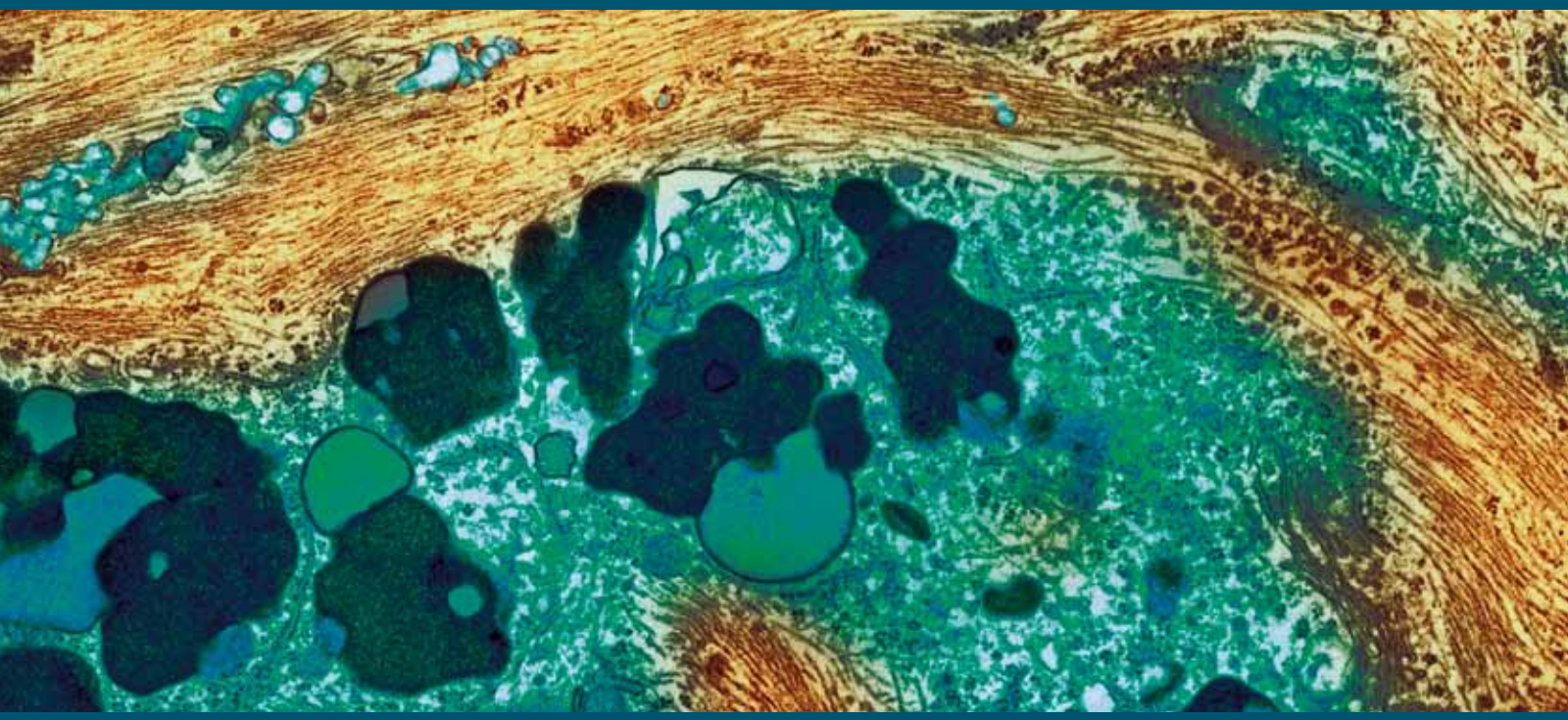
International Journal of Alzheimer's Disease

Microglia in Alzheimer's Disease

Guest Editors: Lee-Way Jin, Colin Combs, Joseph El Khoury, and Akio Suzumura



Microglia in Alzheimer's Disease

International Journal of Alzheimer's Disease

Microglia in Alzheimer's Disease

Guest Editors: Lee-Way Jin, Colin Combs, Joseph El Khoury, and Akio Suzumura

Editorial Board

Contents

Microglia in Alzheimer's Disease, Lee-Way Jin
Volume 2012, Article ID 598371, 2 pages

Microglia, Alzheimer's Disease, and Complement, Helen Crehan, John Hardy, and Jennifer Pocock
Volume 2012, Article ID 983640, 10 pages

A Changing Perspective on the Role of Neuroinflammation in Alzheimer's Disease, Donna M. Wilcock
Volume 2012, Article ID 495243, 7 pages

Is There Inflammatory Synergy in Type II Diabetes Mellitus and Alzheimer's Disease?, Lih-Fen Lue, Cassandra Andrade, Marwan Sabbagh, and Douglas Walker
Volume 2012, Article ID 918680, 9 pages

Microglia in Alzheimer's Disease: It's All About Context, Tara M. Weitz and Terrence Town
Volume 2012, Article ID 314185, 11 pages

Microglial KCa3.1 Channels as a Potential Therapeutic Target for Alzheimer's Disease, Izumi Maezawa, David Paul Jenkins, Benjamin E. Jin, and Heike Wulff
Volume 2012, Article ID 868972, 8 pages

Microglial Scavenger Receptors and Their Roles in the Pathogenesis of Alzheimer's Disease, Kim Wilkinson and Joseph El Khoury
Volume 2012, Article ID 489456, 10 pages

Traumatic Brain Injury, Microglia, and Beta Amyloid, Rebekah C. Mannix and Michael J. Whalen
Volume 2012, Article ID 608732, 5 pages

Sweepers in the CNS: Microglial Migration and Phagocytosis in the Alzheimer Disease Pathogenesis, Mariko Noda and Akio Suzumura
Volume 2012, Article ID 891087, 11 pages

Microglia in Alzheimer Brain: A Neuropathological Perspective, Robert E. Mrak
Volume 2012, Article ID 165021, 6 pages

The Biphasic Role of Microglia in Alzheimer's Disease, Tetsuya Mizuno
Volume 2012, Article ID 737846, 9 pages

Microglial Amyloid-β1-40 Phagocytosis Dysfunction Is Caused by High-Mobility Group Box Protein-1: Implications for the Pathological Progression of Alzheimer's Disease, Kazuyuki Takata, Tetsuya Takada, Aina Ito, Mayo Asai, Manami Tawa, Yuki Saito, Eishi Ashihara, Hidekazu Tomimoto, Yoshihisa Kitamura, and Shun Shimohama
Volume 2012, Article ID 685739, 11 pages

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 598371, 2 pages
doi:10.1155/2012/598371

Editorial
Microglia in Alzheimer's Disease

Lee-Way Jin

Department of Pathology and Laboratory Medicine, Alzheimer's Disease Center and UC Davis MIND Institute, Davis, 2805 50th Street, Sacramento, CA 95618, USA

Correspondence should be addressed to Lee-Way Jin, lee-way.jin@ucdmc.ucdavis.edu

Received 20 November 2012; Accepted 20 November 2012

This special issue describes exciting papers contributed to the International Journal of Alzheimer's Disease issue on microglia in Alzheimer's disease (AD). It has been well recognized that neuroinflammation is an early event in the pathogenesis of AD, in which microglia is a major player. A core theme of the articles presented in this special issue is the dichotomous role played by the microglia—the context-dependent neuroprotective and neurotoxic functions. Recent insights regarding molecular mechanisms that regulate the diverse microglia responses are summarized. The understanding of these molecular pathways would lead to targeted therapies aimed at modulating microlgial behavior toward neuroprotection, as exemplified by approaches targeting the microglial KCa3.1 potassium channel, scavenger receptors, or complements, for example. Microglia also play critical roles in comorbidities of AD, such as diabetes and traumatic brain injury.

The first paper I would suggest the readers to read is R. E. Mrak's neuropathological survey of microglia in Alzheimer brain. Thanks to the pioneer observations by neuropathologists about two decades ago, microglia emerged into the scene of AD research. Although our view of microglia in AD has since substantially changed, mostly based on studies using rodents as models, human neuropathology still provides a "golden standard" or reality check for what is relevant and important to AD. Interesting sets of data presented by R. E. Mrak suggest that microglia actively influence the pattern of development of amyloid plaques as well as neuronal tangle formation in AD brains, rather than simple associations with these lesions. In addition, plaques of different stages, perhaps reflecting different pathological stages of AD, are associated with activated microglia with different morphology or immunohistochemical phenotypes. The readers can consult the article by D. M. Wilcock for a synopsis of various states of microglia that may serve different functions. In principle, microglia possess all the response repertoire of peripheral macrophages; therefore the established classification system for the inflammatory state of macrophages, based on defined sets of molecular markers, can be applied to microglia. D. M. Wilcock further provides a summary about how the microglia inflammatory state can be altered by specific sets of stimuli, how different states influence amyloid load, and how passive immunization with anti-Aβ antibodies alters the microglia inflammatory state prior to significant reductions in amyloid deposition. Again, this line of evidence supports an active role of microglia in the development and dissolution of amyloid plaques. However, how the microglia inflammatory phenotypes related exactly to the pathologies of AD appears to be complex and remains to be explored.

In this issue, M. Noda and A. Suzumura provide a comprehensive review of the molecular players in the quintessential functions of microglia—chemotaxis and phagocytosis. They point out that the lack of microglial phagocytosis worsens the pathology of AD and induces memory impairment. In addition to the well-known players such as phosphatidylserine receptors, scavenger receptors, and complement factors, M. Noda and A. Suzumnura also discuss a less publicized but highly interesting phagocytosis modulator expressed in microglia—γ-secretase, which in neurons is certainly the center of attention for its role in generating Aβ. One novel phagocytosis modulator relevant to AD reported in this special issue is the high mobility group box protein 1 (HMGB1). HMGB1 is a nonhistone chromosomal protein that is released from cells undergoing necrosis and acts as an inflammatory mediator. K. Takata et al. report evidence

to support that microglial Aβ phagocytosis dysfunction may be caused by HMGB1 that accumulates extracellularly on Aβ plaques.

When it comes to the effect of microglial phenotypes on neurons, microglia serve a biphasic role in AD—they are either neurotoxic or neuroprotective. In this issue, T. Mizuno reviews the molecules that trigger and execute the neurotoxic actions of microglia in AD, as well as those able to induce microglia neuroprotective properties. However, the interwoven molecular pathways and the dichotomous behavioral pattern of microglia make it difficult to provide a clear answer regarding if a microglia action is beneficial or harmful. A synthesis of the complex clinical and experimental literature encompassing the debate of "good" and "bad" microglia (and, of course, without excluding the possibility of microglia playing no role in AD) is provided by T. M. Weitz and T. Town. They conclude that an understanding seems possible when considering context—the conditions under which microglia encounter AD-like pathological lesions. Specifically, a model emerges where microglia mount different types of activated responses depending on whether they encounter particular species of misfolded protein (Aβ or tau) and whether this innate recognition occurs early on or after pathology is well established, an insight that echoes the neuropathological observations reviewed by R. E. Mrak.

A group of well-studied and highly significant molecules in microglia is scavenger receptors (SRs), reviewed by K. Wilkinson and J. El Khoury. Although the roles of several members of SRs in AD are diverse and complex, convincing evidence shows that SCARA-1-mediated microglia interactions with Aβ are beneficial and promote phagocytosis and clearance of Aβ, whereas CD36-Aβ interactions are harmful and together with TLR-4 and TLR-6 lead to the production of neurotoxins and proinflammatory molecules. The other group of molecules reviewed in this issue is complements. H. Crehan et al. review evidence indicating that the ability of microglia to adopt the "good" or "bad" role in AD is influenced by complement factors. Furthermore, microglia appear to be a major player in the dysregulated complement cascade in AD, in which microglia are aberrantly activated by disruptive complement signaling and Aβ to enhance their secretion of cytokines, which can lead to further complement cascades.

