligomeric forms of $A\beta_{42}$ in the culture medium. At 1 and 5 μM, fibrillar forms of $A\beta_{42}$ were detected by the ThT assay. It is reasonable to assume that higher concentrations of $A\beta_{42}$ correlate to the density of the band, and to the fluorescence signal determined by the ThT assay. However, the aggregation of $A\beta_{42}$ is also dependent on concentration [222], making comparisons between different concentrations of $A\beta_{42}$ in relation to their aggregational form difficult.

To analyse the aggregational forms of $A\beta_{42}$ at the time of harvesting the cells, the cross-linker glutaraldehyde was used, in order to 'freeze' the forms of $A\beta_{42}$. As shown previously [223], without fixing with glutaraldehyde, only the monomeric form of $A\beta_{42}$ was found. However, it cannot be excluded that glutaraldehyde itself may have an influence on the aggregation of $A\beta_{42}$.

5.1.4 Phagocytosis of $A\beta_{42}$

In **Paper I** and **III**, the effects of n-3 FAs and SPMs on microglial phagocytosis of $A\beta_{42}$ were studied. The results showed that DHA, EPA and one of the DHA derivatives, MaR1, increased the phagocytosis of $A\beta_{42}$, whereas neither LXA_4 nor RvD1 nor PDX had an effect. Altogether, these results indicate that the increased phagocytosis of $A\beta_{42}$ induced by DHA (**Paper I)** is mediated by its downstream product MaR1, but not by anyone of the other derivatives of DHA, *i.e.* RvD1 or PD1 (for which PDX is an analogue). This is contradictory to previously published results showing that also these SPMs are potent in stimulating phagocytosis by macrophages [124, 224, 225]. To our knowledge, the effects of SPMs on phagocytosis of Aβ in microglial cells have not been reported earlier. However, RvD1 was shown to increase the phagocytosis of fibrillar Aβ by PBMC cells from AD-patients [226]. It is possible that microglia and PBMCs have different ability to phagocytose Aβ, or that their responses to SPMs differ. Studies on the effects on phagocytosis by macrophages have used other objects such as zymosan or apoptotic neutrophils [225, 227, 228]. Since phagocytosis of different objects or molecules can be mediated by distinct mechanisms, involving different phagocytosis receptors, it can be speculated that the signals that stimulate Aβ phagocytosis are different from those stimulating phagocytosis of zymosan.

Microglial cells express various phagocytosis receptors that can recognize Aβ and cause Aβ internalization. It has been suggested that phagocytosis can be divided into inflammation-associated and non-inflammation-associated (non-phlogistic) phagocytosis. Receptors such as CD36, CD14 and CR3 are linked to inflammation-associated phagocytosis, while receptors such as triggering receptor expressed on myeloid cells (TREM2) and phophatidylserine (PS) receptors are linked to non-phlogistic phagocytosis [229]. Therefore, to modulate

phagocytosis by activating anti-inflammatory receptors and thereby increasing non-phlogistic phagocytosis, and at the same time reducing inflammation, is an interesting perspective, and is exemplified in this thesis by resolution signalling.

Furthermore, various immune-modulatory substances that exist in the biological environment can modulate the phagocytosis activity. It has been reported that pro-inflammatory cytokines suppress the phagocytosis of fibrillar Aβ, and that this can be restored by co-incubation with anti-inflammatory molecules, such as anti-inflammatory cytokines and the COX inhibitor ibuprofen [230]. It is well documented that AD involves a chronic inflammation in the brain [231, 232], and hence reducing the inflammation and promoting non-phlogistic phagocytosis represent a potential treatment. The findings from **Paper I** and **III** indicate that n-3 FAs and SPMs represent such treatments.

However, phagocytosis is not the end of the story, the fate of Aβ after internalization is also an important aspect to consider. We have previously shown that Aβ taken up by microglia is co-localized with lysosome, indicating that it is destined for degradation [233]. However, delivery to the lysosome does not equal degradation, as sufficient lysosome acidification and lysosome proteinases are required for a successful degradation [234].

Whether pro-resolving treatments as performed in **Paper I** and **III** are sufficient to induce the degradation of Aβ is a question that needs to be addressed in future studies.

5.2 MODIFYING MICROGLIAL PHENOTYPE AS TREATMENT FOR DISEASE (PAPER I, III)

5.2.1 Microglial phenotype

Monitoring microglial activation has been proposed as a way to monitor the inflammation in AD. Mitochondrial translocator protein (TSPO) is predominantly expressed by activated microglia, thus representing a potential marker for neuroinflammation, and has been developed recently as a PET tracer [235]. However, since there are both good and bad aspects linked to microglial activation, monitoring activation without taking into account the different phenotypes may not give the whole picture of inflammation in the brain. Furthermore, *in situ* microglia exhibit a continuous spectrum of activation, and the M1 and M2 phenotypes can only be viewed as two extremes of activation. Even though microglia have been reported to be activated towards predominantly one direction in the pro- to anti-inflammatory spectrum in *in vitro* cultures, it is at present impossible to reliably assess which phenotype of activation microglia have in the *in vivo* situation, since a mixture of pro- and anti-inflammatory markers may be expressed [236]. Furthermore, microglial cells express an abundance of markers, which are adapted to their different functions in different scenarios. The tendency of us

researchers to force markers into categories of pro- or anti-inflammatory activation may make research easier, but may obscure the more complex reality.

5.2.2 Immunomodulatory effects of n-3 FAs and SPMs on microglia

In **Paper I** and **III**, we have also analysed the immunomodulatory effects of n-3 FAs (**Paper I**) and SPMs (**Paper III**) on $A\beta_{42}$-induced changes in microglial phenotype. Both DHA and EPA down-regulated the pro-inflammatory M1 markers CD40 and CD86, and EPA up-regulated the anti-inflammatory M2 marker CD206. Both RvD1 and MaR1 down-regulated the $A\beta_{42}$-induced up-regulation of CD40 and CD11b (activated). It may be speculated that the anti-inflammatory effect of SPMs is stronger than that of n-3 FAs, as SPMs are downstream derivatives of n-3 FAs. However, no studies have directly compared the efficacy of their anti-inflammatory actions, and adequate comparisons between **Paper I** and **Paper III** are not possible since different concentrations of $A\beta_{42}$ were used. However, DHA, EPA and MaR1 seemed to be most effective in promoting phagocytosis in the 10 – 100 nM range (**Paper I**, Fig. 2; **Paper III**, Fig. 7). In the case of the immunomodulatory effects of n-3 FAs and SPMs, however, the effective concentrations seemed to have a broader range, and the dose-dependency is not as clear as that seen with regard to phagocytosis (**Paper I**, Fig. 3-4; **Paper III**, Fig. 6). The broad range of effective concentrations can be due to multiple pathways involved in mediating the anti-inflammatory action of n-3 FAs and SPMs. DHA and EPA have been shown bind to GPR120 with low affinity [176]. Similarly to the SPM receptors, GPR120 has been shown as a receptor for pro-inflammatory ligands such as saturated FAs [237]. In addition, PPAR-γ has been shown to be a nuclear receptor for both n-3 FAs and SPMs [155, 175, 238-240], with a variety of ligands such as eicosanoids [238, 241]. Whether these ligands counter-regulate each other, or work synergistically, is a question that should be addressed.

5.3 HOW TO TARGET INFLAMMATION AS TREATMENT FOR AD?

The main finding in **Paper II** is that Aβ-induced inflammatory responses differ from a conventional infectious stimulus as in that induced by bacterial LPS. Both Aβ and LPS induced pro-inflammatory activation of the microglia, but the factors related to resolution of inflammation were suppressed by Aβ and not by LPS, indicating a difference in the inflammation induced by these between the two causative agents. Inflammation is a common feature for a variety of very different diseases and may present itself differently. In the following two paragraphs the question regarding different types of inflammation and their progression to resolution will be discussed.

5.3.1 Different types of inflammation

Is inflammation different dependent on the disease, and is the pathogenesis of these diseases affected by inflammation in different ways? In a study where the authors compared inflammation-related gene changes in neuroinflammatory diseases such as AD, Parkinson's disease (PD), schizophrenia (SCZ) and multiple sclerosis (MS), with peripheral inflammatory diseases such as ulcerative colitis (UC) and inflammatory bowel disease (IBD), using unsupervised hierarchical clustering analysis, they found that MS had an inflammatory gene profile that was clustered with peripheral inflammatory diseases, but not with the other neuroinflammatory diseases [242]. A plausible explanation for this is that MS is an autoimmune disease that involves the infiltration of peripheral immune cells such as T-cells, whereas there is no clear evidence for infiltration of peripheral blood cells in neurodegenerative disorders such as AD. Together with our findings in **Paper II**, this suggests that different types of inflammation do exist, and exploring the differences between these common inflammatory diseases may help us to understand the etiology of the diseases, and to design disease-specific drugs modifying the inflammation.

5.3.2 Inflammation at different stages of AD

The question whether inflammation is a cause or the consequence of the pathology in AD is controversial, and little is known regarding the role of resolution in AD. To pinpoint the role of inflammation and resolution of inflammation in AD, we need to know how they change during the pathological course of the disease in comparison with normal aging.

Aging is associated with a gradual increase in inflammation as shown by increased microglial activation [243, 244] and increased plasma levels of pro-inflammatory cytokines such as IL-6 and TNF-α [245, 246]. Resolution of inflammation in normal human aging has not been investigated. However, in a senescence-accelerated mouse model (SAMP8), resolution of inflammation was suggested to be insufficient to cope with the increased levels of inflammation associated with aging [247].

AD is associated with increased inflammation compared to age-matched controls [248]. However, in general, longitudinal studies that monitor inflammation in the pathological course of AD are lacking. MCI is considered as the prodromal stages of AD, and therefore comparing inflammation-related changes in MCI with AD and with healthy controls can give some hints about changes of inflammation during the progression AD. It seems that the changes of inflammatory cytokines during the disease are very dynamic. At the individual cytokine level, there are data showing an increase, a temporary increase, unchanged or decreased levels [101] during the pathological course of AD, indicating the complexity of the inflammatory process in AD.

The differentially altered inflammatory cytokines during the progression of AD may play distinct roles at different stages of the disease. The levels of LXA_4 in CSF were found to be lower in AD compared to both MCI and control subjects. It may be speculated that the pro-resolution signal steadily decreases as AD-pathology progresses [101].

As indicated in **Paper II**, Aβ induces inflammation but suppresses resolution. However, inflammatory changes have been reported to precede the plaque deposition [25, 249]. In addition, persons with a family history of late-onset AD have been reported to be hyper-responsive to inflammatory stimuli [250]. The real picture is conceivably more complex, and more research needed before we can fully understand the role of inflammation in AD.