Along this line of research on "good" and "bad" microglia, I. Maezawa et al. actually propose a molecular target that is ready for manipulation by pharmacologically well-characterized small molecules. They provide evidence that targeting the microglia potassium channel KCa3.1 could harness calcium signaling resulting from microglia activation, thus "fine tune" the microglia response toward neuroprotection—phagocytosis of Aβ with reduced release of harmful inflammatory mediators and oxidative species. Because the pharmacology of KCa3.1 has been well studied and highly selective inhibitors are available and safe, targeting KCa3.1 may hold promise as a specific anti-inflammatory therapy for AD.

Also included in this special issue are two articles discussing the microglia link in two significant comorbidities in AD: traumatic brain injury (TBI) and type II diabetes mellitus (T2DM). R. C. Mannix and M. J. Whalen point out that Aβ, which is elevated acutely after TBI, may be a key mediator of microglial activation in TBI. TBI-induced Aβ alters long-term microglial function and Aβ-clearance and could therefore contribute to the development of AD. In this regard, the advancement of drug discoveries in the field of TBI, such as sex steroids or Apoe mimetics which alter both microglial function and Aβ metabolism, may have potentially important roles in TBI as well as AD. Microglia-mediated neuroinflammation appears also to provide a shared mechanism between AD and T2DM. L. F. Lue et al. review evidence supporting that T2DM could accentuate microglial activation, neuroinflammation, and vascular inflammatory/oxidative injury in AD brains through mechanisms mediated by RAGE and other pattern recognition receptors and the cascade of cytokine and chemokines. Because a wide range of inflammatory mediators and receptors are involved in these two diseases, it is important to characterize the patterns of microglial activation in AD patients with T2DM and AD patients without T2DM for future anti-inflammatory approaches. One interesting difference pointed out by L. F. Lue et al. is that complement activation is a prominent feature in AD, but not in T2DM.

In summary, common themes emerge in the articles presented in this special issue. Microglia response is highly complex in AD and is context dependent. Aging, the most significant risk factor for AD, and other risk factors such as TBI and T2DM may contribute to the development of AD via altered microglia-mediated neuroinflammation (microglia dysfunction). The task ahead is to be able to manipulate microglia responses to therapeutic advantage. Further understanding of the triggers and molecular pathways of various microglia responses will help us to design microglia-targeted therapies to enhance the neuroprotective or the "good" behavior and at the same time dampen the neurotoxic or the "bad" behavior of microglia.

Lee-Way Jin

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 983640, 10 pages
doi:10.1155/2012/983640

Review Article

Microglia, Alzheimer's Disease, and Complement

Helen Crehan,[1,2] John Hardy,[1] and Jennifer Pocock[2]

[1] *Department of Molecular Neurosciences, University College London Institute of Neurology, 1 Wakefield Street, London WC1N 1PJ, UK*
[2] *Department of Neuroinflammation, University College London Institute of Neurology, 1 Wakefield Street, London WC1N 1PJ, UK*

Correspondence should be addressed to Jennifer Pocock, j.pocock@ucl.ac.uk

Received 6 February 2012; Revised 1 May 2012; Accepted 7 May 2012

Academic Editor: Colin Combs

Microglia, the immune cell of the brain, are implicated in cascades leading to neuronal loss and cognitive decline in Alzheimer's disease (AD). Recent genome-wide association studies have indicated a number of risk factors for the development of late-onset AD. Two of these risk factors are an altered immune response and polymorphisms in complement receptor 1. In view of these findings, we discuss how complement signalling in the AD brain and microglial responses in AD intersect. Dysregulation of the complement cascade, either by changes in receptor expression, enhanced activation of different complement pathways or imbalances between complement factor production and complement cascade inhibitors may all contribute to the involvement of complement in AD. Altered complement signalling may reduce the ability of microglia to phagocytose apoptotic cells and clear amyloid beta peptides, modulate the expression by microglia of complement components and receptors, promote complement factor production by plaque-associated cytokines derived from activated microglia and astrocytes, and disrupt complement inhibitor production. The evidence presented here indicates that microglia in AD are influenced by complement factors to adopt protective or harmful phenotypes and the challenge ahead lies in understanding how this can be manipulated to therapeutic advantage to treat late onset AD.

1. Introduction

The complement system is composed of a series of soluble and membrane-associated proteins present in the blood, which play a role in host defence through the identification, opsonisation, and lysis of pathogenic targets [1–3]. Activation of complement leads to an enzymatic cascade whereby one protein promotes the sequential binding of the following protein [4]. There are three pathways through which complement activation can occur, namely, *classical, lectin,* and *alternative*. Although these pathways depend on different binding molecules for their initiation, they all ultimately lead to the production of complement 3 (C3) convertase which is responsible for the actions of complement [5] (Figure 1). The initiation of the classical pathway involves the binding of C1q, the first protein in the complement cascade, to an antigen-bound antibody complex (IgG or IgM) to either the pathogen surface or to the C-reactive protein bound to the pathogen leading to the generation of the protease C3 convertase through C4 and C2 cleavage [3]. Lectin pathway activation involves carbohydrate binding proteins such as mannose-binding lectin (MBL) or Ficolin binding to carbohydrate elements present on the surface of pathogens, further leading to the production of C3 convertase [6]. The third pathway, the alternative pathway, is different in that there is a constant low level of activation due to the spontaneous hydrolysis of C3 to C3(H_2O), and this forms C3 convertase through the cleavage of Factor B by Factor D [7]. C3 convertase, the protease formed by all three complement pathways, further binds to the pathogen surface to cleave C3, generating C3b which serves as a ligand for complement receptor 1 (CR1) [8].

The membrane attack complex (MAC) is a macromolecular complex consisting of a number of complement components: C5b, C6, C7, C8, and several C9 molecules whose function is to allow the influx of calcium ions through its ring-like structure resulting in lysis of the target cell [9]. Most cells express protecting/complement regulatory protein/membrane inhibitor of reactive lysis (MIRL or CD59), and this provides protection against MAC as the glycophosphoinositol (GPI-) anchored membrane protein

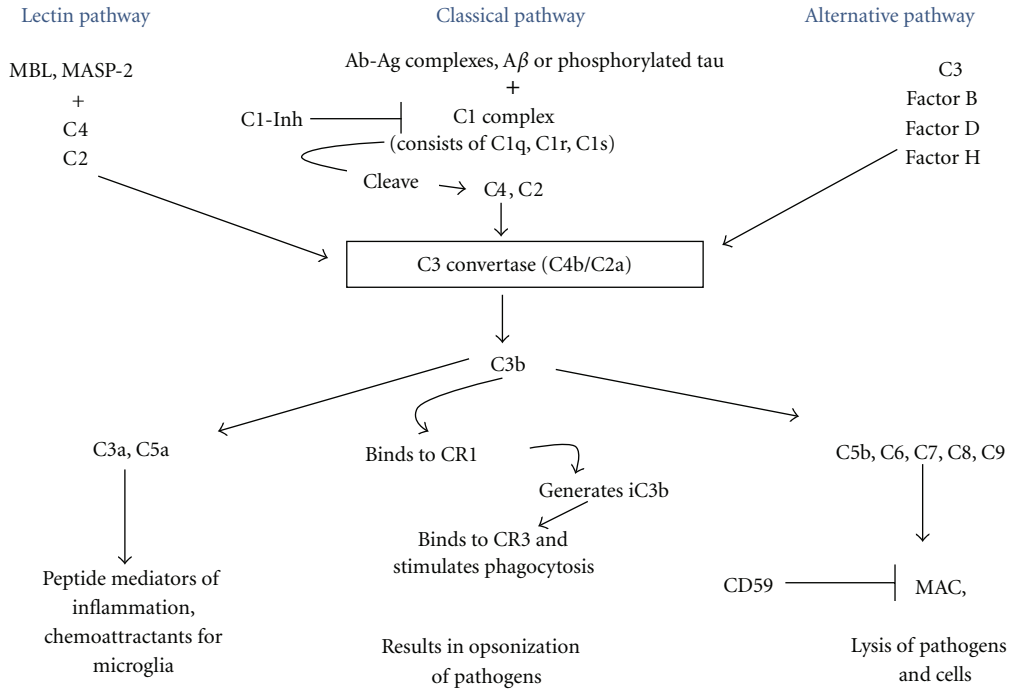


Figure 1: Pathways activating and inhibiting complement. The three complement activation pathways converge at the formation of the enzyme C3 convertase (or C4b/C2a), activation of which leads to the formation of C3b, the ligand of complement receptor 1 (CR1, also known as CD35). Activation of the complement pathway can ultimately lead to the release of inflammatory mediators, opsonisation of pathogens, and the membrane attack complex (MAC). The C1 complex of the classical complement pathway is comprised of C1q, C1r, and C1s. The endogenous complement C1 inhibitor/C1-esterase inhibitor (C1-Inh), which regulates the activation of the C1 complex, is decreased in AD. C5b, C6, C7, C8, and C9 form the MAC complex in the alternative complement activation pathway. CD59, an endogenous regulator of the MAC complex, is decreased in AD whilst C9 may be increased. Levels of Factor H, a regulatory glycoprotein of the alternative complement cascade, may also be perturbed in AD.

prevents the complete assembly and insertion of the complex into the membrane [10].

The role of complement in the elimination of pathogens by phagocytic recruitment and opsonisation occurs through binding with complement receptors [11, 12]. To date, the family of complement receptors consists of four known types: CR1 (CD35), CR2 (CD21), CR3 (CD11b/CD18), and CR4 (CD11c/CD18). However CR1 has been the best characterized of these since it was discovered almost 60 years ago when it was found that human erythrocytes bound to bacteria treated with complement and a specific antibody [13]. The single-chain glycoprotein that comprises CR1 functions as the receptor to C3b and C4b and as a regulator of complement activation [14]. It is positioned on chromosome 1, band q32, and is composed of a series of short consensus repeats [15]. It is expressed on a number of cells including erythrocytes, B cells, polymorphonuclear leukocytes, monocytes, follicular dendritic cells, and podocytes [16, 17]. Multiple forms of CR1 exist with varying molecular weights ranging from 190 to 260 kDa and the expression of CR1 is under the control of two codominant alleles which code for high (H) and low (L) receptor number, of which the L allele appears to be associated with AD (see review of Crehan et al. [18]). The main role of CR1 on erythrocytes is to remove complement-activating particles and immune complexes from the blood [19].

2. Complement Factors and Signalling in Alzheimer's Disease

Studies carried out on mono- and dizygotic twins demonstrate a significant genetic role in the susceptibility to late onset AD (as reviewed by Ertekin-Taner [20]), and until recently APOE was the only known genetic variant to influence the risk of AD development. Recent genomewide association studies (GWASs) have indicated common genetic variations in CLU, CR1, PICALM, ABCA7, BIN1, EPHA1, CD33, CD2AP, and the MSA4 gene cluster as additional risk factors for the development of late-onset AD [21–24]. The genetic variation at CR1 has been confirmed by a meta-analysis with a completely independent data set [25, 26]. CR1 loci demonstrated association with MRI characteristics of AD [27]. The association of the CR1 polymorphism, rs6656401, and cognitive function was measured in 1380 elderly people by Mini Mental State Examination (MMSE), and a cognitive composite score indicated an association between the CR1 polymorphism and poorer performance in the cognitive composite score in males [28]. Recent studies aimed at identifying AD biomarkers have correlated elevated CSF levels of complement factors C3 and C4 in AD patients (with dementia) compared with patients with mild cognitive impairment (MCI) which did not progress to AD [29]. Furthermore CSF levels of CR1 were elevated in patients

with MCI progressing to AD and in AD patients, supporting aberrant complement regulation in AD [29].

Classical markers of immune-mediated damage have been identified in AD brains including major histocompatibility complex class I and II positive microglia [30–32], glial cells expressing inflammatory cytokines [33], and the acute phase protein α1-antichymotrypsin [34]. Early studies identified complement proteins of the classical pathway, such as factor C1q, in AD brains [35], and subsequent studies established the presence of all of the native complement proteins as well as their activation products C4d, C3d, and MAC in AD brain [2]. However the lack of classical immune complexes led to the search for other complement activators. Rogers et al. [36] demonstrated that, in the absence of antibody, Aβ bound to and activated factor C1q, part of the classical complement cytolytic pathway, and, furthermore, that factors of this complement activation pathway were localised to areas of AD pathology. C1q was subsequently shown to be intimately associated with Aβ plaques [37, 38] as, indeed, have other complement factors such as C3c/d, C4c/d, and C5-9, [38, 39]. The search for antibody-independent activators of the complement pathway continued with the investigation of tau, the major protein component of neurofibrillary tangles. Shen et al. [40] demonstrated complement activation by neurofibrillary tangle material extracted from AD brains and furthermore by human recombinant tau. Whilst most research on complement activation in AD has focussed on the classical pathway, alternative pathway activation also occurs, since the presence of mRNA of the essential alternative pathway element, factor B, has been observed in the frontal cortex of the AD brain [41].