5.3.3 Anti-inflammation as a therapy for AD

Epidemiological studies showed an association between the intake of NSAIDs and a lower prevalence of AD [251]. A similar association was found for the intake of n-3 FAs [252]. Inspired by the epidemiological studies, several studies have been carried out to investigate the possibility to treat and prevent AD by NSAIDs [253] and n-3 FAs [254-256]. However, these studies have rendered varying results. With regard to n-3 FAs, beneficial effects have been found in subpopulations with very mild cognitive impairment or only in ApoE4 non-carriers [256, 257]. Heterogeneous study populations may mask small improvements in cognition, and it may be that there are forms of pathology in AD that inhibit the beneficial effects of n-3 FAs and NSAIDs, as indicated by the results from **Paper IV** where 6 months n-3 FA supplementation increased the levels of DHA, but not its down-stream product RvD1. Adding current knowledge on NSAIDs and n-3 FAs and analysis of common features may render new ideas, and the resolution of inflammation may represent a converging point.

5.4 HOW TO TARGET RESOLUTION OF INFLAMMATION FOR AD?

Resolution of inflammation represents a novel strategy that differs from treatments with anti-inflammatory drugs, which inhibit a pathway or block the synthesis of pro-inflammatory mediators. Instead, stimulating pro-resolving activities represents a way to end inflammation in a similar fashion as under normal physiological conditions. In the following, three possible means to stimulate the resolution of inflammation will be discussed, *i.e.* treatment with precursors of SPMs (**Paper I**), direct treatment with SPMs (**Paper III**), and stimulation of the endogenous production of SPMs (**Paper I** and **IV**).

5.4.1 n-3 FAs (precursors of SPMs)

Several epidemiological studies suggest that an increased intake of n-3 FAs is associated with reduced risk of dementia [258-260]. However, clinical trials that treat AD patients with n-3 FAs have not been clearly successful [254, 255, 261]. As discussed above, an explanation could be that there are factors that prohibit beneficial effects in late-stage AD patients. A lack of conversion from n-3 FAs to SPMs can be one of the factors (**Paper IV**). This led us to hypothesize that SPMs represent the effective molecular components that mediate the beneficial effects of n-3 FAs, as observed in epidemiological studies, *i.e.* n-3 FAs are less effective if they are not converted to SPMs. A consecutive hypothesis is that this conversion can be inhibited in states of pathology such as found in AD. This can explain the gap between the epidemiological studies and the clinical trials on AD patients. In Paper IV it was found that although AD patients respond to n-3 FA supplementation with increased plasma levels of DHA and EPA (see **Paper IV)**, this increase is not accompanied with an increase in important down-stream effectors - SPMs.

5.4.2 SPMs

With a normal diet, n-3 FAs can be converted from other essential FAs, and it is hypothesized that this conversion is disturbed in AD, which together with a decreased capacity of conversion to SPMs can lead to decreased levels of SPMs. Supporting this hypothesis, lower levels of NPD1 and LXA_4 have been found in the hippocampus [125, 136], and CSF[136] of AD patients, indicating that increasing the levels of SPMs may represent a better choice than treatment with n-3 FAs. Furthermore, the levels of both LXA_4 and RvD1 in CSF samples correlated with MMSE scores [136], suggesting an association between these SPMs and cognitive function. In studies on the ENT (**Paper III)**, using an LC-MS-MS approach, we found that the levels of PD1 as well as MaR1 were lower in AD. Furthermore, RvD5 was detected in the human brain for the first time, and exhibited lower levels in AD patients as compared to age-matched controls. In contrast, the levels of the pro-inflammatory PGD_2 were higher in AD. Thus, together with previous findings our data indicate a disturbance in the resolution of inflammation in AD, and counteracting this by stimulation of pro-resolving activites may be a therapeutic target for AD.

SPMs has been shown to exert beneficial effects in various disease models (see Table 1), and chronic administration of AT-LXA_4 has been shown to ameliorate AD pathology in the 3xTgAD mouse model [164, 165], supporting that treatment with SPMs may be a successful therapeutic strategy. However, before it can be tried on AD patients, several questions need to be addressed.

Safety is one of the most important aspects to consider. n-3 FAs have been shown to contribute to an increased risk of bleeding and hemorrhagic stroke, due to their effects on

platelet aggregation [262]. n-3 FA supplementation has also been associated with suppressed immune responses to infections [263-265]. No side effects of SPMs have been reported so far, but being the downstream products of n-3 FAs, this needs to be investigated. Considering that SPMs are relatively new subjects of research, more studies focused on safety are needed to rule out potential harmful effects. The administration route also needs to be considered. There is no direct evidence showing that SPMs can pass the BBB. However, since SPMs are small molecular weight lipophilic molecules, it is conceivable that they cross the BBB, similarly to their precursors DHA and EPA. In the OmegAD study**,** 6 months n-3 FA supplementation increased the levels of DHA, EPA and DPA in plasma (**Paper IV**), and in CSF [123]. However, the efficacy of n-3 supplementation in increasing brain levels of n-3 FAs has not been fully clarified.

The choice of SPMs for use in treatments is another important question to consider, in **Paper III** we showed that SPMs have different efficacy regarding neuroprotection, anti-inflammation and phagocytosis, even though SPMs have similar biological functions such as anti-inflammation and promoting phagocytosis (see introduction), the difference of action between different SPMs have not been reported. In addition, each SPM has different analogues with similar chemical structures, and the biological differences between them are largely unknown. Whether one SPM is more effective than another, or if a combination effect is stronger, need to be further investigated.

5.4.3 Promoting endogenous production – class-switching

A treatment strategy based on overcoming the potential inhibitory effect of AD pathology on SPM production represents an alternative approach. By stimulating the endogenous production and promoting class-switching, the levels of SPMs may be increased. In addition to α7nAchR activation, discussed in the Introduction, non-selective COX-inhibitors such as aspirin, PPAR-γ agonists, and anti-inflammatory cytokines have been indicated in the literature to be involved in class-switching.

Aspirin is a well-known NSAID, widely used for treatment of inflammation. Low doses of aspirin are prescribed for the prevention of cardiovascular events. The mechanism of action for aspirin is through acetylation of COX-1 and thereby affecting its catalytic activity and inhibiting the synthesis of PGs and thromboxanes (TXs) [200]. In addition, aspirin has been found to stimulate resolution of inflammation by acetylating COX-2 [202], which gives rise to the production of aspirin-triggered form of SPMs. Due to side effects of aspirin, such as gastrointestinal (GI) irritation [266], the development of more specific modulators of COXs could help promoting the production of SPMs without causing side effects.

The PPAR-γ agonist rosiglitazone has been shown to increase the production of LXA_4 [155]. However, due to its multi-ligand binding capacity, e.g. in glucose-lowering, used in treatment

of diabetes [267], the mechanism for its effect on LXA_4 production needs to be investigated in order to target this pathway. Anti-inflammatory cytokines such as IL-13 have been shown to increase the expression of 15-LOX-1 [268], and may therefore help stimulating the production of SPMs, although the balance between the beneficial and detrimental effects of 15-LOX-1 must be taken into account. Lastly, phosphorylation of 5-LOX at Serine 523 has been shown to increase the production of LXA_4 and to reduce the production of LTB_4 [182, 183], indicating another possible target for stimulating the endogenous resolution of inflammation.

5.5 INDIVIDUALIZED THERAPY FOR AD BASED ON GENOTYPE AND DISEASE STAGE

In **Paper IV** we investigate the effects of n-3 FA supplementation on the release of SPMs in relation to the presence of an ApoE4 genotype in AD patients. Patients from the OmegAD study were selected according to the ApoE genotype. With the hypothesis that a possible influence of the E4 allele would be larger in ApoE4 double carriers, the study was based on these and ApoE4 non-carriers. In non-carriers, but not in ApoE4 carriers, the proportion of patients with unchanged/improved cognition was higher in patients with n-3 FA supplementation compared to placebo-treated patients. Thus, ApoE4 non-carriers may benefit more from the n-3 FA supplementation compared to the ApoE4 carriers, consistent with previous reports [257, 269, 270]. ApoE4 affects lipid metabolism in many different ways including FA processing to their down-stream products, increased lipid peroxidation, etc. Our data seem to support that treatment with the end products, SPMs, would be preferable over n-3 FAs in the case of ApoE4 carriers.

In a wider perspective, *i.e.* in addition to treating patients according to their genetic background, treatments should be designed to suit other individual pathological changes that may prohibit beneficial effects, as indicated from findings in **Paper IV** and discussed above. If AD pathology inhibits the conversion of n-3 FAs, patients with advanced stage of pathology may benefit from SPMs. Additional examples are from the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT), a large randomized clinical trial (2388 patients were included) started in 2001 and designed to test the ability of the NSAIDs naproxen and celecoxib to delay or prevent the onset of AD and age-related cognitive decline [271]. The trial was designed to run for 5-7 years, but was prematurely terminated due to safety concerns. However, the patients were followed after the suspension of the trial. The primary outcome was negative [109], but re-analysis of the data from the pre-clinical group of patients according to their cognitive performance [272], showed that the fast-decliners (patients who have the strongest risk of developing AD) had beneficial effects from the non-selective COX inhibitor naproxen, while slow-decliners (patients who have relatively lower risk of developing AD) had beneficial effects from the selective COX-2 inhibitor celecoxib,

indicating that patients with different cognitive characteristics, and at different stages of disease pathology, may respond to different types of NSAIDs.

6 CONCLUDING REMARKS AND FUTURE PERSPECTIVES

6.1 CONCLUDING REMARKS

The main aim of this thesis is to investigate stimulation of resolution of inflammation as a therapeutic strategy for AD. We have utilized cellular models (**Paper I-III**), and clinical materials from AD patients (**Paper III, IV**) to address this hypothesis. Resolution signalling was stimulated by increasing the levels of resolution mediators, including the n-3 FAs DHA and EPA (precursors of SPMs), or by direct addition of SPMs. Besides treatment studies, **Paper II** characterizes the effects of different inflammatory stimuli on resolving pathways in microglia, comparing AD-related pathology with an infectious stimulus.

The key findings of this thesis are summarized as follows:

- $A\beta_{42}$ induced pro-inflammatory activation and suppressed resolution in microglia.
- Levels of MaR1, PD1, and RvD5 were lower in ENT of AD patients as compared to age-matched controls.

DHA, EPA and SPMs are beneficial for AD as:

- DHA, EPA and one of the DHA derivatives MaR1 increased the phagocytosis of $A\beta_{42}$.
- DHA and EPA down-regulated the pro-inflammatory markers CD40 and CD86; EPA up-regulated the anti-inflammatory marker CD206.
- RvD1 and MaR1 down-regulated $A\beta_{42}$-induced up-regulation of CD40 and the activated form of CD11b.
- LXA_4, MaR1 and RvD1 were neuroprotective against STS-induced apoptosis.
- n-3 FA supplementation in AD patients did not increase the downstream pro-resolving product RvD1.
- n-3 FA supplementation reduced plasma LXA_4 levels in ApoE4 double carriers, but not in non-carriers, which showed an increased RvD1/LTB_4 ratio upon n-3 FA supplementation.

To conclude, stimulating resolution of inflammation is beneficial by increasing removal of Aβ via phagocytosis, counter-regulating Aβ-induced pro-inflammatory microglial activation, and neuronal cell death. However, factors affecting resolution such as ApoE4 genotype and AD-related pathology need to be taken into consideration when translating the results from cellular studies to the clinical situation.