The role of C1q in AD has been experimentally addressed in studies using animal models deficient in the protein. One such study involved the crossing of C1q-deficient mice with a Tg2576 mouse model which exhibits an age-dependent increase in Aβ, dystrophic neuritis, and activated glial cells (microglia and astrocytes) [42]. These authors reported that the number of activated glia surrounding Aβ plaques was lower in the C1q-deficient mice compared with the AD mouse model. In addition there was a reduction in the loss of synaptophysin and MAP2 compared with Tg2576 control mice [42], leading to the conclusion that C1q may have a harmful effect on the integrity of the neuron through initiating an inflammatory response. C1q-deficient mice also exhibited reduced retinal synapse elimination in mouse models of glaucoma, leading to the proposal that C1q mediates synapse loss in other neurodegenerative diseases [43]. Sárvári et al. [44], investigating the effects on hippocampal cells of the C1 complex inhibitor, C1-Inh, showed that inhibition of C1q protected hippocampal cells from Aβ-induced complement lysis. Neurons in the hippocampus and in the cortex are more vulnerable to complement-mediated damage as they are low in the complement inhibitors which usually protect host tissue from complement lysis [45] but conversely are abundant sources of complement [46]. Since these are the two brain areas which correlate with AD pathology, this may explain why analysis of cerebrospinal fluid (CSF) of AD patients indicated significantly lower C1q levels compared with control CSF, and decreased levels of C1q correlate with a diminished cognitive function [47].

3. Complement Sources, Inhibitors, and Function within the AD Brain

Complement factors can enter the brain via a compromised blood-brain barrier (BBB). Increasing evidence suggests BBB dysfunction is an early event in AD [48–52]. This may potentiate the triggering of detrimental brain parenchymal signalling cascades by blood components including complement proteins. Additionally the presence of MAC component mRNA and proteins in the AD brain has been reported, suggesting a possible CNS origin of synthesis [53–55]. Whilst neurones are an abundant source of complement proteins [46], the expression of complement protein mRNA has also been observed on murine astrocytes and microglia [56] and in postmortem-derived human CNS microglia [57]. Furthermore, astrocytoma cell lines, astrocytes, and oligodendroglia have been shown to produce complement proteins, indicating glial cells as another potential source of complement factors within the brain [58–61].

Inhibitors of the complement cascade have been shown in biomarker analyses to be reduced in AD. Thus complement C1 inhibitor/C1-esterase inhibitor (C1-Inh), which regulates the activation of complement in the classical activation pathway, has been shown to be reduced in plasma from AD patients [62, 63] and may be the result of the inability of neurons and astrocytes within the AD brain to secrete the active form of C1-Inh [64]. Additionally, deficiencies in the regulation of the alternative pathway of complement activation are also reported in AD. Hence the hippocampus and frontal cortex of AD patients have been shown to display significantly less CD59 expression but more complement factor 9, compared with nondemented control brains [65]. Since CD59 negatively controls MAC assembly, and activity, these data suggest that a deficiency in this control and subsequent damage may contribute to neuronal loss in AD. Factor H is a plasma glycoprotein which regulates the alternative pathway. Factor H is present in Aβ plaques in AD and may bind to CR3 receptors expressed on microglia to generate iC3a [66].

4. Microglia and AD

Microglia, resident in normal brain as sentinel cells [67–69], become reactive in AD [70]. In AD, microglia surround damaged or dead cells, clear cellular debris, and predominate around amyloid beta (Aβ) plaques [71]. Microglia proliferate around neurons prior to their loss in murine models of AD [72]. A positron emission tomography (PET) study detecting both activated microglia and an increase in amyloid load correlated the increase in activated microglia with cognitive impairment [73]. Microglia in animal models of AD show reactivity before obvious amyloid plaque deposition [74], indicating an early, "silent" (preclinical and asymptomatic) response of microglia may occur in AD by

as yet unconfirmed triggers. These may include amyloid oligomers and hypoperfusion [75, 76], but also complement. Complement activation and activated microglia are early neuropathological events in AD brains [77], and microglial responses show similarity to the peripheral immune system reaction of the macrophage. Activation products of the early complement components C1, C4, and C3 are found within neuritic plaques but there is little evidence of late complement components C7 and C9 or of MAC in the neuropathological lesions in AD brains [78]. This finding leads to the suggestion that in AD the complement system does not act as an inflammatory mediator through MAC formation, but through the actions of the early complement products which fuel the inflammatory responses, leading to neurotoxicity [78].

It is thus increasingly the accepted dogma that inflammation can actively cause neuronal damage and ultimately death of the neuron [79]. Recent data demonstrating that the responsiveness of the innate immune system is higher in offspring with a parental history of late-onset AD indicate heritable traits for AD that are related to inflammatory processes [80]. Furthermore the correlation of higher serum levels of certain acute-phase proteins with cognitive decline or dementia provides additional evidence for the early involvement of inflammation in AD pathogenesis [80]. Microglial reactivity is generally beneficial but the prolonged and progressive nature of the microglial response in AD can promote neurodegeneration. Pathogenic input to microglia, including the enhanced deposition of Aβ peptides, can result in the production of excessive free radicals, proinflammatory cytokines, complement proteins, and glutamate [81–83]. Consequences of the attenuation of inflammation in AD are seen clearly in animal studies. Craft et al. [84] demonstrated that inhibition of glial inflammation in an animal AD model resulted in reduced neurotoxicity. Advanced glycation end product (AGE) accumulation is accelerated in AD as it accumulates on plaques, and AGE-positive neurons and glia both increase with age and dramatically so with AD progression [85]. Activation of the receptor for AGE, (RAGE), on microglia with one of its ligands, such as AGE or Aβ, results in the release of proinflammatory mediators (free radicals and cytokines) [86]. A combination of both these ligands (AGE and Aβ) can lead to an enhanced microglial inflammatory response [87].

5. Complement, Phagocytosis, and Microglia

Rat microglia constitutively express C1q and its corresponding receptor CR1 [88, 89] and activated and amoeboid rat microglia, but not ramified microglia, can express CR3, C3 mRNA and shed C3 protein [61, 90]. Human monocytes and macrophages express three known receptors, CR1, CR3, and CR4 that bind complement proteins or their degradation products [91]. CR1 (CD35) binds mainly C3b, C4b, and C1q [16, 89] whereas CR3 (CD11b/CD18) and CR4 (CD11c/CD18) are relatively specific for iC3b [92]. Human macrophages require activation by both alternative and classical complement pathways in order to phagocytose apoptotic cells [93]. Exposure of phosphatidylserine on the apoptotic cell surface is a partial requirement for complement activation and results in coating the apoptotic cell surface with iC3b (Figure 2). The macrophage receptors for iC3b, CR3 (CD11b/CD18), and CR4 (CD11c/CD18) are implicated in this phagocytosis of apoptotic cells and appear more effective compared with other phagocytic receptors, such as scavenger receptors, implicated in clearance [93]. C1q binds directly and specifically to surface blebs of apoptotic cells [94]. Immune complexes coated with C3b and C4b bind to CR1 which leads to their elimination through endocytosis by the CR3 containing phagocytes in the liver [95]. CR1 also functions to regulate complement activation by acting as a cofactor in the Factor-1 mediated cleavage of C3b and C4b [96]. Complement C3 deficiency leads to accelerated Aβ plaque deposition, neurodegeneration, and promotion of a nonphagocytic microglial phenotype in APP transgenic mice [97].

Since complement activation is required for efficient phagocytosis [11] and removal of apoptotic cells within the systemic circulation, early component deficiencies could predispose to systemic autoimmunity by enhanced exposure to and/or aberrant deposition of apoptotic cells [93]. Apoptotic cells promote autoimmunity and defects in the clearance of self-antigens and chronic stimulation of type 1 interferons lead to the systemic autoimmunity seen in C1q deficiency [98]. Null mutations in complement proteins underlie the autoimmune disease systemic lupus erythematosus (SLE), and the severest forms of the disease are those associated with C1q deficiency [99]. Knock-out studies using mice deficient in the complement component, C1q, and patients with systemic lupus erythematosus (SLE) show increased mortality and multiple apoptotic cell bodies and immune deposits, compatible with the hypothesis that C1q deficiency causes autoimmunity by an impaired clearance of apoptotic cells, thought to be a major source of autoantigens in SLE [99, 100]. Further studies have demonstrated the importance of complement in AD. Human-APP transgenic mice expressing the soluble form of the C3 convertase inhibitor Crry (sCrry), (thus hAPP/sCrry mice), showed a 2- to 3-fold higher deposition of Aβ deposits and an accumulation of degenerating neurons compared with the hAPP mice [101]. This suggests that, as in SLE, the impairment of apoptosis in AD and subsequent immune cell responses may fuel disease progression. Changes in complement activation or in CR1 expression [22] might thus lead to a disruption in the clearance of cellular debris and Aβ by microglia. In addition microglial CR3 (CD11b/CD18) is implicated in the phagocytosis of Aβ peptides, acting alongside the low-density lipoprotein receptor-related protein (LRP) [102], and deficiencies in C3bi signalling might thus reduce microglial Aβ phagocytosis.

The signalling pathways triggered by complement factors in microglia have attracted modest attention [103–105]. CD88, otherwise known as the complement component C5a receptor 1, plays a role in the calcium signalling required for phagocytosis in microglia ([105], reviewed by [106]). Complement 5a (C5a), a chemotactic agent for macrophages and microglia, transiently activates an outwardly rectifying

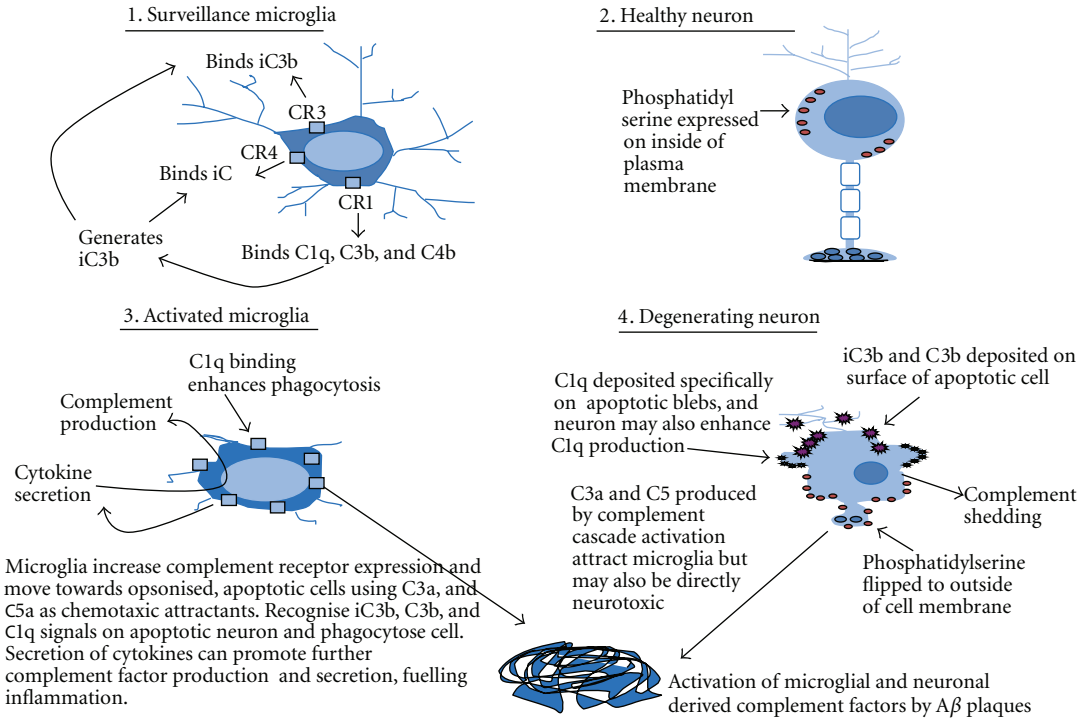


Figure 2: Interactions between microglia and neurons mediated by complement. Surveillance microglia may express low levels of CR1, CR3, and iC3b whilst healthy neurones do not express or produce significant complement. Phosphatidylserine is mainly expressed on the internal surface of the neuronal plasma membrane, preventing it acting as an "eat-me" signal, and complement production by the cell is low. During neuroinflammation and neurodegeneration, activated microglia, responding to the generation of complement factors, increase their expression of complement receptors, produce complement factors, and migrate towards the chemotaxic signals of C3a and C5a. Microglia may exacerbate the secretion of complement factors by secreting cytokines (following exposure to Aβ plaques), which can feed onto astrocytes or form a feedback loop with microglia themselves, promoting glial complement factors secretion. Exposure of secreted complement factors to Aβ plaques can lead to complement activation. Apoptotic neurones become opsonised with iC3b, C3b, and C1q deposition, the latter on apoptotic blebs, and the neurons may also shed additional complement factors. Phosphatidylserine flips to the outside of the plasma membrane where it can potentiate expression of "eat-me" signals by promoting the expression of iC3b on the cell surface [93].

K^+ conductance, mediates intracellular calcium mobilisation, and serves to increase microglial motility and to direct these cells by a G-protein-dependent pathway to damaged areas [103, 104]. Recently data suggest that the microglial expression of C5aR/CD88 correlates with Aβ deposition in murine transgenic models of AD, with C5aR/CD88 showing enhanced expression in microglia adjacent to Aβ plaques [107]. Antagonism of microglial C5aR resulted in a significant reduction in pathology in the AD mouse model Tg2576 and reduced hyperphosphorylated tau in 3xTg mice. [107, 108], suggesting a possible therapeutic target for the treatment of AD. Exposure of microglia to complement fragment C3a also induces a calcium response mediated by PTX-sensitive G-proteins [103]. Complement factors have also been shown to increase microglial glutamate transporter GLT-1 expression and promote increased glutamate uptake, without affecting glutamate release [109].

Recent evidence suggests that deletion of C3 convertase regulator complement receptor 1-related protein y (Crry) on microglia results in microglial priming, a microglial state which controversially may precipitate a neurotoxic microglial phenotype and predispose the brain to neurodegeneration [110]. These authors observed that mice that were double-knockout for Crry and either C3 or factor B did not show priming, demonstrating dependence on alternative pathway activation. Colocalization of C3b/iC3b and CR3 implicated the CR3/iC3b interaction in priming, and similar expression patterns were observed in microglia in human multiple sclerosis. In the rodent MS model, EAE was accelerated and exacerbated in Crry-deficient mice and was dependent on complement activation.

Microglia are a source of cytokines, which may, in the AD brain, result in the alteration of complement cascade inhibitors and complement factors. Thus cytokines detected in AD plaques, such as IL-1, IL-6, and TNF-α, have been found to differentially stimulate the secretion of C1 subcomponents, C1-Inh, C3, and C4 from glial cells including microglia [39]. Microglia constitutively express C1q, whilst the cytokines IL-1α, IL-1β, TNFα, and IL-6 can stimulate the secretion of C1r, C1s, and C3 from microglia, astrocytes, and neuroblastoma cells, and C4 can be secreted in response to IFNγ and IL-6, but complement inhibitor C1-Inh is only secreted in response to IFNγ. Since this cytokine is not present in Aβ plaques, there is the potential for an imbalance

between the generation of complement factors and their inactivation by C1-Inh [39], allowing unregulated activation of complement cascades.

6. Complement: A Protective Role in AD and Microglial Responses?

Complement activation in the CNS has been mainly discussed so far with regard to its damaging effects when in fact a number of components of the complement pathway have demonstrated protective effects [111–113]. For example the inflammatory and phagocytotic mediator, C5a, has a protective effect against glutamate neurotoxicity through regulation of the ionotropic Glu2 receptor subunit and protects against neuronal apoptosis [114]. C5a also protected human neuroblastoma cells and normal rodent hippocampal neurons from Aβ-induced neurotoxicity by triggering rapid activation of protein kinase C and activation and nuclear translocation of the NF-kappa B transcription factor [115]. Furthermore, C5a-deficient animals are more susceptible to damage from excitotoxic lesions in the hippocampus [116].

We have previously summarized the detrimental effects of C1q in relation to neurodegenerative diseases. However in contrast, inherited deficiency of this component of the classical complement pathway is associated with systemic lupus erythematosus (SLE) [117, 118] as discussed earlier. C1q mRNA has been reported to be increased in the neurons of patients with AD [119] and other neurodegenerative diseases such as Huntington's disease [120]. Also neuronal C1q synthesis has been demonstrated in the brain of rodent models of ischaemia and excitotoxic insult [121]. Furthermore, an increase in gene expression of C1q has been also demonstrated during normal brain ageing in mice which may be due to an oxidative stress response [122]. This could imply that C1q synthesis may be a response to injury and in fact play a protective role by promoting clearance of apoptotic cells which might otherwise pose an autoimmunity risk [99, 123]. A study in which C1q was incubated with primary neuronal cell cultures revealed a neuroprotective role of this complement factor, when the neurons were exposed to toxic concentrations of Aβ and serum amyloid-P (SAP) [124]. The neuroprotective properties of another complement factor generated from C1q, namely, C3, have also been investigated; a complement C3-deficient amyloid precursor protein (APP) transgenic AD mouse model (APP; C3(−/−)) exhibited accelerated plaque burden in the cortex and hippocampus, increased plasma Aβ levels, and significant hippocampal neuronal loss [97]. Interestingly, the microglia were present in the so-called alternative activation phenotype, displaying significantly increased CD45 immunoreactivity, together with increased brain levels of IL-4 and IL-10 and reduced levels of CD68, F4/80, inducible nitric oxide synthase, and TNFα [97]. This would suggest a protective role for C3 in terms of plaque clearance and for its triggering of Aβ phagocytosis by microglia, as shown by Choucair-Jaafar et al. [102] and Fu et al. [125] together with an overall neuroprotection. The findings also reveal that the alternative activation phenotype of microglia in AD may not be a particularly desirous state to aim for as a therapeutic endpoint. Instead it could be argued that some form of microglial response to complement in AD is essential.

7. Conclusion

In summary, there is now a substantial body of work implicating alternations in complement signalling in AD. Dysregulation of the complement cascade, either by changes in receptor expression, enhanced activation of different complement pathways or imbalances between complement factor levels, and complement cascade inhibitors may all contribute to the involvement of complement in AD. With regard to microglia and complement, the evidence presented here indicates that microglia can be manipulated by complement factors to adopt protective or harmful phenotypes. Thus, in AD, microglia may be activated by disruptive complement signalling and the presence of Aβ plaques to enhance their secretion of cytokines, which can fuel secretion of further complement factors, leading to a chronic inflammatory response. The task ahead is to unravel further the complex interactions between complement, AD, and microglia as an essential prerequisite to understanding, and manipulating to therapeutic advantage, the role of complement and microglia in AD.

Acknowledgments

The authors wish to thank the Corsan Family Foundation and Alzheimer's Research UK for funding.

References

[1] B. P. Morgan and P. Gasque, "Expression of complement in the brain: role in health and disease," *Immunology Today*, vol. 17, no. 10, pp. 461–466, 1996.

[2] K. Yasojima, C. Schwab, E. G. McGeer, and P. L. McGeer, "Up-regulated production and activation of the complement system in Alzheimer's disease brain," *American Journal of Pathology*, vol. 154, no. 3, pp. 927–936, 1999.

[3] J. R. Dunkelberger and W. C. Song, "Complement and its role in innate and adaptive immune responses," *Cell Research*, vol. 20, no. 1, pp. 34–50, 2010.

[4] J. V. Sarma and P. A. Ward, "The complement system," *Cell and Tissue Research*, vol. 343, no. 1, pp. 227–235, 2011.

[5] K. Murphy, P. Travers, and M. Walport, *Janeway's Immuno Biology*, Garland Science, Taylor & Francis group, LLC, New York, NY, USA, 7th edition, 2008.

[6] R. Sørensen, S. Thiel, and J. C. Jensenius, "Mannan-binding-lectin-associated serine proteases, characteristics and disease associations," *Springer Seminars in Immunopathology*, vol. 27, no. 3, pp. 299–319, 2005.

[7] H. Qu, D. Ricklin, and J. D. Lambris, "Recent developments in low molecular weight complement inhibitors," *Molecular Immunology*, vol. 47, no. 2-3, pp. 185–195, 2009.

[8] D. Liu and Z. X. Niu, "The structure, genetic polymorphisms, expression and biological functions of complement receptor type 1 (CR1/CD35)," *Immunopharmacology and Immunotoxicology*, vol. 31, no. 4, pp. 524–535, 2009.

[9] Sang-Ho Kim, D. F. Carney, C. H. Hammer, and M. L. Shin, "Nucleated cell killing by complement: effects of C5b-9 channel size and extracellular Ca^{2+} on the lytic process," *Journal of Immunology*, vol. 138, no. 5, pp. 1530–1536, 1987.

[10] S. Meri, B. P. Morgan, A. Davies et al., "Human protectin (CD59), an 18,000–20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers," *Immunology*, vol. 71, no. 1, pp. 1–9, 1990.

[11] M. M. Frank and L. F. Fries, "The role of complement in inflammation and phagocytosis," *Immunology Today*, vol. 12, no. 9, pp. 322–326, 1991.

[12] E. McGreal and P. Gasque, "Structure-function studies of the receptors for complement C1q," *Biochemical Society Transactions*, vol. 30, no. 6, pp. 1010–1014, 2002.

[13] R. A. Nelson Jr., "The immune-adherence phenomenon; an immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis," *Science*, vol. 118, no. 3077, pp. 733–737, 1953.

[14] V. M. Holers, D. D. Chaplin, and J. F. Leykam, "Human complement C3b/C4b receptor (CRI) mRNA polymorphism that correlates with the CRI allelic molecular weight polymorphism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 8, pp. 2459–2463, 1987.

[15] M. Krych-Goldberg and J. P. Atkinson, "Structure-function relationships of complement receptor type 1," *Immunological Reviews*, vol. 180, pp. 112–122, 2001.

[16] D. T. Fearon, "Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte," *Journal of Experimental Medicine*, vol. 152, no. 1, pp. 20–30, 1980.

[17] M. D. Appary, M. D. Kazatchkine, M. Levi-Strauss, N. Hinglais, and J. Bariety, "Expression of CR1 (CD35) mRNA in podocytes from adult and fetal human kidneys," *Kidney International*, vol. 38, no. 2, pp. 289–293, 1990.

[18] H. Crehan, P. Holton, S. Wray, J. Pocock, R. Guerreiro, and J. Hardy, "Complement receptor 1 (CR1) and Alzheimer's disease," *Immunobiology*, vol. 217, no. 2, pp. 244–250, 2012.

[19] J. A. Schifferli, Y. C. Ng, J. Estreicher, and M. J. Walport, "The clearance of tetanus toxoid/anti-tetanus toxoid immune complexes from the circulation of humans. Complement- and erythrocyte complement receptor 1-dependent mechanisms," *Journal of Immunology*, vol. 140, no. 3, pp. 899–904, 1988.

[20] N. Ertekin-Taner, "Genetics of Alzheimer's disease: a centennial review," *Neurologic Clinics*, vol. 25, no. 3, pp. 611–667, 2007.

[21] D. Harold, R. Abraham, P. Hollingworth et al., "Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease," *Nature Genetics*, vol. 41, no. 10, pp. 1088–1093, 2009.