6.2 FUTURE PERSPECTIVES

Promoting resolution of inflammation represents a novel strategy for treatment of inflammatory disorders. SPMs have been shown to be beneficial in various inflammatory cellular and animal disease models, and results from this thesis further strengthen the idea that stimulating resolution of inflammation towards a return of homeostasis can be a therapeutic target for AD. However, there is a long way to go, and we need to better understand the mechanisms before it can be successfully translated to the clinic:

- Resolution of inflammation include two aspects: anti-inflammation and repair. The repair mechanisms are less explored. In the case of diseases with a chronic inflammation, such as AD, in which the tissue has already been damaged, it is important to investigate whether stimulating pro-resolving activties can initiate repair and neuronal regeneration.
- Promoting endogenous production of SPMs, *i.e.* by increasing class-switching mechanisms is of great interest since the stability of SPMs in the brain is not fully known. Modulators of COXs and LOXs, as well as PPAR-γ agonists, are of potential interest.
- SPMs are produced during physiological conditions, but do they have functions separate from their role in resolving inflammation?
- SPMs are end products in the metabolic pathways of COXs and LOXs from PUFAs, and there are many intermediates in these pathways. The functions of these molecules are largely unknown, and need further investigation.

7 ACKNOWLEDGEMENTS

The moment when I realized my studies were close to finish I had a feeling that I would like to continue. The PhD journey has been a great experience to me for being exposed to the scientific world, and for having chance to meet people that I would like to thank here:

Professor **Marianne Schultzberg**, my main supervisor, for accepting me as a PhD student and guiding me all the way through my PhD journey, for your great support whenever needed both scientifically and emotionally, for your encouragement and understanding when I thought there was a problem, for being very patient and letting me learn things in my own way, and for the freedom that you gave me to explore the scientific questions, for all the work you did for setting up collaborations that made our projects go further, and for the good atmosphere that you create in our group.

Dr. **Erik Hjorth**, my co-supervisor, for supervising me, a very naïve scientist, starting from very basic things such as how to culture cells, plan experiments and statistics, etc. I feel lucky to be your student and I think I have learnt a lot from you, including to come to the office early in the morning and to go to the gym. Thank you for always being available whenever needed, and for being very knowledgeable, which always makes me feel inspired after our discussions.

Professor **Maria Eriksdotter**, my co-supervisor, thank you for accepting me as your student, for always being encouraging and for all your work you did as the head of our department.

Professor **Jiachun Feng**, my supervisor and mentor in China, for encouraging me to go abroad for my PhD studies, for your understanding and support during the past years, for your expertise in the clinical work, which I need to learn a lot in the future.

Associate Professor **Jie Zhu**, for introducing me to Karolinska Institutet and being supportive all these years.

All our group members: **Heela Sarlus**, for your warm and beautiful heart under your beautiful face. **Xiuzhe Wang,** for being helpful all these years, for the collaboration of our projects, for your calm personality and wise advices. **Veronica Cortés-Toro**, for your hard work and rigorous research attitude. **Mircea Oprica**, for being humorous and creating a relaxed atmosphere during the lab-meetings. **Ann-Charlotte Granholm**, for your positive energy and encouragement. **Lisa Schröder**, for your contributions to our paper and for the weekends together. **Stefen Spulber, Catharina Lindberg** and **Åsa Forslin-Aronsson**, for the outside lab activities and Christmas tables together.

All the co-authors in my papers: **Inger Vedin, Jan Palmblad, Tommy Cederholm, Yvonne Freund-Levi, Gerd Faxen-Irving, Lars-Olof Wahlund** and **Hans Basun** from **the OmegAD study group,** for providing materials for the projects and contribution to the papers. **Romain A. Colas** and **Charles N. Serhan**, Harvard University, for the lipidomic analyses of human samples and valuable advice for our joint papers.

All the colleagues and friends in the Division of Neurodegeneration: **Erik Sundström** for information during division meetings and journal clubs. **Ning Xu**, for your great support during past years. **Elisabeth Åkesson**, **Per Henrik Vincent**, **Lena Holmberg** and **Eva-Britt Samuelsson**, **Mahmod Panahi, Dang Wang** and **Bo Zhang** for creating a nice atmosphere in the lab. Special thanks to **Maria Roos** for all the information and document work.

All senior researchers in NVS: **Åke Seiger**, for organizing the Kandel seminars. **Homira Behbahani** and **Taher Darreh-Shori** for the PhD seminars. **Helena Karlström**, **Susanne Frykman, Elisabeth Åkesson**, **Lars Tjernberg, Lars-Olof Wahlund**, **Agneta Nordberg**, **Maria Ankarcrona, Abdul H. Mohammed, Dag Aarsland, Jan Johansson, Caroline Graff**, **Erikur Benedikz**, **Eric Westman** and **Ronnie Folkesson** for a nice working environment and all the activities within the department.

The Swedish Brain Power organization: especially **Bengt Winblad**, for the research grants you brought in, your enthusiasm for work and the SBP workshop, and **Gunilla Johansson**, for your administrative and organization work.

All my dear colleagues and friends at department of NVS: **Xiaozhen Li**, for your wise advice, **Ruiqing Ni**, for exercising together, **Qiupin Jia**, for shopping together. **Zhi Tang, Hongliang Zhang, Jia Liu, Xiangyu Zheng, Geifei Chen, Hong Yu, Xu Wang，Chi Ma and Siqin Wu,** for the celebration of Chinese New Years together. **Alina Codita** for helping me with the cover of the thesis, **Azadeh Karami,** for all the pictures you took. **Daniela Enache**, for your kind support. **Kevin Grimes** and **Seyed-Mohammad Fereshtehnejad** for philosophy discussions together, **Anna Sandebring**, **Gabriella Spulber**, **Erika Bereczki, Olga Voevodskaya**, **Silvia Maioli, Muhammad Al Mustafa Ismail**, **Annelie Pamrén**, **Elena Rodriguez-Vieitez**, **Bernadette Schreiner**, **Swetha Vijayaraghavan**, **Soheil Damangir**, **Farshad Falahati**, **Seyed Mohammad Fereshtehnejad, Daniel Padilla Ferreira, Carlos Aguilar**, **Nuninho Leal**, **Hue-Hsin Chen**, **Walid Tajeddinn**, **Torbjörn Persson**, **Médoune Sarr**, **Axel Leppert, Oihan Basabe Burgos**, **Nina Kronqvist**, **Kostantinos Chiotis**, **Antoine Leuzy**, **Jolanta Lundgren**, **Erica Lana**, **Lisa Dolfe**, **Laetitia Lemoine**, **Pavla Cermakova, Annette Karlsson**, **Annette Eidehall** and **Maggie Lukasiewicz**, for making the NVS environment very friendly.

All my friends in Stockholm that I didn't mention above: **Jing Lyu**, for your company and support, I wish you good luck with your future. **Yan Li**, for your warm heart and help. **Yang Ruan**, for the time we spent together. **Lisa Gan**, for all your help and for sharing your stories. **Shaohua Xu** & **Lu Zhang**, I am so sorry that I could not attend your wedding, I think you two make a nice couple and wish you all the best for your future. **Shouting Zhang** for your encouragement. **Xingmei Zhang**, for always being positive. **Qingda Meng, Zhenjiang Liu, Huijie Xiao, Jia Sun, Meng Li, Yuan Zhang, Xintong Jiang** and **Yali Miao**, for the nice memories that you gave.

My dear **mother** and **father**, for your endless love and support. My **sisters** and my little **brother**, for your love and support, for the lovely memories that you gave, I hope we will have more chances to

meet in the future. 我亲爱的爸爸妈妈, 感谢你们的无尽的爱与支持。我兄弟姐妹， 感谢你们关心和支持，希望我们以后有更多的时间在一起。

My dear **husband** and my **son**, thank you for being part of my life, for your great love and support. 我亲爱的老公和儿子， 很遗憾和你们分开了这么长时间， 希望以后的日子我们都能快快乐乐的在一起。

My dear **mother-in-law**, thank you for all your love and support, for your warm heart and being positive whatever life gives us. 我亲爱的岳母， 非常感谢你对家和的照顾，让我专心的完成我的学业，我们以后会有更多的时间在一起！

8 REFERENCES

[1] Alzheimer A, Stelzmann RA, Schnitzlein HN, Murtagh FR (1995) An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clin Anat* **8**, 429-431.

[2] Hardy J (2006) A hundred years of Alzheimer's disease research. *Neuron* **52**, 3-13.

[3] Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP (2013) The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement* **9**, 63-75 e62.

[4] Wimo A, Jonsson L, Bond J, Prince M, Winblad B, Alzheimer Disease I (2013) The worldwide economic impact of dementia 2010. *Alzheimers Dement* **9**, 1-11 e13.

[5] Hebert LE, Weuve J, Scherr PA, Evans DA (2013) Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology* **80**, 1778-1783.

[6] Schneider JA, Arvanitakis Z, Bang W, Bennett DA (2007) Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* **69**, 2197-2204.

[7] Bird TD (1993) Early-Onset Familial Alzheimer Disease In *GeneReviews(R)*, Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, eds., Seattle (WA).

[8] Raber J, Huang Y, Ashford JW (2004) ApoE genotype accounts for the vast majority of AD risk and AD pathology. *Neurobiol Aging* **25**, 641-650.

[9] Fratiglioni L, Ahlbom A, Viitanen M, Winblad B (1993) Risk factors for late-onset Alzheimer's disease: a population-based, case-control study. *Ann Neurol* **33**, 258-266.

[10] Rusanen M, Kivipelto M, Quesenberry CP, Jr., Zhou J, Whitmer RA (2011) Heavy smoking in midlife and long-term risk of Alzheimer disease and vascular dementia. *Arch Intern Med* **171**, 333-339.

[11] Pendlebury ST, Rothwell PM (2009) Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. *Lancet Neurol* **8**, 1006-1018.

[12] Xu WL, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L (2011) Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. *Neurology* **76**, 1568-1574.

[13] Yaffe K, Hoang TD, Byers AL, Barnes DE, Friedl KE (2014) Lifestyle and health-related risk factors and risk of cognitive aging among older veterans. *Alzheimers Dement* **10**, S111-121.

[14] Helzner EP, Scarmeas N, Cosentino S, Tang MX, Schupf N, Stern Y (2008) Survival in Alzheimer disease: a multiethnic, population-based study of incident cases. *Neurology* **71**, 1489-1495.

[15] Xie J, Brayne C, Matthews FE, Medical Research Council Cognitive F, Ageing Study c (2008) Survival times in people with dementia: analysis from population based cohort study with 14 year follow-up. *BMJ* **336**, 258-262.

[16] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) 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* **34**, 939-944.

[17] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[18] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.

[19] Doody RS, Gavrilova SI, Sano M, Thomas RG, Aisen PS, Bachurin SO, Seely L, Hung D, dimebon i (2008) Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. *Lancet* **372**, 207-215.

[20] Imbimbo BP, Solfrizzi V, Panza F (2010) Are NSAIDs useful to treat Alzheimer's disease or mild cognitive impairment? *Front Aging Neurosci* **2.** , PMID: 20725517.

[21] Mullane K, Williams M (2013) Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? *Biochem Pharmacol* **85**, 289-305.

[22] Burke V, Beilin LJ, Cutt HE, Mansour J, Williams A, Mori TA (2007) A lifestyle program for treated hypertensives improved health-related behaviors and cardiovascular risk factors, a randomized controlled trial. *J Clin Epidemiol* **60**, 133-141.

[23] McGeer PL, Itagaki S, Tago H, McGeer EG (1987) Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. *Neurosci Lett* **79**, 195-200.

[24] McGeer PL, Itagaki S, Boyes BE, McGeer EG (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* **38**, 1285-1291.

[25] Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White CL, 3rd, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci U S A* **86**, 7611-7615.
[26] Cacabelos R, Alvarez XA, Fernandez-Novoa L, Franco A, Mangues R, Pellicer A, Nishimura T (1994) Brain interleukin-1β in Alzheimer's disease and vascular dementia. *Methods Find Exp Clin Pharmacol* **16**, 141-151.
[27] Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, et al. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704-706.
[28] Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Perkicak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754-760.
[29] Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, Chi H, Lin C, Holman K, Tsuda T, et al. (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376**, 775-778.
[30] Guerreiro R, Hardy J (2014) Genetics of Alzheimer's disease. *Neurotherapeutics* **11**, 732-737.
[31] Chouraki V, Seshadri S (2014) Genetics of Alzheimer's disease. *Adv Genet* **87**, 245-294.
[32] Zheng WH, Bastianetto S, Mennicken F, Ma W, Kar S (2002) Aβ peptide induces tau phosphorylation and loss of cholinergic neurons in rat primary septal cultures. *Neuroscience* **115**, 201-211.
[33] Busciglio J, Lorenzo A, Yeh J, Yankner BA (1995) β-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron* **14**, 879-888.
[34] Franco R, Cedazo-Minguez A (2014) Successful therapies for Alzheimer's disease: why so many in animal models and none in humans? *Front Pharmacol* **5**, 146.
[35] Del Bo R, Angeretti N, Lucca E, De Simoni MG, Forloni G (1995) Reciprocal control of inflammatory cytokines, IL-1 and IL-6, and Aβ production in cultures. *Neurosci Lett* **188**, 70-74.
[36] Quintanilla RA, Orellana DI, Gonzalez-Billault C, Maccioni RB (2004) Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. *Exp Cell Res* **295**, 245-257.
[37] Golde TE, Estus S, Younkin LH, Selkoe DJ, Younkin SG (1992) Processing of the amyloid protein precursor to potentially amyloidogenic derivatives. *Science* **255**, 728-730.
[38] Hardy J (2009) The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *J Neurochem* **110**, 1129-1134.
[39] Wicklund L, Leao RN, Stromberg AM, Mousavi M, Hovatta O, Nordberg A, Marutle A (2010) $A\beta_{42}$ oligomers impair function of human embryonic stem cell-derived forebrain cholinergic neurons. *PLoS ONE* **5**, e15600.
[40] Combs CK, Karlo JC, Kao SC, Landreth GE (2001) β-amyloid stimulation of microglia and monocytes results in TNFα-dependent expression of inducible nitric oxide synthase and neuronal apoptosis. *J Neurosci* **21**, 1179-1188.
[41] Selkoe DJ (2001) Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid-β protein. *J Alzheimers Dis* **3**, 75-80.
[42] Goto Y, Yagi H, Yamaguchi K, Chatani E, Ban T (2008) Structure, formation and propagation of amyloid fibrils. *Curr Pharm Des* **14**, 3205-3218.
[43] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med* **1**, a006189.
[44] Furst AJ, Rabinovici GD, Rostomian AH, Steed T, Alkalay A, Racine C, Miller BL, Jagust WJ (2012) Cognition, glucose metabolism and amyloid burden in Alzheimer's disease. *Neurobiol Aging* **33**, 215-225.
[45] Lesne SE, Sherman MA, Grant M, Kuskowski M, Schneider JA, Bennett DA, Ashe KH (2013) Brain amyloid-β oligomers in ageing and Alzheimer's disease. *Brain* **136**, 1383-1398.
[46] Kayed R, Lasagna-Reeves CA (2013) Molecular mechanisms of amyloid oligomers toxicity. *J Alzheimers Dis* **33 Suppl 1**, S67-78.
[47] Hersh LB, Rodgers DW (2008) Neprilysin and amyloid β peptide degradation. *Curr Alzheimer Res* **5**, 225-231.
[48] Saido T, Leissring MA (2012) Proteolytic degradation of amyloid-β protein. *Cold Spring Harb Perspect Med* **2**, a006379.
[49] Qiu WQ, Folstein MF (2006) Insulin, insulin-degrading enzyme and amyloid-β peptide in Alzheimer's disease: review and hypothesis. *Neurobiol Aging* **27**, 190-198.
[50] Yan P, Hu X, Song H, Yin K, Bateman RJ, Cirrito JR, Xiao Q, Hsu FF, Turk JW, Xu J, Hsu CY, Holtzman DM, Lee JM (2006) Matrix metalloproteinase-9 degrades amyloid-β fibrils in vitro and compact plaques in situ. *J Biol Chem* **281**, 24566-24574.

[51] Donahue JE, Flaherty SL, Johanson CE, Duncan JA, 3rd, Silverberg GD, Miller MC, Tavares R, Yang W, Wu Q, Sabo E, Hovanesian V, Stopa EG (2006) RAGE, LRP-1, and amyloid-β protein in Alzheimer's disease. *Acta Neuropathol* **112**, 405-415.
[52] Vogelgesang S, Jedlitschky G, Brenn A, Walker LC (2011) The role of the ATP-binding cassette transporter P-glycoprotein in the transport of β-amyloid across the blood-brain barrier. *Curr Pharm Des* **17**, 2778-2786.
[53] Zlokovic BV (2004) Clearing amyloid through the blood-brain barrier. *J Neurochem* **89**, 807-811.
[54] Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Stern A, Schmidt AM, Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D, Zlokovic B (2003) RAGE mediates amyloid-β peptide transport across the blood-brain barrier and accumulation in brain. *Nat Med* **9**, 907-913.
[55] Karran E, Hardy J (2014) A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. *Ann Neurol* **76**, 185-205.
[56] Iqbal K, Liu F, Gong CX, Grundke-Iqbal I (2010) Tau in Alzheimer disease and related tauopathies. *Curr Alzheimer Res* **7**, 656-664.
[57] Alonso AC, Li B, Grundke-Iqbal I, Iqbal K (2008) Mechanism of tau-induced neurodegeneration in Alzheimer disease and related tauopathies. *Curr Alzheimer Res* **5**, 375-384.
[58] Sigurdsson EM (2014) Tau immunotherapy and imaging. *Neurodegener Dis* **13**, 103-106.
[59] Gong CX, Iqbal K (2008) Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Curr Med Chem* **15**, 2321-2328.
[60] Scott A, Khan KM, Cook JL, Duronio V (2004) What is "inflammation"? Are we ready to move beyond Celsus? *Br J Sports Med* **38**, 248-249.
[61] Tracy RP (2006) The five cardinal signs of inflammation: Calor, Dolor, Rubor, Tumor ... and Penuria (Apologies to Aulus Cornelius Celsus, De medicina, c. A.D. 25). *J Gerontol A Biol Sci Med Sci* **61**, 1051-1052.
[62] Noble M (2004) The possible role of myelin destruction as a precipitating event in Alzheimer's disease. *Neurobiol Aging* **25**, 25-31.
[63] Lyros E, Bakogiannis C, Liu Y, Fassbender K (2014) Molecular links between endothelial dysfunction and neurodegeneration in Alzheimer's disease. *Curr Alzheimer Res* **11**, 18-26.
[64] Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res* **117**, 145-152.
[65] Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841-845.
[66] Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* **10**, 1538-1543.
[67] Elmore MR, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA, Kitazawa M, Matusow B, Nguyen H, West BL, Green KN (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron* **82**, 380-397.
[68] Ladeby R, Wirenfeldt M, Garcia-Ovejero D, Fenger C, Dissing-Olesen L, Dalmau I, Finsen B (2005) Microglial cell population dynamics in the injured adult central nervous system. *Brain Res Brain Res Rev* **48**, 196-206.
[69] Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. *Science* **333**, 1456-1458.
[70] Hjorth E, Frenkel D, Weiner H, Schultzberg M (2010) Effects of immunomodulatory substances on phagocytosis of $A\beta_{42}$ by human microglia. *Int J Alzheimers Dis* **10.4061/2010/798424**.
[71] Durafourt BA, Moore CS, Zammit DA, Johnson TA, Zaguia F, Guiot MC, Bar-Or A, Antel JP (2012) Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia* **60**, 717-727.
[72] Perry VH, Teeling J (2013) Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Semin Immunopathol* **35**, 601-612.
[73] Gomez-Nicola D, Perry VH (2015) Microglial Dynamics and Role in the Healthy and Diseased Brain: A Paradigm of Functional Plasticity. *Neuroscientist* **21**, 169-184.
[74] Eikelenboom P, Stam FC (1982) Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. *Acta Neuropathol* **57**, 239-242.
[75] Coraci IS, Husemann J, Berman JW, Hulette C, Dufour JH, Campanella GK, Luster AD, Silverstein SC, El-Khoury JB (2002) CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to β-amyloid fibrils. *Am J Pathol* **160**, 101-112.
[76] Strohmeyer R, Ramirez M, Cole GJ, Mueller K, Rogers J (2002) Association of factor H of the alternative pathway of complement with agrin and complement receptor 3 in the Alzheimer's disease brain. *J Neuroimmunol* **131**, 135-146.