[22] J. C. Lambert, S. Heath, G. Even et al., "Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease," *Nature Genetics*, vol. 41, no. 10, pp. 1094–1099, 2009.

[23] P. Hollingworth, D. Harold, R. Sims et al., "Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease," *Nature Genetics*, vol. 43, no. 5, pp. 429–436, 2011.

[24] A. C. Naj, G. Jun, G. W. Beecham et al., "Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease," *Nature Genetics*, vol. 43, no. 5, pp. 436–441, 2011.

[25] G. Jun, A. C. Naj, G. W. Beecham et al., "Meta-analysis confirms CR1, CLU, and PICALM as Alzheimer disease risk loci and reveals interactions with APOE genotypes," *Archives of Neurology*, vol. 67, no. 12, pp. 1473–1484, 2010.

[26] M. M. Carrasquillo, O. Belbin, T. A. Hunter et al., "Replication of CLU, CR1, and PICALM associations with Alzheimer disease," *Archives of Neurology*, vol. 67, no. 8, pp. 961–964, 2010.

[27] A. Biffi, C. D. Anderson, R. S. Desikan et al., "Genetic variation and neuroimaging measures in Alzheimer disease," *Archives of Neurology*, vol. 67, no. 6, pp. 677–685, 2010.

[28] J. Mengel-From, K. Christensen, M. McGue, and L. Christiansen, "Genetic variations in the CLU and PICALM genes are associated with cognitive function in the oldest old," *Neurobiology of Aging*, vol. 32, no. 3, article 554, 2011.

[29] J. Daborg, U. Andreasson, M. Pekna et al., "Cerebrospinal fluid levels of complement proteins C3, C4 and CR1 in Alzheimer's disease," *Journal of Neural Transmission*, vol. 119, no. 7, pp. 789–797, 2012.

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

[31] I. Tooyama, H. Kimura, H. Akiyama, and P. L. McGeer, "Reactive microglia express class I and class II major histocompatibility complex antigens in Alzheimer's disease," *Brain Research*, vol. 523, no. 2, pp. 273–280, 1990.

[32] L. S. Perlmutter, S. A. Scott, E. Barrón, and H. C. Chui, "Erratum: MHC class II-positive microglia in human brain: association with Alzheimer lesions," *Journal of Neuroscience Research*, vol. 35, no. 3, p. 346, 1992.

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

[34] C. R. Abraham, D. J. Selkoe, and H. Potter, "Immunochemical identification of the serine protease inhibitor α1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease," *Cell*, vol. 52, no. 4, pp. 487–501, 1988.

[35] P. Eikelenboom and F. C. Stam, "Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study," *Acta Neuropathologica*, vol. 57, no. 2-3, pp. 239–242, 1982.

[36] J. Rogers, N. R. Cooper, S. Webster et al., "Complement activation by β-amyloid in Alzheimer disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 21, pp. 10016–10020, 1992.

[37] A. Afagh, B. J. Cummings, D. H. Cribbs, C. W. Cotman, and A. J. Tenner, "Localization and cell association of C1q in Alzheimer's disease brain," *Experimental Neurology*, vol. 138, no. 1, pp. 22–32, 1996.

[38] A. J. M. Rozemuller, P. Eikelenboom, J. W. Theeuwes, E. N. H. Jansen Steur, and R. A. I. de Vos, "Activated microglial cells and complement factors are unrelated to cortical Lewy bodies," *Acta Neuropathologica*, vol. 100, no. 6, pp. 701–708, 2000.

[39] R. Veerhuis, I. Janssen, C. J. A. de Groot, F. L. van Muiswinkel, C. E. Hack, and P. Eikelenboom, "Cytokines associated with amyloid plaques in Alzheimer's disease brain stimulate human glial and neuronal cell cultures to secrete early complement proteins, but not C1-inhibitor," *Experimental Neurology*, vol. 160, no. 1, pp. 289–299, 1999.

[40] Y. Shen, L. F. Lue, L. B. Yang et al., "Complement activation by neurofibrillary tangles in Alzheimer's disease," *Neuroscience Letters*, vol. 305, no. 3, pp. 165–168, 2001.

[41] R. Strohmeyer, Y. Shen, and J. Rogers, "Detection of complement alternative pathway mRNA and proteins in the Alzheimer's disease brain," *Molecular Brain Research*, vol. 81, no. 1-2, pp. 7–18, 2000.

[42] M. I. Fonseca, J. Zhou, M. Botto, and A. J. Tenner, "Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease," *Journal of Neuroscience*, vol. 24, no. 29, pp. 6457–6465, 2004.

[43] B. Stevens, N. J. Allen, L. E. Vazquez et al., "The classical complement cascade mediates CNS synapse elimination," *Cell*, vol. 131, no. 6, pp. 1164–1178, 2007.

[44] M. Sárvári, I. Vágó, C. S. Wéber et al., "Inhibition of C1q-β-amyloid binding protects hippocampal cells against complement mediated toxicity," *Journal of Neuroimmunology*, vol. 137, no. 1-2, pp. 12–18, 2003.

[45] S. K. Singhrao, J. W. Neal, N. K. Rushmere, B. P. Morgan, and P. Gasque, "Spontaneous classical pathway activation and deficiency of membrane regulators render human neurons susceptible to complement lysis," *American Journal of Pathology*, vol. 157, no. 3, pp. 905–918, 2000.

[46] K. Terai, D. G. Walker, E. G. McGeer, and P. L. McGeer, "Neurons express proteins of the classical complement pathway in Alzheimer disease," *Brain Research*, vol. 769, no. 2, pp. 385–390, 1997.

[47] M. D. Smyth, D. H. Cribbs, A. J. Tenner et al., "Decreased levels of Clq in cerebrospinal fluid of living Alzheimer patients correlate with disease state," *Neurobiology of Aging*, vol. 15, no. 5, pp. 609–614, 1994.

[48] D. Gay and M. Esiri, "Blood-brain barrier damage in acute multiple sclerosis plaques. An immunocytological study," *Brain*, vol. 114, no. 1 B, pp. 557–572, 1991.

[49] I. Alafuzoff, R. Adolfsson, I. Grundke-Iqbal, and B. Winblad, "Blood-brain barrier in Alzheimer dementia and in non-demented elderly. An immunocytochemical study," *Acta Neuropathologica*, vol. 73, no. 2, pp. 160–166, 1987.

[50] L. Claudio, "Ultrastructural features of the blood-brain barrier in biopsy tissue from Alzheimer's disease patients," *Acta Neuropathologica*, vol. 91, no. 1, pp. 6–14, 1996.

[51] M. Fiala, Q. N. Liu, J. Sayre et al., "Cyclooxygenase-2-positive macrophages infiltrate the Alzheimer's disease brain and damage the blood-brain barrier," *European Journal of Clinical Investigation*, vol. 32, no. 5, pp. 360–371, 2002.

[52] A. Minagar and J. S. Alexander, "Blood-brain barrier disruption in multiple sclerosis," *Multiple Sclerosis*, vol. 9, no. 6, pp. 540–549, 2003.

[53] Y. Shen, R. Li, E. G. McGeer, and P. L. McGeer, "Neuronal expression of mRNAs for complement proteins of the classical pathway in Alzheimer brain," *Brain Research*, vol. 769, no. 2, pp. 391–395, 1997.

[54] J. van Beek, M. Bernaudin, E. Petit et al., "Expression of receptors for complement anaphylatoxins C3a and C5a following permanent focal cerebral ischemia in the mouse," *Experimental Neurology*, vol. 161, no. 1, pp. 373–382, 2000.

[55] D. G. Walker and P. L. McGeer, "Complement gene expression in human brain: comparison between normal and Alzheimer disease cases," *Molecular Brain Research*, vol. 14, no. 1-2, pp. 109–116, 1992.

[56] N. Davoust, S. Nataf, V. M. Holers, and S. R. Barnum, "Expression of the murine complement regulatory protein Crry by glial cells and neurons," *Glia*, vol. 27, no. 2, pp. 162–170, 1999.

[57] D. G. Walker, S. U. Kim, and P. L. McGeer, "Complement and cytokine gene expression in cultured microglia derived from postmortem human brains," *Journal of Neuroscience Research*, vol. 40, no. 4, pp. 478–493, 1995.

[58] P. Gasque, A. Ischenko, J. Legoedec, C. Mauger, M. T. Schouft, and M. Fontaine, "Expression of the complement classical pathway by human glioma in culture. A model for complement expression by nerve cells," *The Journal of Biological Chemistry*, vol. 268, no. 33, pp. 25068–25074, 1993.

[59] D. G. Walker, S. U. Kim, and P. L. McGeer, "Expression of complement C4 and C9 genes by human astrocytes," *Brain Research*, vol. 809, no. 1, pp. 31–38, 1998.

[60] M. Hosokawa, A. Klegeris, J. Maguire, and P. L. McGeer, "Expression of complement messenger RNAs and proteins by human oligodendroglial cells," *Glia*, vol. 42, no. 4, pp. 417–423, 2003.

[61] M. Rutar, R. Natoli, P. Kozulin, K. Valter, P. Gatenby, and J. M. Provis, "Analysis of complement expression in light-induced retinal degeneration: synthesis and deposition of C3 by microglia/macrophages is associated with focal photoreceptor degeneration," *Investigative Ophthalmology and Visual Science*, vol. 52, no. 8, pp. 5347–5358, 2011.

[62] R. Zhang, L. Barker, D. Pinchev et al., "Mining biomarkers in human sera using proteomic tools," *Proteomics*, vol. 4, no. 1, pp. 244–256, 2004.

[63] E. L. Akuffo, J. B. Davis, S. M. Fox et al., "The discovery and early validation of novel plasma biomarkers in mild-to-moderate Alzheimer's disease patients responding to treatment with rosiglitazone," *Biomarkers*, vol. 13, no. 6, pp. 618–636, 2008.

[64] R. Veerhuis, I. Janssen, J. J. M. Hoozemans, C. J. A. de Groot, C. E. Hack, and P. Eikelenboom, "Complement C1-inhibitor expression in Alzheimer's disease," *Acta Neuropathologica*, vol. 96, no. 3, pp. 287–296, 1998.

[65] L. B. Yang, R. Li, S. Meri, J. Rogers, and Y. Shen, "Deficiency of complement defense protein CD59 may contribute to neurodegeneration in Alzheimer's disease," *Journal of Neuroscience*, vol. 20, no. 20, pp. 7505–7509, 2000.

[66] R. Strohmeyer, M. Ramirez, G. J. Cole, K. Mueller, and J. Rogers, "Association of factor H of the alternative pathway of complement with agrin and complement receptor 3 in the Alzheimer's disease brain," *Journal of Neuroimmunology*, vol. 131, no. 1-2, pp. 135–146, 2002.

[67] G. Raivich, "Like cops on the beat: the active role of resting microglia," *Trends in Neurosciences*, vol. 28, no. 11, pp. 571–573, 2005.

[68] F. Vilhardt, "Microglia: phagocyte and glia cell," *International Journal of Biochemistry and Cell Biology*, vol. 37, no. 1, pp. 17–21, 2005.

[69] K. Helmut, U. K. Hanisch, M. Noda, and A. Verkhratsky, "Physiology of microglia," *Physiological Reviews*, vol. 91, no. 2, pp. 461–553, 2011.

[70] J. M. Pocock, A. C. Liddle, C. Hooper, D. L. Taylor, C. M. Davenport, and S. C. Morgan, "Activated microglia in Alzheimer's disease and stroke," *Ernst Schering Research Foundation workshop*, no. 39, pp. 105–132, 2002.

[71] L. Fetler and S. Amigorena, "Brain under surveillance: the microglia patrol," *Science*, vol. 309, no. 5733, pp. 392–393, 2005.

[72] M. Fuhrmann, T. Bittner, C. K. E. Jung et al., "Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of

Alzheimer's disease," *Nature Neuroscience*, vol. 13, no. 4, pp. 411–413, 2010.

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

[74] J. J. Rodríguez, J. Witton, M. Olabarria, H. N. Noristani, and A. Verkhratsky, "Increase in the density of resting microglia precedes neuritic plaque formation and microglial activation in a transgenic model of Alzheimer's disease," *Cell Death and Disease*, vol. 1, no. 1, article e1, 2010.

[75] C. Huang, L. O. Wahlund, L. Svensson, B. Winblad, and P. Julin, "Cingulate cortex hypoperfusion predicts Alzheimer's disease in mild cognitive impairment," *BMC Neurology*, vol. 2, no. 1, article 9, 2002.

[76] M. T. Ferretti and A. C. Cuello, "Does a pro-inflammatory process precede Alzheimer's disease and mild cognitive impairment?" *Current Alzheimer Research*, vol. 8, no. 2, pp. 164–174, 2011.

[77] P. Eikelenboom, E. van Exel, R. Veerhuis, A. J. M. Rozemuller, W. A. van Gool, and J. J. M. Hoozemans, "Innate immunity and the etiology of late-onset Alzheimer's disease," *Neurodegenerative Diseases*, vol. 10, no. 1–4, pp. 271–273, 2012.

[78] P. Eikelenboom and R. Veerhuis, "The role of complement and activated microglia in the pathogenesis of Alzheimer's disease," *Neurobiology of Aging*, vol. 17, no. 5, pp. 673–680, 1996.

[79] M. L. Block and J. S. Hong, "Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism," *Progress in Neurobiology*, vol. 76, no. 2, pp. 77–98, 2005.

[80] P. Eikelenboom, R. Veerhuis, E. van Exel, J. J. M. Hoozemans, A. J. M. Rozemuller, and W. A. van Gool, "The early involvement of the innate immunity in the pathogenesis of lateonset Alzheimer's disease: neuropathological, epidemiological and genetic evidence," *Current Alzheimer Research*, vol. 8, no. 2, pp. 142–150, 2011.

[81] S. W. Barger and A. D. Harmon, "Microglial activation by alzhelmer amyloid precursor protein and modulation by apolipoprotein E," *Nature*, vol. 388, no. 6645, pp. 878–881, 1997.

[82] C. M. Davenport, I. G. Sevastou, C. Hooper, and J. M. Pocock, "Inhibiting p53 pathways in microglia attenuates microglial-evoked neurotoxicity following exposure to Alzheimer peptides," *Journal of Neurochemistry*, vol. 112, no. 2, pp. 552–563, 2010.

[83] I. Morales, G. Farías, and R. B. MacCioni, "Neuroimmunomodulation in the pathogenesis of Alzheimer's disease," *NeuroImmunoModulation*, vol. 17, no. 3, pp. 202–204, 2010.

[84] J. M. Craft, D. M. Watterson, S. A. Frautschy, and L. J. van Eldik, "Aminopyridazines inhibit β-amyloid-induced glial activation and neuronal damage in vivo," *Neurobiology of Aging*, vol. 25, no. 10, pp. 1283–1292, 2004.

[85] H. J. Lüth, V. Ogunlade, B. Kuhla et al., "Age- and stage-dependent accumulation of advanced glycation end products in intracellular deposits in normal and Alzheimer's disease brains," *Cerebral Cortex*, vol. 15, no. 2, pp. 211–220, 2005.

[86] K. Berbaum, K. Shanmugam, G. Stuchbury, F. Wiede, H. Körner, and G. Münch, "Induction of novel cytokines and chemokines by advanced glycation endproducts determined with a cytometric bead array," *Cytokine*, vol. 41, no. 3, pp. 198–203, 2008.

[87] J. Gasic-Milenkovic, S. Dukic-Stefanovic, W. Deuther-Conrad, U. Gärtner, and G. Münch, "β-Amyloid peptide potentiates inflammatory responses induced by lipopolysaccharide, interferon-γ and "advanced glycation endproducts" in a murine microglia cell line," *European Journal of Neuroscience*, vol. 17, no. 4, pp. 813–821, 2003.

[88] A. R. Korotzer, J. Watt, D. Cribbs et al., "Cultured rat microglia express C1q and receptor for C1q: implications for amyloid effects on microglia," *Experimental Neurology*, vol. 134, no. 2, pp. 214–221, 1995.

[89] L. B. Klickstein, S. F. Barbashov, T. Liu, R. M. Jack, and A. Nicholson-Weller, "Complement receptor type 1 (CR1, CD35) is a receptor for C1q," *Immunity*, vol. 7, no. 3, pp. 345–355, 1997.

[90] E. A. Ling, C. Kaur, T. Y. Yick, and W. C. Wong, "Immunocytochemical localization of CR3 complement receptors with OX-42 in amoeboid microglia in postnatal rats," *Anatomy and Embryology*, vol. 182, no. 5, pp. 481–486, 1990.

[91] U. Martin, D. Bock, L. Arseniev et al., "The human C3a receptor is expressed on neutrophils and monocytes, but not on B or T lymphocytes," *Journal of Experimental Medicine*, vol. 186, no. 2, pp. 199–207, 1997.

[92] M. A. Arnaout, "Structure and function of the leukocyte adhesion molecule CD11/CD18," *Blood*, vol. 75, no. 5, pp. 1037–1050, 1990.

[93] D. Mevorach, J. O. Mascarenhas, D. Gershov, and K. B. Elkon, "Complement-dependent clearance of apoptotic cells by human macrophages," *Journal of Experimental Medicine*, vol. 188, no. 12, pp. 2313–2320, 1998.

[94] L. C. Korb and J. M. Ahearn, "C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited," *Journal of Immunology*, vol. 158, no. 10, pp. 4525–4528, 1997.

[95] Z. Bánki, D. Wilflingseder, C. G. Ammann et al., "Factor I-mediated processing of complement fragments on HIV immune complexes targets HIV to CR2-expressing B cells and facilitates B cell-mediated transmission of opsonized HIV to T cells," *Journal of Immunology*, vol. 177, no. 5, pp. 3469–3476, 2006.

[96] D. E. Hourcade, L. Mitchell, L. A. Kuttner-Kondo, J. P. Atkinson, and M. E. Medof, "Decay-accelerating factor (DAF), complement receptor 1 (CR1), and factor H dissociate the complement AP C3 convertase (C3bBb) via sites on the type A domain of Bb," *The Journal of Biological Chemistry*, vol. 277, no. 2, pp. 1107–1112, 2002.

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

[98] M. H. Cheng and M. S. Anderson, "Monogenic autoimmunity," *Annual Review of Immunology*, vol. 30, pp. 393–427, 2012.

[99] P. R. Taylor, A. Carugati, V. A. Fadok et al., "A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo," *Journal of Experimental Medicine*, vol. 192, no. 3, pp. 359–366, 2000.

[100] M. Botto, C. Dell'Agnola, A. E. Bygrave et al., "Homozygous C1q deficiency causes glomerulonephritis associated with

multiple apoptotic bodies," *Nature Genetics*, vol. 19, no. 1, pp. 56–59, 1998.

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

[102] N. Choucair-Jaafar, V. Laporte, R. Levy, P. Poindron, Y. Lombard, and J. P. Gies, "Complement receptor 3 (CD11b/CD18) is implicated in the elimination of β-amyloid peptides," *Fundamental and Clinical Pharmacology*, vol. 25, no. 1, pp. 115–122, 2011.

[103] S. Ilschner, C. Nolte, and H. Kettenmann, "Complement factor C5a and epidermal growth factor trigger the activation of outward potassium currents in cultured murine microglia," *Neuroscience*, vol. 73, no. 4, pp. 1109–1120, 1996.

[104] C. Nolte, T. Möller, T. Walter, and H. Kettenmann, "Complement 5a controls motility of murine microglial cells in vitro via activation of an inhibitory G-protein and the rearrangement of the actin cytoskeleton," *Neuroscience*, vol. 73, no. 4, pp. 1091–1107, 1996.

[105] A. Hoffmann, O. Kann, C. Ohlemeyer, U. K. Hanisch, and H. Kettenmann, "Elevation of basal intracellular calcium as a central element in the activation of brain macrophages (microglia): suppression of receptor-evoked calcium signaling and control of release function," *Journal of Neuroscience*, vol. 23, no. 11, pp. 4410–4419, 2003.

[106] P. Nunes and N. Demaurex, "The role of calcium signalling in phagocytosis," *Journal of Leukocyte Biology*, vol. 88, no. 1, pp. 57–68, 2010.

[107] R. R. Ager, M. I. Fonseca, S. H. Chu et al., "Microglial C5aR (CD88) expression correlates with amyloid-β deposition in murine models of Alzheimer's disease," *Journal of Neurochemistry*, vol. 113, no. 2, pp. 389–401, 2010.

[108] M. I. Fonseca, R. R. Ager, S. H. Chu et al., "Treatment with a C5aR antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease," *Journal of Immunology*, vol. 183, no. 2, pp. 1375–1383, 2009.

[109] M. Persson, M. Pekna, E. Hansson, and L. Rönnbäck, "The complement-derived anaphylatoxin C5a increases microglial GLT-1 expression and glutamate uptake in a TNF-α-independent manner," *European Journal of Neuroscience*, vol. 29, no. 2, pp. 267–274, 2009.

[110] V. Ramaglia, T. R. Hughes, R. M. Donev et al., "C3-dependent mechanism of microglial priming relevant to multiple sclerosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 3, pp. 965–970, 2012.

[111] D. Mastellos, J. C. Papadimitriou, S. Franchini, P. A. Tsonis, and J. D. Lambris, "A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration," *Journal of Immunology*, vol. 166, no. 4, pp. 2479–2486, 2001.

[112] S. M. Drouin, M. Sinha, G. Sfyroera, J. D. Lambris, and R. A. Wetsel, "A protective role for the fifth complement component (C5) in allergic airway disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 173, no. 8, pp. 852–857, 2006.

[113] Z. Wang, S. Zhang, Z. Tong, L. Li, and G. Wang, "Maternal transfer and protective role of the alternative complement components in zebrafish Danio rerio," *PLoS ONE*, vol. 4, no. 2, Article ID e4498, 2009.

[114] P. Mukherjee, S. Thomas, and G. M. Pasinetti, "Complement anaphylatoxin C5a neuroprotects through regulation of glutamate receptor subunit 2 in vitro and in vivo," *Journal of Neuroinflammation*, vol. 5, article 5, 2008.

[115] S. A. O'Barr, J. Caguioa, D. Gruol et al., "Neuronal expression of a functional receptor for the C5a complement activation fragment," *Journal of Immunology*, vol. 166, no. 6, pp. 4154–4162, 2001.

[116] G. M. Pasinetti, "Inflammatory mechanisms in neurodegeneration and Alzheimer's disease: the role of the complement system," *Neurobiology of Aging*, vol. 17, no. 5, pp. 707–716, 1996.

[117] R. Topaloglu, A. Bakkaloglu, J. H. Slingsby et al., "Molecular basis of hereditary C1q deficiency associated with SLE and IgA nephropathy in a Turkish family," *Kidney International*, vol. 50, no. 2, pp. 635–642, 1996.

[118] H. V. Marquart, L. Schejbel, A. Sjoholm et al., "C1q deficiency in an Inuit family: identification of a new class of C1q disease-causing mutations," *Clinical Immunology*, vol. 124, no. 1, pp. 33–40, 2007.

[119] S. A. Johnson, M. Lampert-Etchells, G. M. Pasinetti, I. Rozovsky, and C. E. Finch, "Complement mRNA in the mammalian brain: responses to Alzheimer's disease and experimental brain lesioning," *Neurobiology of Aging*, vol. 13, no. 6, pp. 641–648, 1992.

[120] S. K. Singhrao, J. W. Neal, B. P. Morgan, and P. Gasque, "Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease," *Experimental Neurology*, vol. 159, no. 2, pp. 362–376, 1999.

[121] J. Huang, L. J. Kim, R. Mealey et al., "Neuronal protection in stroke by an sLe(x)-glycosylated complement inhibitory protein," *Science*, vol. 285, no. 5427, pp. 595–599, 1999.

[122] C. K. Lee, R. Weindruch, and T. A. Prolla, "Gene-expression profile of the ageing brain in mice," *Nature Genetics*, vol. 25, no. 3, pp. 294–297, 2000.