[77] Zhang D, Hu X, Qian L, Chen SH, Zhou H, Wilson B, Miller DS, Hong JS (2011) Microglial MAC1 receptor and PI3K are essential in mediating β-amyloid peptide-induced microglial activation and subsequent neurotoxicity. *J Neuroinflammation* **8**, 3.
[78] Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, Hooli B, Choi SH, Hyman BT, Tanzi RE (2013) Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid-β. *Neuron* **78**, 631-643.
[79] Bradshaw EM, Chibnik LB, Keenan BT, Ottoboni L, Raj T, Tang A, Rosenkrantz LL, Imboywa S, Lee M, Von Korff A, Alzheimer Disease Neuroimaging I, Morris MC, Evans DA, Johnson K, Sperling RA, Schneider JA, Bennett DA, De Jager PL (2013) CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nat Neurosci* **16**, 848-850.
[80] Takuma K, Fang F, Zhang W, Yan S, Fukuzaki E, Du H, Sosunov A, McKhann G, Funatsu Y, Nakamichi N, Nagai T, Mizoguchi H, Ibi D, Hori O, Ogawa S, Stern DM, Yamada K, Yan SS (2009) RAGE-mediated signaling contributes to intraneuronal transport of amyloid-β and neuronal dysfunction. *Proc Natl Acad Sci U S A* **106**, 20021-20026.
[81] Takano T, Fiore S, Maddox JF, Brady HR, Petasis NA, Serhan CN (1997) Aspirin-triggered 15-epi-lipoxin A4 (LXA4) and LXA4 stable analogues are potent inhibitors of acute inflammation: evidence for anti-inflammatory receptors. *J Exp Med* **185**, 1693-1704.
[82] Verkhratsky A, Olabarria M, Noristani HN, Yeh CY, Rodriguez JJ (2010) Astrocytes in Alzheimer's disease. *Neurotherapeutics* **7**, 399-412.
[83] Thangavel R, Stolmeier D, Yang X, Anantharam P, Zaheer A (2012) Expression of glia maturation factor in neuropathological lesions of Alzheimer's disease. *Neuropathol Appl Neurobiol* **38**, 572-581.
[84] Pike CJ, Cummings BJ, Cotman CW (1995) Early association of reactive astrocytes with senile plaques in Alzheimer's disease. *Exp Neurol* **132**, 172-179.
[85] Shao Y, Gearing M, Mirra SS (1997) Astrocyte-apolipoprotein E associations in senile plaques in Alzheimer disease and vascular lesions: a regional immunohistochemical study. *J Neuropathol Exp Neurol* **56**, 376-381.
[86] DeWitt DA, Perry G, Cohen M, Doller C, Silver J (1998) Astrocytes regulate microglial phagocytosis of senile plaque cores of Alzheimer's disease. *Exp Neurol* **149**, 329-340.
[87] Bartfai T, Schultzberg M (1993) Cytokines in neuronal cell types. *Neurochem Int* **22**, 435-444.
[88] Ek M, Kurosawa M, Lundeberg T, Ericsson A (1998) Activation of vagal afferents after intravenous injection of interleukin-1β: role of endogenous prostaglandins. *J Neurosci* **18**, 9471-9479.
[89] Patterson PH, Nawa H (1993) Neuronal differentiation factors/cytokines and synaptic plasticity. *Cell* **72 Suppl**, 123-137.
[90] Kelley KW, Bluthe RM, Dantzer R, Zhou JH, Shen WH, Johnson RW, Broussard SR (2003) Cytokine-induced sickness behavior. *Brain Behav Immun* **17 Suppl 1**, S112-118.
[91] Holmannova D, Kolackova M, Kondelkova K, Kunes P, Krejsek J, Andrys C (2012) CD200/CD200R paired potent inhibitory molecules regulating immune and inflammatory responses; Part I: CD200/CD200R structure, activation, and function. *Acta Medica (Hradec Kralove)* **55**, 12-17.
[92] Walker DG, Dalsing-Hernandez JE, Campbell NA, Lue LF (2009) Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation. *Exp Neurol* **215**, 5-19.
[93] Togo T, Akiyama H, Kondo H, Ikeda K, Kato M, Iseki E, Kosaka K (2000) Expression of CD40 in the brain of Alzheimer's disease and other neurological diseases. *Brain Res* **885**, 117-121.
[94] Tan J, Town T, Paris D, Mori T, Suo Z, Crawford F, Mattson MP, Flavell RA, Mullan M (1999) Microglial activation resulting from CD40-CD40L interaction after β-amyloid stimulation. *Science* **286**, 2352-2355.
[95] Trinchieri G (2004) Cytokines and cytokine receptors. *Immunol Rev* **202**, 5-7.
[96] Schneider H, Pitossi F, Balschun D, Wagner A, del Rey A, Besedovsky HO (1998) A neuromodulatory role of interleukin-1β in the hippocampus. *Proc Natl Acad Sci U S A* **95**, 7778-7783.
[97] Avital A, Goshen I, Kamsler A, Segal M, Iverfeldt K, Richter-Levin G, Yirmiya R (2003) Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity. *Hippocampus* **13**, 826-834.
[98] Spulber S, Mateos L, Oprica M, Cedazo-Minguez A, Bartfai T, Winblad B, Schultzberg M (2009) Impaired long term memory consolidation in transgenic mice overexpressing the human soluble form of IL-1ra in the brain. *J Neuroimmunol* **208**, 46-53.
[99] Bellinger FP, Madamba S, Siggins GR (1993) Interleukin 1β inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Res* **628**, 227-234.
[100] Barrientos RM, Frank MG, Hein AM, Higgins EA, Watkins LR, Rudy JW, Maier SF (2009) Time course of hippocampal IL-1β and memory consolidation impairments in aging rats following peripheral infection. *Brain Behav Immun* **23**, 46-54.
[101] Brosseron F, Krauthausen M, Kummer M, Heneka MT (2014) Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. *Mol Neurobiol* **50**, 534-544.
[102] Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, Jones T, Banati RB (2001) In-vivo measurement of activated microglia in dementia. *Lancet* **358**, 461-467.

[103] Tosto G, Reitz C (2013) Genome-wide association studies in Alzheimer's disease: a review. *Curr Neurol Neurosci Rep* **13**, 381.
[104] Meda L, Cassatella MA, Szendrei GI, Otvos L, Jr., Baron P, Villalba M, Ferrari D, Rossi F (1995) Activation of microglial cells by β-amyloid protein and interferon-γ. *Nature* **374**, 647-650.
[105] Hu J, Akama KT, Krafft GA, Chromy BA, Van Eldik LJ (1998) Amyloid-β peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release. *Brain Res* **785**, 195-206.
[106] Blasko I, Veerhuis R, Stampfer-Kountchev M, Saurwein-Teissl M, Eikelenboom P, Grubeck-Loebenstein B (2000) Costimulatory effects of interferon-γ and interleukin-1β or tumor necrosis factor α on the synthesis of $A\beta_{1-40}$ and $A\beta_{1-42}$ by human astrocytes. *Neurobiol Dis* **7**, 682-689.
[107] Dash PK, Moore AN (1995) Enhanced processing of APP induced by IL-1β can be reduced by indomethacin and nordihydroguaiaretic acid. *Biochem Biophys Res Commun* **208**, 542-548.
[108] McGeer PL, McGeer E, Rogers J, Sibley J (1990) Anti-inflammatory drugs and Alzheimer disease. *Lancet* **335**, 1037.
[109] Group AR, Lyketsos CG, Breitner JC, Green RC, Martin BK, Meinert C, Piantadosi S, Sabbagh M (2007) Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. *Neurology* **68**, 1800-1808.
[110] Reines SA, Block GA, Morris JC, Liu G, Nessly ML, Lines CR, Norman BA, Baranak CC, Rofecoxib Protocol 091 Study G (2004) Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. *Neurology* **62**, 66-71.
[111] Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N (1999) A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. *Neurology* **53**, 197-201.
[112] O'Bryant Sea (2014) A proinflammatory endophenotype predicts treatment response in a multicenter trial of NSAIDs in AD. *Alzheimer's & Dementia* **10**, P273 - P274.
[113] Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, van Meer G, Wakelam MJ, Dennis EA (2009) Update of the LIPID MAPS comprehensive classification system for lipids. *J Lipid Res* **50 Suppl**, S9-14.
[114] Andersen OS, Koeppe RE, 2nd (2007) Bilayer thickness and membrane protein function: an energetic perspective. *Annu Rev Biophys Biomol Struct* **36**, 107-130.
[115] Spite M (2013) Deciphering the role of n-3 polyunsaturated fatty acid-derived lipid mediators in health and disease. *Proc Nutr Soc* **72**, 441-450.
[116] Shimizu T (2009) Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. *Annu Rev Pharmacol Toxicol* **49**, 123-150.
[117] Farooqui AA (2011) *Lipid mediators and their metabolism in the brain*, Springer, New York.
[118] Robinson PG (1982) Common names and abbreviated formulae for fatty acids. *J Lipid Res* **23**, 1251-1253.
[119] Whitney E (2014) *Understanding nutrition*, Cengage Learning, San Francisco, CA.
[120] Hansen AE, Burr GO (1946) Essential fatty acids and human nutrition. *J Am Med Assoc* **132**, 855-859.
[121] Holman RT (1960) Essential fatty acids in nutrition and metabolism. *Arch Intern Med* **105**, 33-38.
[122] Williams CM, Burdge G (2006) Long-chain n-3 PUFA: plant v. marine sources. *Proc Nutr Soc* **65**, 42-50.
[123] Freund Levi Y, Vedin I, Cederholm T, Basun H, Faxen Irving G, Eriksdotter M, Hjorth E, Schultzberg M, Vessby B, Wahlund LO, Salem N, Jr., Palmblad J (2014) Transfer of omega-3 fatty acids across the blood-brain barrier after dietary supplementation with a docosahexaenoic acid-rich omega-3 fatty acid preparation in patients with Alzheimer's disease: the OmegAD study. *J Intern Med* **275**, 428-436.
[124] Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. *Nature* **510**, 92-101.
[125] Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG (2005) A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J Clin Invest* **115**, 2774-2783.
[126] Kang J, Rivest S (2012) Lipid metabolism and neuroinflammation in Alzheimer's disease: a role for liver X receptors. *Endocr Rev* **33**, 715-746.
[127] Kanekiyo T, Xu H, Bu G (2014) ApoE and Aβ in Alzheimer's disease: accidental encounters or partners? *Neuron* **81**, 740-754.
[128] Namba Y, Tomonaga M, Kawasaki H, Otomo E, Ikeda K (1991) Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res* **541**, 163-166.
[129] Pirttila T, Soininen H, Heinonen O, Lehtimaki T, Bogdanovic N, Paljarvi L, Kosunen O, Winblad B, Riekkinen P, Sr., Wisniewski HM, Mehta PD (1996) Apolipoprotein E (apoE) levels in brains from Alzheimer disease patients and controls. *Brain Res* **722**, 71-77.
[130] Beffert U, Cohn JS, Petit-Turcotte C, Tremblay M, Aumont N, Ramassamy C, Davignon J, Poirier J (1999) Apolipoprotein E and β-amyloid levels in the hippocampus and frontal cortex of Alzheimer's disease subjects are disease-related and apolipoprotein E genotype dependent. *Brain Res* **843**, 87-94.