[123] A. J. Tenner, "Complement in Alzheimer's disease: opportunities for modulating protective and pathogenic events," *Neurobiology of Aging*, vol. 22, no. 6, pp. 849–861, 2001.

[124] K. Pisalyaput and A. J. Tenner, "Complement component C1q inhibits β-amyloid- and serum amyloid P-induced neurotoxicity via caspase- and calpain-independent mechanisms," *Journal of Neurochemistry*, vol. 104, no. 3, pp. 696–707, 2008.

[125] H. Fu, B. Liu, J. L. Frost et al., "Complement compoment C3 and complement receptor type 3 contribute to the phagocytosis and clearance of fibrillar Aβ by microglia," *Glia*, vol. 60, no. 6, pp. 993–1003, 2012.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 495243, 7 pages
doi:10.1155/2012/495243

Review Article

A Changing Perspective on the Role of Neuroinflammation in Alzheimer's Disease

Donna M. Wilcock

Department of Physiology, Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, USA

Correspondence should be addressed to Donna M. Wilcock, donna.wilcock@uky.edu

Received 27 January 2012; Revised 31 May 2012; Accepted 1 June 2012

Academic Editor: Colin Combs

Alzheimer's disease (AD) is a complex, neurodegenerative disorder characterized by the presence of amyloid plaques and neurofibrillary tangles in the brain. Glial cells, particularly microglial cells, react to the presence of the amyloid plaques and neurofibrillary tangles producing an inflammatory response. While once considered immunologically privileged due to the blood-brain barrier, it is now understood that the glial cells of the brain are capable of complex inflammatory responses. This paper will discuss the published literature regarding the diverse roles of neuroinflammation in the modulation of AD pathologies. These data will then be related to the well-characterized macrophage phenotypes. The conclusion is that the glial cells of the brain are capable of a host of macrophage responses, termed M1, M2a, M2b, and M2c. The relationship between these states and AD pathologies remains relatively understudied, yet published data using various inflammatory stimuli provides some insight. It appears that an M1-type response lowers amyloid load but exacerbates neurofibrillary tangle pathology. In contrast, M2a is accompanied by elevated amyloid load and appears to ameliorate, somewhat, neurofibrillary pathology. Overall, it is clear that more focused, cause-effect studies need to be performed to better establish how each inflammatory state can modulate the pathologies of AD.

1. Introduction

Alzheimer's disease (AD) is defined as the presence of amyloid plaques composed of amyloid-beta (Aβ) peptide aggregates and neurofibrillary tangles composed of hyperphosphorylated and aggregated tau protein. Neuroinflammation has been known to be present in AD since the original description of the histopathology of AD by Alois Alzheimer in 1907, who described "gliose," inflammation of the glia. Since then, it has been shown in numerous studies, both mouse and human, that glial cells respond to the presence of AD pathological lesions (plaques and tangles) by morphologically changing their characteristics, expressing numerous cell surface receptors and surrounding the lesions [1, 2]. The prevailing view of neuroinflammation in AD for many years has been that it is an on-off phenomenon that contributes to the cytotoxicity of AD lesions and therefore contributes to the neurodegeneration in AD [3]. It is only within the recent decade that neuroinflammation has come to the forefront of AD research, not only with respect to its contribution to the neurodegenerative process, but also for its role in the clearance of AD lesions and beneficial contribution to AD progression. The dichotomy of the research findings on the role(s) of neuroinflammation in AD may be explained by the capacity of glial cells to generate multiple distinct phenotypes dependent upon the stimuli present.

Glial cells describe the nonneuronal cells of the brain and include microglia, astrocytes, oligodendrocytes, and pericytes. These accessory cells are critical for the maintenance of an appropriate environment in which the neuronal cells can function optimally. This includes, but not limited to, ionic and osmotic homeostasis, myelination, debris removal, and neurotransmitter uptake and recycling. All glial cells are capable of achieving some degree of inflammatory response; however, the key cell type for the initiation, regulation, and resolution of the inflammatory response is considered to be the microglial cell. Derived from the macrophage cell lineage, microglia are specialized tissue macrophages in the brain and are capable of a broad range

of inflammatory responses dependent upon the stimulus. This paper will summarize the published literature regarding different activation states of microglia and their subsequent impact on AD pathologies. This paper will then discuss how the field can use data from the peripheral macrophage body of literature to better characterize microglial activation states and begin to predict what impact each state will have on the progression of AD.

2. Microglia in AD

For many years the body of literature regarding microglial cells and their role in AD focused on the negative influence inflammation would be thought to have on the progression of AD. This primarily focused on the concept of the autotoxic loop. Described in 1998 by E. G. McGeer and P. L. McGeer as a "vicious cycle," the autotoxic loop is the description of the microglial activation in response to cellular debris in the AD brain, this microglial activation is then thought to result in the release of cytotoxic cytokines that then leads to a more rapid neuronal death, thus providing more cellular debris to further accelerate this process [3]. Evidence for this process stemmed from the finding that there are increased cytokine levels in the brains and CSF of AD patients. These cytokines, primarily IL-1β and TNFα, are known to be toxic to cells in culture and also toxic in the brain if injected into the brain parenchyma (reviewed in [4]). While there has been some evidence for the presence of an autotoxic loop, much of the body of literature suggests that the levels of cytokines are not great enough, or sustained enough in the AD brain to cause significant neuronal damage. Attempts to recreate the autotoxic loop led to some surprising findings, mostly that the initiation of an inflammatory response in the brain often leads to the clearance of amyloid plaques in transgenic mouse models.

Table 1 summarizes some of the studies that have stimulated inflammatory responses. It is initially apparent in Table 1 that most studies that have stimulated inflammation and activated microglia result in reduced amyloid load and do not present evidence of exacerbated neuronal degeneration. These data do not disprove the autotoxic loop, instead they suggest a much greater complexity to the inflammatory response of the brain than originally considered possible.

Lipopolysaccharide (LPS) is a gram-negative bacterial cell-surface proteoglycan that stimulates an innate immune response. Injection of LPS into the brain parenchyma of aged APP/PS1 mice originally aimed at stimulating the autotoxic loop resulted in microglial activation and the rapid reduction of amyloid deposits in the brain [5]. A later study identified the types of microglial activation occurring with LPS in wildtype mice using intraparenchymal LPS injections over a time course of 1, 6, and 24 hours as well as an extended time course of 3, 7, 14, and 28 days [6]. Over this time course, it was found that most gene expression changes in inflammatory markers peaked around the 3-day time point and slowly declined to normal levels by 14 days. The inflammatory markers examined included TNFα and IL1β as well as Fcγ receptors and scavenger receptors. Histologically, the same report found that microglial expression of cell-surface proteins including complement receptor 3 (also known as CD11b), CD45, scavenger receptor A, and Fcγ receptors II and III also peak around three days and then decline; however, some markers did not decline to control levels. Importantly, performance of a similar time course in APP/PS1 mice demonstrated that the majority of amyloid removal occurred between the time zero and three days, a small further decrease occurred at 7 and 14 days, while amyloid levels, surprisingly, rebounded to near time zero levels by 28 days [7].

In contrast to the amyloid data, LPS injection into tau transgenic mice showed opposite effects. Intraparenchymal injection of LPS into the rTg4510 tau transgenic mice resulted in exacerbation of tau pathology seven days after the injection [8]. This was determined by examining several phosphoepitopes of tau as well as Gallyas's silver staining-positive neurofibrillary tangles. In addition to the standard microglial cell surface markers including CD45, this study identified additional markers of microglial activation stimulated by LPS; these were arginase 1 and YM1. The importance of these markers will be discussed later in this paper. Additionally, LPS injection into the 3XTg mouse model of amyloid and tau pathology exacerbated the tau hyperphosphorylation [9]. These data suggest that tau and amyloid pathologies have opposite responses to the same inflammatory stimuli, in this case LPS. Whether this is the case for all inflammatory stimuli remains to be determined; however, these data should provide significant caution to the extrapolation of findings in amyloid depositing mice to the overall condition of AD.

Anti-Aβ immunotherapy is a potential therapeutic approach to the treatment of AD that uses either a vaccination approach [10] or passive immunotherapy approach [11] to increase levels of circulating anti-Aβ IgG molecules. There have now been numerous studies showing that this approach significantly lowers amyloid pathology and enhances behavioral performance in amyloid depositing transgenic mice (reviewed in [12]). Importantly, we previously performed a series of studies showing that anti-Aβ antibodies stimulate an inflammatory response in the brain. This occurs whether the anti-Aβ antibodies are directly injected into the brain parenchyma [13] or systemically administered in a passive immunization protocol [14]. We were also able to show that inhibition of this inflammatory response attenuates the amyloid reductions significantly [15, 16]. In contrast to the LPS studies, we were able to show in a different transgenic mouse model, the APPSw/NOS2$^{-/-}$ mice that develop amyloid and tau pathologies, that anti-Aβ immunotherapy is able to lower both amyloid and tau pathologies while improving behavioral performance [17]. We examined the breadth of the inflammatory response in both passively immunized APP transgenic mice and actively vaccinated APPSw/NOS2$^{-/-}$ transgenic mice, and we found that anti-Aβ immunotherapy stimulates the gene expression of IL-1β, TNFα, and IL-6 while concomitantly reducing the expression of inflammatory markers associated with wound repair; YM1 and arginase 1 [18]. These data contrast with those found

Table 1: Summary of some transgenic mouse studies that have modulated inflammation and the effects these modulations had on the pathology.

Mode of inflammatory modulation	Genetic model of AD	Pathological changes observed				References
		Amyloid load	Tau pathology	Neuronal degeneration	Microglial "activation"	
LPS intracranial	APP/PS1 amyloid	↓			↑	[5, 7]
LPS intracranial	rTg4510 tau		↑		↑	[8]
Anti-Aβ immunotherapy	APP amyloid	↓			↑	[13, 14]
Anti-Aβ immunotherapy	APP/NOS$^{-/-}$ amyloid, tau, neuron loss	↓	↓	↓	↑	[17]
IL-1β overexpression in brain	APP/PS1 amyloid	↓			↑	[22]
TGFβ overexpression in brain	APP amyloid	↓			↑	[19]
TNFR1 and R2 deletion	3Xtg amyloid and tau	↑			↓	[21]

with LPS injection, where IL-1β, TNFα, YM1, and arginase 1 were all significantly elevated.

Genetic overexpression of individual inflammatory cytokines has yielded data similar to those observed with LPS and anti-Aβ immunotherapy. Increased expression of TGFβ by astrocytes results in reduced amyloid deposition and increased microglial activation in APP amyloid depositing transgenic mice [19]. In addition, an interesting finding in this study showed that while parenchymal amyloid deposition decreased, vascular amyloid deposition (cerebral amyloid angiopathy (CAA)) increased in a correlative manner. We observed a similar phenomenon with the anti-Aβ immunotherapy passive immunization studies, where we found increased CAA despite significantly decreased parenchymal amyloid deposition [20]. The data from Wyss-Coray et al. would suggest that inflammatory mechanisms may at least in part, be responsible for the shifted distribution of amyloid from the brain parenchyma to the cerebrovasculature.

TNFα and IL-1β are considered the major proinflammatory cytokines and are studied as classical markers of neuroinflammation. Individually, both have been implicated in an autotoxic loop as both are capable of inducing cell death in vitro and in vivo. Yet, when these pathways are targeted in amyloid depositing transgenic mice the data show that these cytokine pathways may have some beneficial action by ameliorating amyloid deposition. One study that genetically deleted TNF receptors I and II in the 3XTg mouse model of amyloid deposition and tau pathology showed that blocking TNFα signaling actually increases amyloid deposition and tau pathology [21]. Increased expression of IL-1β in the hippocampus of APP/PS1 amyloid depositing transgenic mice by genetic means resulted in reduced amyloid deposition and enhanced microglial activation [22]. The author suggest that IL-1β-mediated activation of microglia is the mechanism for the reductions in amyloid deposition. However, in contrast to these studies, other studies have shown a clear relationship between IL-1β and neurodegeneration. In a similar way to the LPS studies, IL-1β has been shown to be responsible for tau hyperphosphorylation in an in vitro coculture system of microglia and neurons [23]. Also, a positive correlation was observed when examining IL-1β levels compared to neurodegeneration in the APPV717F transgenic mice [24]. Therefore, while IL-1β may ameliorate amyloid pathology, it seems that the same pathways may also enhance tau pathology and neurodegeneration.