[131] Chouinard-Watkins R, Rioux-Perreault C, Fortier M, Tremblay-Mercier J, Zhang Y, Lawrence P, Vohl MC, Perron P, Lorrain D, Brenna JT, Cunnane SC, Plourde M (2013) Disturbance in uniformly 13C-labelled DHA metabolism in elderly human subjects carrying the apoE epsilon4 allele. *Br J Nutr* **110**, 1751-1759.
[132] Smith JD, Miyata M, Poulin SE, Neveux LM, Craig WY (1998) The relationship between apolipoprotein E and serum oxidation-related variables is apolipoprotein E phenotype dependent. *Int J Clin Lab Res* **28**, 116-121.
[133] Martin V, Fabelo N, Santpere G, Puig B, Marin R, Ferrer I, Diaz M (2010) Lipid alterations in lipid rafts from Alzheimer's disease human brain cortex. *J Alzheimers Dis* **19**, 489-502.
[134] Ehehalt R, Keller P, Haass C, Thiele C, Simons K (2003) Amyloidogenic processing of the Alzheimer β-amyloid precursor protein depends on lipid rafts. *J Cell Biol* **160**, 113-123.
[135] Ikonomovic MD, Abrahamson EE, Uz T, Manev H, Dekosky ST (2008) Increased 5-lipoxygenase immunoreactivity in the hippocampus of patients with Alzheimer's disease. *J Histochem Cytochem* **56**, 1065-1073.
[136] Wang X, Zhu M, Hjorth E, Cortes-Toro V, Eyjolfsdottir H, Graff C, Nennesmo I, Palmblad J, Eriksdotter M, Sambamurti K, Fitzgerald JM, Serhan CN, Granholm AC, Schultzberg M (2015) Resolution of inflammation is altered in Alzheimer's disease. *Alzheimers Dement* **11**, 40-50 e42.
[137] Mohri I, Kadoyama K, Kanekiyo T, Sato Y, Kagitani-Shimono K, Saito Y, Suzuki K, Kudo T, Takeda M, Urade Y, Murayama S, Taniike M (2007) Hematopoietic prostaglandin D synthase and DP1 receptor are selectively upregulated in microglia and astrocytes within senile plaques from human patients and in a mouse model of Alzheimer disease. *J Neuropathol Exp Neurol* **66**, 469-480.
[138] Bate C, Kempster S, Williams A (2006) Prostaglandin D2 mediates neuronal damage by amyloid-β or prions which activates microglial cells. *Neuropharmacology* **50**, 229-237.
[139] Hedqvist P, Raud J, Palmertz U, Haeggstrom J, Nicolaou KC, Dahlen SE (1989) Lipoxin A4 inhibits leukotriene B4-induced inflammation in the hamster cheek pouch. *Acta Physiol Scand* **137**, 571-572.
[140] Chiang N, Takano T, Clish CB, Petasis NA, Tai HH, Serhan CN (1998) Aspirin-triggered 15-epi-lipoxin A4 (ATL) generation by human leukocytes and murine peritonitis exudates: development of a specific 15-epi-LXA4 ELISA. *J Pharmacol Exp Ther* **287**, 779-790.
[141] Chiang N, Gronert K, Clish CB, O'Brien JA, Freeman MW, Serhan CN (1999) Leukotriene B4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J Clin Invest* **104**, 309-316.
[142] Serhan CN (2006) Novel chemical mediators in the resolution of inflammation: resolvins and protectins. *Anesthesiol Clin* **24**, 341-364.
[143] Serhan CN, Petasis NA (2011) Resolvins and protectins in inflammation resolution. *Chem Rev* **111**, 5922-5943.
[144] Miyata J, Fukunaga K, Iwamoto R, Isobe Y, Niimi K, Takamiya R, Takihara T, Tomomatsu K, Suzuki Y, Oguma T, Sayama K, Arai H, Betsuyaku T, Arita M, Asano K (2013) Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma. *J Allergy Clin Immunol* **131**, 353-360 e351-352.
[145] Planaguma A, Kazani S, Marigowda G, Haworth O, Mariani TJ, Israel E, Bleecker ER, Curran-Everett D, Erzurum SC, Calhoun WJ, Castro M, Chung KF, Gaston B, Jarjour NN, Busse WW, Wenzel SE, Levy BD (2008) Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma. *Am J Respir Crit Care Med* **178**, 574-582.
[146] Fredman G, Oh SF, Ayilavarapu S, Hasturk H, Serhan CN, Van Dyke TE (2011) Impaired phagocytosis in localized aggressive periodontitis: rescue by Resolvin E1. *PLoS ONE* **6**, e24422.
[147] Aoki H, Hisada T, Ishizuka T, Utsugi M, Kawata T, Shimizu Y, Okajima F, Dobashi K, Mori M (2008) Resolvin E1 dampens airway inflammation and hyperresponsiveness in a murine model of asthma. *Biochem Biophys Res Commun* **367**, 509-515.
[148] Levy BD, Lukacs NW, Berlin AA, Schmidt B, Guilford WJ, Serhan CN, Parkinson JF (2007) Lipoxin A4 stable analogs reduce allergic airway responses via mechanisms distinct from CysLT1 receptor antagonism. *FASEB J* **21**, 3877-3884.
[149] Flesher RP, Herbert C, Kumar RK (2014) Resolvin E1 promotes resolution of inflammation in a mouse model of an acute exacerbation of allergic asthma. *Clin Sci* **126**, 805-814.
[150] Fiorucci S, Wallace JL, Mencarelli A, Distrutti E, Rizzo G, Farneti S, Morelli A, Tseng JL, Suramanyam B, Guilford WJ, Parkinson JF (2004) A β-oxidation-resistant lipoxin A4 analog treats hapten-induced colitis by attenuating inflammation and immune dysfunction. *Proc Natl Acad Sci U S A* **101**, 15736-15741.
[151] Marcon R, Bento AF, Dutra RC, Bicca MA, Leite DF, Calixto JB (2013) Maresin 1, a proresolving lipid mediator derived from omega-3 polyunsaturated Fatty acids, exerts protective actions in murine models of colitis. *J Immunol* **191**, 4288-4298.
[152] Yamada T, Tani Y, Nakanishi H, Taguchi R, Arita M, Arai H (2011) Eosinophils promote resolution of acute peritonitis by producing proresolving mediators in mice. *FASEB J* **25**, 561-568.
[153] Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN (2007) Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. *J Immunol* **178**, 3912-3917.

[154] Bazan NG, Eady TN, Khoutorova L, Atkins KD, Hong S, Lu Y, Zhang C, Jun B, Obenaus A, Fredman G, Zhu M, Winkler JW, Petasis NA, Serhan CN, Belayev L (2012) Novel aspirin-triggered neuroprotectin D1 attenuates cerebral ischemic injury after experimental stroke. *Exp Neurol* **236**, 122-130.
[155] Sobrado M, Pereira MP, Ballesteros I, Hurtado O, Fernandez-Lopez D, Pradillo JM, Caso JR, Vivancos J, Nombela F, Serena J, Lizasoain I, Moro MA (2009) Synthesis of lipoxin A4 by 5-lipoxygenase mediates PPARγ-dependent, neuroprotective effects of rosiglitazone in experimental stroke. *J Neurosci* **29**, 3875-3884.
[156] Wu Y, Wang YP, Guo P, Ye XH, Wang J, Yuan SY, Yao SL, Shang Y (2012) A lipoxin A4 analog ameliorates blood-brain barrier dysfunction and reduces MMP-9 expression in a rat model of focal cerebral ischemia-reperfusion injury. *J Mol Neurosci* **46**, 483-491.
[157] Ye XH, Wu Y, Guo PP, Wang J, Yuan SY, Shang Y, Yao SL (2010) Lipoxin A4 analogue protects brain and reduces inflammation in a rat model of focal cerebral ischemia reperfusion. *Brain Res* **1323**, 174-183.
[158] Wu L, Miao S, Zou LB, Wu P, Hao H, Tang K, Zeng P, Xiong J, Li HH, Wu Q, Cai L, Ye DY (2012) Lipoxin A4 inhibits 5-lipoxygenase translocation and leukotrienes biosynthesis to exert a neuroprotective effect in cerebral ischemia/reperfusion injury. *J Mol Neurosci* **48**, 185-200.
[159] Huang L, Wang CF, Serhan CN, Strichartz G (2011) Enduring prevention and transient reduction of postoperative pain by intrathecal resolvin D1. *Pain* **152**, 557-565.
[160] Hu S, Mao-Ying QL, Wang J, Wang ZF, Mi WL, Wang XW, Jiang JW, Huang YL, Wu GC, Wang YQ (2012) Lipoxins and aspirin-triggered lipoxin alleviate bone cancer pain in association with suppressing expression of spinal proinflammatory cytokines. *J Neuroinflammation* **9**, 278.
[161] Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ, Ji RR, Zhu M, Petasis NA (2012) Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. *FASEB J* **26**, 1755-1765.
[162] Xu ZZ, Liu XJ, Berta T, Park CK, Lu N, Serhan CN, Ji RR (2013) NpD/PD1 protects against neuropathic pain in mice after nerve trauma. *Ann Neurol* **74**, 490-495.
[163] Park CK, Lu N, Xu ZZ, Liu T, Serhan CN, Ji RR (2011) Resolving TRPV1- and TNF-α-mediated spinal cord synaptic plasticity and inflammatory pain with neuroprotectin D1. *J Neurosci* **31**, 15072-15085.
[164] Medeiros R, Kitazawa M, Passos GF, Baglietto-Vargas D, Cheng D, Cribbs DH, LaFerla FM (2013) Aspirin-triggered lipoxin A4 stimulates alternative activation of microglia and reduces Alzheimer disease-like pathology in mice. *Am J Pathol* **182**, 1780-1789.
[165] Dunn HC, Ager RR, Baglietto-Vargas D, Cheng D, Kitazawa M, Cribbs DH, Medeiros R (2015) Restoration of lipoxin A4 signaling reduces Alzheimer's disease-like pathology in the 3xTg-AD mouse model. *J Alzheimers Dis* **43**, 893-903.
[166] Wu SH, Chen XQ, Liu B, Wu HJ, Dong L (2013) Efficacy and safety of 15(R/S)-methyl-lipoxin A(4) in topical treatment of infantile eczema. *Br J Dermatol* **168**, 172-178.
[167] de Paiva CS, Schwartz CE, Gjorstrup P, Pflugfelder SC (2012) Resolvin E1 (RX-10001) reduces corneal epithelial barrier disruption and protects against goblet cell loss in a murine model of dry eye. *Cornea* **31**, 1299-1303.
[168] Li N, He J, Schwartz CE, Gjorstrup P, Bazan HE (2010) Resolvin E1 improves tear production and decreases inflammation in a dry eye mouse model. *J Ocul Pharmacol Ther* **26**, 431-439.
[169] Maddox JF, Hachicha M, Takano T, Petasis NA, Fokin VV, Serhan CN (1997) Lipoxin A4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein-linked lipoxin A4 receptor. *J Biol Chem* **272**, 6972-6978.
[170] Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, Petasis NA, Serhan CN (2010) Resolvin D1 binds human phagocytes with evidence for proresolving receptors. *Proc Natl Acad Sci U S A* **107**, 1660-1665.
[171] Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN (2005) Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J Exp Med* **201**, 713-722.
[172] Le Y, Gong W, Tiffany HL, Tumanov A, Nedospasov S, Shen W, Dunlop NM, Gao JL, Murphy PM, Oppenheim JJ, Wang JM (2001) $A\beta_{42}$ activates a G-protein-coupled chemoattractant receptor, FPR-like-1. *J Neurosci* **21**, RC123.
[173] Bondue B, Wittamer V, Parmentier M (2011) Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism. *Cytokine Growth Factor Rev* **22**, 331-338.
[174] Fulop P, Seres I, Lorincz H, Harangi M, Somodi S, Paragh G (2014) Association of chemerin with oxidative stress, inflammation and classical adipokines in non-diabetic obese patients. *J Cell Mol Med* **18**, 1313-1320.
[175] Zhao Y, Calon F, Julien C, Winkler JW, Petasis NA, Lukiw WJ, Bazan NG (2011) Docosahexaenoic acid-derived neuroprotectin D1 induces neuronal survival via secretase- and PPARγ-mediated mechanisms in Alzheimer's disease models. *PLoS ONE* **6**, e15816. PMID: 21246057.
[176] Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, Olefsky JM (2010) GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* **142**, 687-698.

[177] Radmark O, Werz O, Steinhilber D, Samuelsson B (2015) 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease. *Biochim Biophys Acta* **1851**, 331-339.
[178] Zhang L, Zhang WP, Hu H, Wang ML, Sheng WW, Yao HT, Ding W, Chen Z, Wei EQ (2006) Expression patterns of 5-lipoxygenase in human brain with traumatic injury and astrocytoma. *Neuropathology* **26**, 99-106.
[179] Firuzi O, Zhuo J, Chinnici CM, Wisniewski T, Pratico D (2008) 5-Lipoxygenase gene disruption reduces Aβ pathology in a mouse model of Alzheimer's disease. *FASEB J* **22**, 1169-1178.
[180] Ferretti MT, Allard S, Partridge V, Ducatenzeiler A, Cuello AC (2012) Minocycline corrects early, pre-plaque neuroinflammation and inhibits BACE-1 in a transgenic model of Alzheimer's disease-like amyloid pathology. *J Neuroinflammation* **9**, 62.
[181] Radmark O, Werz O, Steinhilber D, Samuelsson B (2007) 5-Lipoxygenase: regulation of expression and enzyme activity. *Trends Biochem Sci* **32**, 332-341.
[182] Ye Y, Lin Y, Perez-Polo JR, Uretsky BF, Ye Z, Tieu BC, Birnbaum Y (2008) Phosphorylation of 5-lipoxygenase at ser523 by protein kinase A determines whether pioglitazone and atorvastatin induce proinflammatory leukotriene B4 or anti-inflammatory 15-epi-lipoxin A4 production. *J Immunol* **181**, 3515-3523.
[183] Luo M, Jones SM, Phare SM, Coffey MJ, Peters-Golden M, Brock TG (2004) Protein kinase A inhibits leukotriene synthesis by phosphorylation of 5-lipoxygenase on serine 523. *J Biol Chem* **279**, 41512-41520.
[184] Yao Y, Clark CM, Trojanowski JQ, Lee VM, Pratico D (2005) Elevation of 12/15 lipoxygenase products in AD and mild cognitive impairment. *Ann Neurol* **58**, 623-626.
[185] Berger M, Schwarz K, Thiele H, Reimann I, Huth A, Borngraber S, Kuhn H, Thiele BJ (1998) Simultaneous expression of leukocyte-type 12-lipoxygenase and reticulocyte-type 15-lipoxygenase in rabbits. *J Mol Biol* **278**, 935-948.
[186] Joshi YB, Giannopoulos PF, Pratico D (2015) The 12/15-lipoxygenase as an emerging therapeutic target for Alzheimer's disease. *Trends Pharmacol Sci* **36**, 181-186.
[187] Pratico D, Zhukareva V, Yao Y, Uryu K, Funk CD, Lawson JA, Trojanowski JQ, Lee VM (2004) 12/15-lipoxygenase is increased in Alzheimer's disease: possible involvement in brain oxidative stress. *Am J Pathol* **164**, 1655-1662.
[188] Bhatia B, Maldonado CJ, Tang S, Chandra D, Klein RD, Chopra D, Shappell SB, Yang P, Newman RA, Tang DG (2003) Subcellular localization and tumor-suppressive functions of 15-lipoxygenase 2 (15-LOX2) and its splice variants. *J Biol Chem* **278**, 25091-25100.
[189] Moussalli MJ, Wu Y, Zuo X, Yang XL, Wistuba, II, Raso MG, Morris JS, Bowser JL, Minna JD, Lotan R, Shureiqi I (2011) Mechanistic contribution of ubiquitous 15-lipoxygenase-1 expression loss in cancer cells to terminal cell differentiation evasion. *Cancer Prev Res (Phila)* **4**, 1961-1972.
[190] Wu Y, Fang B, Yang XQ, Wang L, Chen D, Krasnykh V, Carter BZ, Morris JS, Shureiqi I (2008) Therapeutic molecular targeting of 15-lipoxygenase-1 in colon cancer. *Mol Ther* **16**, 886-892.
[191] Zhu H, Glasgow W, George MD, Chrysovergis K, Olden K, Roberts JD, Eling T (2008) 15-lipoxygenase-1 activates tumor suppressor p53 independent of enzymatic activity. *Int J Cancer* **123**, 2741-2749.
[192] Hsi LC, Wilson LC, Eling TE (2002) Opposing effects of 15-lipoxygenase-1 and -2 metabolites on MAPK signaling in prostate. Alteration in peroxisome proliferator-activated receptor gamma. *J Biol Chem* **277**, 40549-40556.
[193] Ivanov I, Heydeck D, Hofheinz K, Roffeis J, O'Donnell VB, Kuhn H, Walther M (2010) Molecular enzymology of lipoxygenases. *Arch Biochem Biophys* **503**, 161-174.
[194] Chen B, Tsui S, Boeglin WE, Douglas RS, Brash AR, Smith TJ (2006) Interleukin-4 induces 15-lipoxygenase-1 expression in human orbital fibroblasts from patients with Graves disease. Evidence for anatomic site-selective actions of Th2 cytokines. *J Biol Chem* **281**, 18296-18306.
[195] Succol F, Pratico D (2007) A role for 12/15 lipoxygenase in the amyloid-β precursor protein metabolism. *J Neurochem* **103**, 380-387.
[196] Yang H, Zhuo JM, Chu J, Chinnici C, Pratico D (2010) Amelioration of the Alzheimer's disease phenotype by absence of 12/15-lipoxygenase. *Biol Psychiatry* **68**, 922-929.
[197] Hoozemans JJ, Rozemuller AJ, Janssen I, De Groot CJ, Veerhuis R, Eikelenboom P (2001) Cyclooxygenase expression in microglia and neurons in Alzheimer's disease and control brain. *Acta Neuropathol* **101**, 2-8.
[198] Yermakova AV, O'Banion MK (2001) Downregulation of neuronal cyclooxygenase-2 expression in end stage Alzheimer's disease. *Neurobiol Aging* **22**, 823-836.
[199] Hoozemans JJ, Rozemuller JM, van Haastert ES, Veerhuis R, Eikelenboom P (2008) Cyclooxygenase-1 and -2 in the different stages of Alzheimer's disease pathology. *Curr Pharm Des* **14**, 1419-1427.
[200] Botting RM (2006) Inhibitors of cyclooxygenases: mechanisms, selectivity and uses. *J Physiol Pharmacol* **57 Suppl 5**, 113-124.
[201] Claria J, Serhan CN (1995) Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc Natl Acad Sci U S A* **92**, 9475-9479.

[202] Serhan CN (2005) Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. *Prostaglandins Leukot Essent Fatty Acids* **73**, 141-162.
[203] Schliebs R, Arendt T (2011) The cholinergic system in aging and neuronal degeneration. *Behav Brain Res* **221**, 555-563.
[204] Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**, 1237-1239.
[205] Bowen DM, Smith CB, White P, Davison AN (1976) Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain* **99**, 459-496.
[206] Davies P, Maloney AJ (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**, 1403.
[207] Oz M, Lorke DE, Yang KH, Petroianu G (2013) On the interaction of β-amyloid peptides and α7-nicotinic acetylcholine receptors in Alzheimer's disease. *Curr Alzheimer Res* **10**, 618-630.
[208] Perumal D, Pillai S, Nguyen J, Schaal C, Coppola D, Chellappan SP (2014) Nicotinic acetylcholine receptors induce c-Kit ligand/Stem Cell Factor and promote stemness in an ARRB1/ β-arrestin-1 dependent manner in NSCLC. *Oncotarget* **5**, 10486-10502.
[209] Shytle RD, Mori T, Townsend K, Vendrame M, Sun N, Zeng J, Ehrhart J, Silver AA, Sanberg PR, Tan J (2004) Cholinergic modulation of microglial activation by α7 nicotinic receptors. *J Neurochem* **89**, 337-343.
[210] Yang J, Shi SQ, Shi L, Fang D, Liu H, Garfield RE (2014) Nicotine, an α7 nAChR agonist, reduces lipopolysaccharide-induced inflammatory responses and protects fetuses in pregnant rats. *Am J Obstet Gynecol* **211**, 538 e531-537.
[211] Moon JH, Kim SY, Lee HG, Kim SU, Lee YB (2008) Activation of nicotinic acetylcholine receptor prevents the production of reactive oxygen species in fibrillar $A\beta_{42}$-stimulated microglia. *Exp Mol Med* **40**, 11-18.
[212] Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN (2001) Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* **2**, 612-619.
[213] Janabi N, Peudenier S, Heron B, Ng KH, Tardieu M (1995) Establishment of human microglial cell lines after transfection of primary cultures of embryonic microglial cells with the SV40 large T antigen. *Neurosci Lett* **195**, 105-108.
[214] Biedler JL, Helson L, Spengler BA (1973) Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. *Cancer Res* **33**, 2643-2652.
[215] Xie HR, Hu LS, Li GY (2010) SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. *Chin Med J (Engl)* **123**, 1086-1092.
[216] Agholme L, Lindstrom T, Kagedal K, Marcusson J, Hallbeck M (2010) An in vitro model for neuroscience: differentiation of SH-SY5Y cells into cells with morphological and biochemical characteristics of mature neurons. *J Alzheimers Dis* **20**, 1069-1082.
[217] Norden B, Broberg P, Lindberg C, Plymoth A (2005) Analysis and understanding of high-dimensionality data by means of multivariate data analysis. *Chem Biodivers* **2**, 1487-1494.
[218] Xia W, Yang T, Shankar G, Smith IM, Shen Y, Walsh DM, Selkoe DJ (2009) A specific enzyme-linked immunosorbent assay for measuring β-amyloid protein oligomers in human plasma and brain tissue of patients with Alzheimer disease. *Arch Neurol* **66**, 190-199.
[219] Steinerman JR, Irizarry M, Scarmeas N, Raju S, Brandt J, Albert M, Blacker D, Hyman B, Stern Y (2008) Distinct pools of β-amyloid in Alzheimer disease-affected brain: a clinicopathologic study. *Arch Neurol* **65**, 906-912.
[220] Morris JC (2005) Early-stage and preclinical Alzheimer disease. *Alzheimer Dis Assoc Disord* **19**, 163-165.
[221] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr., Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 280-292.
[222] Mansson C, Arosio P, Hussein R, Kampinga HH, Hashem RM, Boelens WC, Dobson CM, Knowles TP, Linse S, Emanuelsson C (2014) Interaction of the molecular chaperone DNAJB6 with growing $A\beta_{42}$ aggregates leads to sub-stoichiometric inhibition of amyloid formation. *J Biol Chem* **289**, 31066-31076.
[223] Lindberg C, Selenica ML, Westlind-Danielsson A, Schultzberg M (2005) β-amyloid protein structure determines the nature of cytokine release from rat microglia. *J Mol Neurosci* **27**, 1-12.
[224] Dalli J, Zhu M, Vlasenko NA, Deng B, Haeggstrom JZ, Petasis NA, Serhan CN (2013) The novel 13S,14S-epoxy-maresin is converted by human macrophages to maresin 1 (MaR1), inhibits leukotriene A4 hydrolase (LTA4H), and shifts macrophage phenotype. *FASEB J* **27**, 2573-2583.
[225] Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M (2009) Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* **206**, 15-23.

[226] Mizwicki MT, Liu G, Fiala M, Magpantay L, Sayre J, Siani A, Mahanian M, Weitzman R, Hayden EY, Rosenthal MJ, Nemere I, Ringman J, Teplow DB (2013) 1α,25-dihydroxyvitamin D3 and resolvin D1 retune the balance between amyloid-β phagocytosis and inflammation in Alzheimer's disease patients. *J Alzheimers Dis* **34**, 155-170.
[227] Ariel A, Serhan CN (2012) New lives given by cell death: macrophage differentiation following their encounter with apoptotic leukocytes during the resolution of inflammation. *Front Immunol* **3**, 4.
[228] El Kebir D, Gjorstrup P, Filep JG (2012) Resolvin E1 promotes phagocytosis-induced neutrophil apoptosis and accelerates resolution of pulmonary inflammation. *Proc Natl Acad Sci U S A* **109**, 14983-14988.
[229] Noda M, Suzumura A (2012) Sweepers in the CNS: Microglial Migration and Phagocytosis in the Alzheimer Disease Pathogenesis. *Int J Alzheimers Dis* **2012**, 891087.
[230] Koenigsknecht-Talboo J, Landreth GE (2005) Microglial phagocytosis induced by fibrillar β-amyloid and IgGs are differentially regulated by proinflammatory cytokines. *J Neurosci* **25**, 8240-8249.
[231] Obulesu M, Jhansilakshmi M (2014) Neuroinflammation in Alzheimer's disease: an understanding of physiology and pathology. *Int J Neurosci* **124**, 227-235.
[232] Latta CH, Brothers HM, Wilcock DM (2014) Neuroinflammation in Alzheimer's disease; A source of heterogeneity and target for personalized therapy. *Neuroscience*.
[233] Hjorth E, Frenkel D, Weiner H, Schultzberg M (2010) Effects of immunomodulatory substances on phagocytosis of $A\beta_{42}$ by human microglia. *Int J Alzheimers Dis* **2010**.
[234] Majumdar A, Cruz D, Asamoah N, Buxbaum A, Sohar I, Lobel P, Maxfield FR (2007) Activation of microglia acidifies lysosomes and leads to degradation of Alzheimer amyloid fibrils. *Mol Biol Cell* **18**, 1490-1496.
[235] Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M (2010) Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov* **9**, 971-988.
[236] Fuentes-Duculan J, Suarez-Farinas M, Zaba LC, Nograles KE, Pierson KC, Mitsui H, Pensabene CA, Kzhyshkowska J, Krueger JG, Lowes MA (2010) A subpopulation of CD163-positive macrophages is classically activated in psoriasis. *J Invest Dermatol* **130**, 2412-2422.
[237] Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G (2005) Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* **11**, 90-94.
[238] Heneka MT, Reyes-Irisarri E, Hull M, Kummer MP (2011) Impact and Therapeutic Potential of PPARs in Alzheimer's Disease. *Curr Neuropharmacol* **9**, 643-650.
[239] Liao Z, Dong J, Wu W, Yang T, Wang T, Guo L, Chen L, Xu D, Wen F (2012) Resolvin D1 attenuates inflammation in lipopolysaccharide-induced acute lung injury through a process involving the PPARγ/NF-κB pathway. *Respir Res* **13**, 110.
[240] Hajjar T, Meng GY, Rajion MA, Vidyadaran S, Othman F, Farjam AS, Li TA, Ebrahimi M (2012) Omega 3 polyunsaturated fatty acid improves spatial learning and hippocampal peroxisome proliferator activated receptors (PPARα and PPARγ) gene expression in rats. *BMC Neurosci* **13**, 109.
[241] Kummer MP, Heneka MT (2008) PPARs in Alzheimer's Disease. *PPAR Res* **2008**, 403896.
[242] Filiou MD, Arefin AS, Moscato P, Graeber MB (2014) 'Neuroinflammation' differs categorically from inflammation: transcriptomes of Alzheimer's disease, Parkinson's disease, schizophrenia and inflammatory diseases compared. *Neurogenetics* **15**, 201-212.
[243] Perry VH, Matyszak MK, Fearn S (1993) Altered antigen expression of microglia in the aged rodent CNS. *Glia* **7**, 60-67.
[244] Sheffield LG, Berman NE (1998) Microglial expression of MHC class II increases in normal aging of nonhuman primates. *Neurobiol Aging* **19**, 47-55.
[245] Wei J, Xu H, Davies JL, Hemmings GP (1992) Increase of plasma IL-6 concentration with age in healthy subjects. *Life Sci* **51**, 1953-1956.
[246] de Gonzalo-Calvo D, Neitzert K, Fernandez M, Vega-Naredo I, Caballero B, Garcia-Macia M, Suarez FM, Rodriguez-Colunga MJ, Solano JJ, Coto-Montes A (2010) Differential inflammatory responses in aging and disease: TNF-α and IL-6 as possible biomarkers. *Free Radic Biol Med* **49**, 733-737.
[247] Wang X, Puerta E, Cedazo-Minguez A, Hjorth E, Schultzberg M (2015) Insufficient resolution response in the hippocampus of a senescence-accelerated mouse model--SAMP8. *J Mol Neurosci* **55**, 396-405.
[248] Solito E, Sastre M (2012) Microglia function in Alzheimer's disease. *Front Pharmacol* **3**, 14.
[249] Heneka MT, Sastre M, Dumitrescu-Ozimek L, Dewachter I, Walter J, Klockgether T, Van Leuven F (2005) Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP[V717I] transgenic mice. *J Neuroinflammation* **2**, 22.
[250] Eikelenboom P, van Exel E, Hoozemans JJ, Veerhuis R, Rozemuller AJ, van Gool WA (2010) Neuroinflammation - an early event in both the history and pathogenesis of Alzheimer's disease. *Neurodegener Dis* **7**, 38-41.
[251] Beard CM, Kokman E, Kurland LT (1991) Rheumatoid arthritis and susceptibility to Alzheimer's disease. *Lancet* **337**, 1426.

[252] Cunnane SC, Plourde M, Pifferi F, Begin M, Feart C, Barberger-Gateau P (2009) Fish, docosahexaenoic acid and Alzheimer's disease. *Prog Lipid Res* **48**, 239-256.
[253] Imbimbo BP, Solfrizzi V, Panza F (2010) Are NSAIDs useful to treat Alzheimer's disease or mild cognitive impairment? *Front Aging Neurosci* **2**.
[254] Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunnell D, Hayward RC, Heatherley SV, Christian LM, McNaughton SA, Ness AR (2008) No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. *Br J Nutr* **99**, 421-431.
[255] Chiu CC, Su KP, Cheng TC, Liu HC, Chang CJ, Dewey ME, Stewart R, Huang SY (2008) The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. *Prog Neuropsychopharmacol Biol Psychiatry* **32**, 1538-1544.
[256] Freund-Levi Y, Eriksdotter-Jönhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A, Vedin I, Vessby B, Wahlund LO, Palmblad J (2006) Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch Neurol* **63**, 1402-1408.
[257] Daiello LA, Gongvatana A, Dunsiger S, Cohen RA, Ott BR, Alzheimer's Disease Neuroimaging I (2015) Association of fish oil supplement use with preservation of brain volume and cognitive function. *Alzheimers Dement* **11**, 226-235.
[258] Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM (1997) Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol* **42**, 776-782.
[259] Engelhart MJ, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC, Breteler MM (2002) Diet and risk of dementia: Does fat matter?: The Rotterdam Study. *Neurology* **59**, 1915-1921.
[260] Barberger-Gateau P, Letenneur L, Deschamps V, Peres K, Dartigues JF, Renaud S (2002) Fish, meat, and risk of dementia: cohort study. *BMJ* **325**, 932-933.
[261] Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A, Vedin I, Vessby B, Wahlund LO, Palmblad J (2006) Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch Neurol* **63**, 1402-1408.
[262] Bright JM, Sullivan PS, Melton SL, Schneider JF, McDonald TP (1994) The effects of n-3 fatty acid supplementation on bleeding time, plasma fatty acid composition, and in vitro platelet aggregation in cats. *J Vet Intern Med* **8**, 247-252.
[263] Kelley DS, Taylor PC, Nelson GJ, Mackey BE (1998) Dietary docosahexaenoic acid and immunocompetence in young healthy men. *Lipids* **33**, 559-566.
[264] Cooper AL, Gibbons L, Horan MA, Little RA, Rothwell NJ (1993) Effect of dietary fish oil supplementation on fever and cytokine production in human volunteers. *Clin Nutr* **12**, 321-328.
[265] Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J, 3rd, Spur BW, Robinson DR, Corey EJ, Lewis RA, Austen KF (1985) Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* **312**, 1217-1224.
[266] Traversa G, Walker AM, Ippolito FM, Caffari B, Capurso L, Dezi A, Koch M, Maggini M, Alegiani SS, Raschetti R (1995) Gastroduodenal toxicity of different nonsteroidal antiinflammatory drugs. *Epidemiology* **6**, 49-54.
[267] Balfour JA, Plosker GL (1999) Rosiglitazone. *Drugs* **57**, 921-930; discussion 931-922.
[268] Montaner LJ, da Silva RP, Sun J, Sutterwala S, Hollinshead M, Vaux D, Gordon S (1999) Type 1 and type 2 cytokine regulation of macrophage endocytosis: differential activation by IL-4/IL-13 as opposed to IFN-γ or IL-10. *J Immunol* **162**, 4606-4613.
[269] Huang TL, Zandi PP, Tucker KL, Fitzpatrick AL, Kuller LH, Fried LP, Burke GL, Carlson MC (2005) Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. *Neurology* **65**, 1409-1414.
[270] Whalley LJ, Deary IJ, Starr JM, Wahle KW, Rance KA, Bourne VJ, Fox HC (2008) n-3 Fatty acid erythrocyte membrane content, APOE varepsilon4, and cognitive variation: an observational follow-up study in late adulthood. *Am J Clin Nutr* **87**, 449-454.
[271] Martin BK, Meinert CL, Breitner JC, Group AR (2002) Double placebo design in a prevention trial for Alzheimer's disease. *Control Clin Trials* **23**, 93-99.
[272] Beyer I, Njemini R, Bautmans I, Demanet C, Mets T (2012) Immunomodulatory effect of NSAID in geriatric patients with acute infection: effects of piroxicam on chemokine/cytokine secretion patterns and levels of heat shock proteins. A double-blind randomized controlled trial. (ISRCTN58517443). *Cell Stress Chaperones* **17**, 255-265.