The contrasting data in different mouse models, cell culture models and stimulating agents clearly paints the picture of a complex process, one that cannot simply be defined as neuroinflammation.

3. Peripheral Macrophage Inflammatory States

Macrophages are circulating immune effector cells that are prodigious phagocytes essential for the clearance of cellular debris and invading pathogens. The macrophages monitor the tissue environment and respond rapidly to any perturbations that may occur. Both macrophages and microglia originate from bone marrow hematopoietic stem cells that undergo differentiation into monocytes. These monocytes then undergo further differentiation upon reaching their target tissue to become macrophages, of which there are several types based on their tissue occupancy, or microglia if they enter the brain (reviewed in [25]). Macrophages are well understood to generate a variety of responses dependent upon the stimuli they are presented with. For instance, the presence of interferon-γ (IFNγ) or TNFα from T cells, antigen-presenting cells, or natural killer cells will stimulate the macrophage to express secrete proinflammatory cytokines and produce oxygen and nitrogen radicals. This state is termed classically activated or M1-activated macrophages. The M1 state has high microbicidal activity and is important as a defense mechanism, yet can also cause damage to the host if not tightly regulated [26].

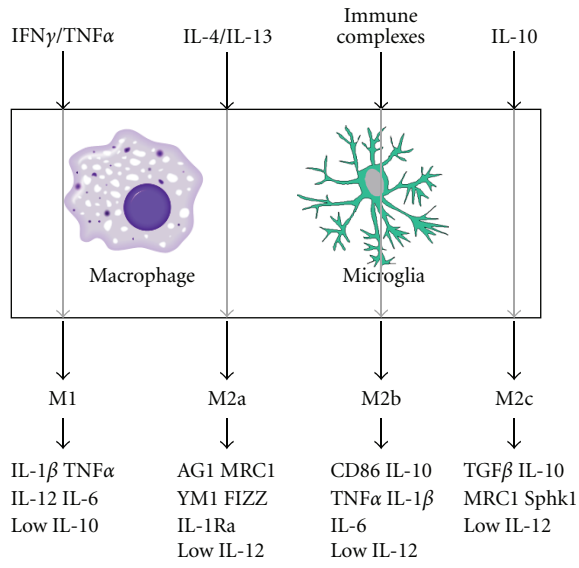


Figure 1: Schematic illustrating the M1, M2a, M2b, and M2c macrophage inflammatory states.

Indeed, classically activated macrophages are implicated in the development of autoimmune pathologies [27].

Stimulation of macrophages by IL-4 and/or IL-13 results in an M2a state, sometimes called a wound-healing macrophage [28]. The M2a macrophage state is characterized by high IL-1 receptor antagonist (IL-1Ra) and high arginase as well as expression of chitinases and other mediators that are known to contribute to the accumulation and reorganization of extracellular matrix [29]. The M2a responses are primarily observed in allergic responses, extracellular matrix deposition, and remodeling. The M2b macrophage state is stimulated by immune complexes (IgG antibody-antigen complexes), toll-like receptor activation, or IL-1 receptor ligands. This state is a combined M1 and M2a state, where arginase is high and IL-12 is low, but IL-1β, IL-6 and TNFα are also high. CD86 also appears to be a relatively specific marker for the M2b state [30]. Finally, the M2c macrophage state is stimulated by IL-10 and is sometimes referred to as a regulatory macrophage with anti-inflammatory activity [30, 31]. These cells express TGFβ and high IL-10 as well as matrix proteins such as pentraxin and versican. The M2c state can also be generated through the hypothalamic-pituitary axis-derived glucocorticoids that inhibit the expression of pro-inflammatory cytokine genes and decrease the mRNA stability of these genes [31]. The M2c macrophages contribute to an environment that results in defective pathogen killing and enhanced survival of organisms. The macrophage states are summarized in Figure 1.

4. Applying the Macrophage Classification to Microglia and AD

Neuroinflammatory markers have been identified in the macrophage literature that can be applied to the study of microglia. Indeed, microglia are capable of expressing many of the macrophage markers identified in Figure 1. My laboratory, and others, has shown that the brains of amyloid-depositing mice, tau transgenic mice, and human AD expressing IL-1β, TNFα, IL-6, YM1, arginase 1, mannose receptor, TGFβ, and IL-1Ra, among others. Most recently, my laboratory has performed in vitro studies on BV2 microglial cells to show that, given the correct stimulus, these microglial cells can generate very specific macrophage-like inflammatory responses using the M1, M2a, M2b, and M2c classifications. Figure 2 shows the gene expression data obtained from BV2 microglial cells treated for 12 hours with IFNγ and TNFα to induce an M1 response, IL-4 and IL-13 to induce an M2a response, anti-Aβ IgG-Aβ immune complexes to induce and M2b response and, finally, IL-10 to induce an M2c response. As can be seen from the graphs in Figure 2, twelve hours of treatment of BV2 cells induced specific responses characterized by the expression of markers matching those described in the macrophage literature. These data suggest that microglia, given the correct stimuli, are capable of generating a range of responses similar to the macrophage. It will be important to follow up these studies in primary microglial cells to confirm that these findings are not unique to the immortalized BV2 microglial cell line.

My laboratory recently showed that passive immunization with anti-Aβ antibodies results in a shift in the inflammatory state of the brains of amyloid depositing transgenic mice. Tg2576 APPSw transgenic mice aged 18 months are normally biased to the M2a and M2c inflammatory states. Following only one month of weekly anti-Aβ antibody injections, the inflammatory state transitioned from M2a and M2c to M1; this was maintained following two and three months of administration [18]. Since the change occurred prior to significant reductions in amyloid deposition, it is likely that this inflammatory state transition is, at least partially, responsible for the reductions in amyloid due to the passive immunotherapy.

The concept that M1 inflammatory state in the brain is associated with lower amyloid burden is supported by several studies that have examined components of this inflammatory state. For instance, IL-1β overexpression in the hippocampus of APP/PS1 transgenic mice results in decreased amyloid burden [22]. IL-1β is an M1 cytokine, so its overexpression may be reproducing the effect of an M1 neuroinflammatory state in the brain. Additionally, inhibition of TNFα signaling by the deletion of TNFRI and II in the 3XTg mice also showed reduced amyloid load [21]. Since TNFα is another M1 cytokine, the deletion of its signaling represents an artificial suppression of the M1 inflammatory state. Finally, LPS stimulates the secretion of IL-1β, TNFα, and IL-6; all M1 cytokines [6, 33]. LPS has been shown in several studies to significantly lower amyloid load in APP/PS1 transgenic mice.

The influence of other inflammatory states (M2) on amyloid load is less established. Few studies have directly targeted any of the M2 inflammatory pathways to establish cause-effect relationships between these states and amyloid deposition. The overexpression of TGFβ may represent a bias toward the M2c inflammatory state however, in the

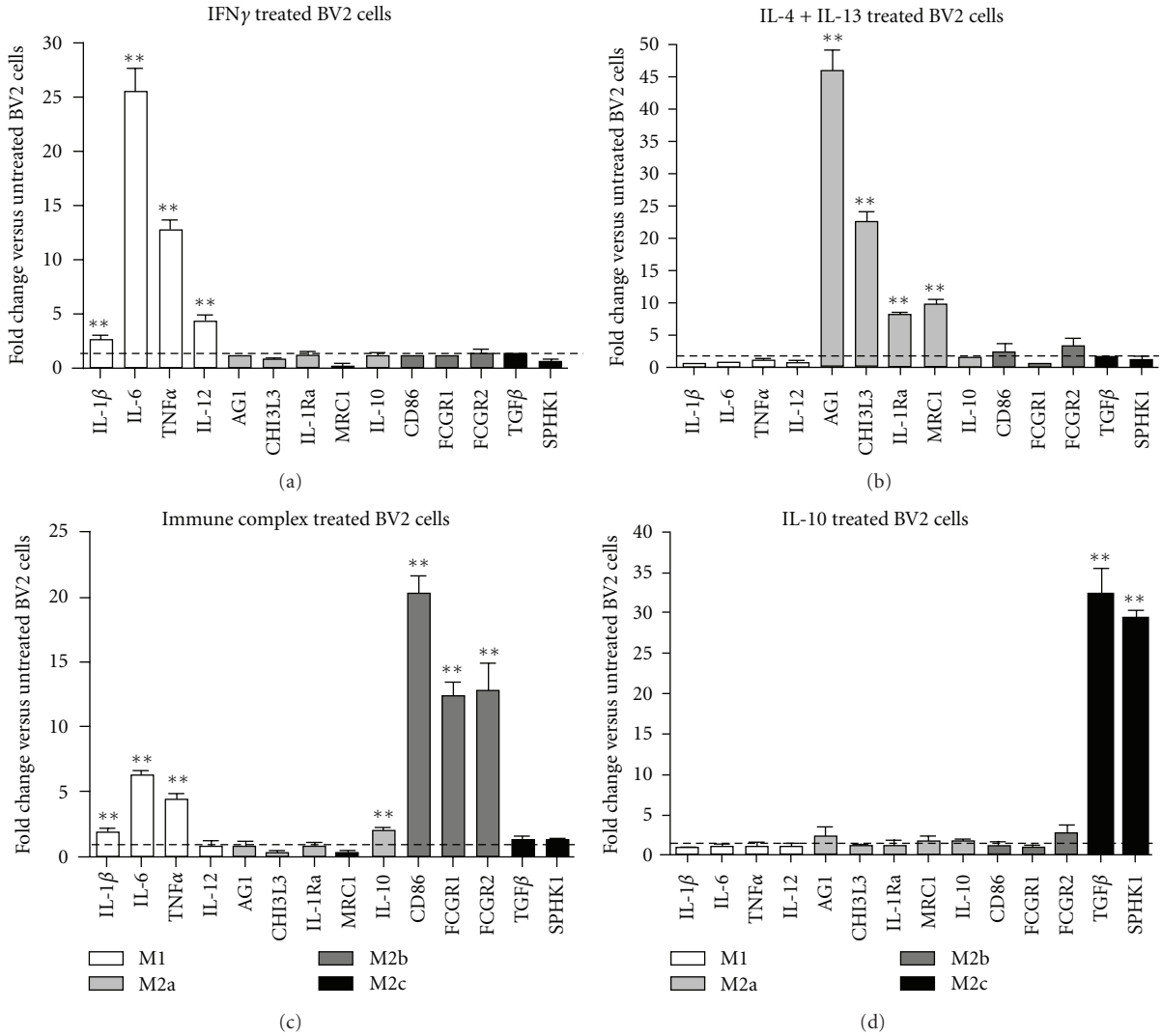


Figure 2: Stimulation of BV2 cultured microglial cells to polarize the response to M1, M2a, M2b, or M2c. BV2 microglial cells were cultured in normal DMEM media. When confluent, cell media was changed to serum-free DMEM media for 24 hours. Then media was changed to either DMEM plus IFNγ (2.5 ng/mL) to stimulate an M1 response (a), DMEM plus IL-4 (20 ng/mL) and IL-13 (20 ng/mL) to stimulate an M2a response (b), DMEM plus immune complexes prepared as described in [32] (5 μg/mL Aβ coated with IgG) to stimulate an M2b response (c), DMEM plus IL-10 (10 ng/mL) to stimulate an M2c response (d), or DMEM alone to act as an untreated control. Cells were then harvested 12 hours after the start of treatment. We repeated the experiments 3 times, each on separate cultures of different passage numbers. Data are shown as fold change compared to untreated control BV2 cells. $^{*}P < 0.05$ and $^{**}P < 0.01$.

absence of a characterization of the inflammatory milieu in these mice it is impossible to draw conclusions. It is apparent, however, from studies performed in aged transgenic mice that as amyloid accumulates in the brains of mice, the inflammatory state becomes increasingly polarized to the M2a inflammatory state. Also, my laboratory recently showed that lithium treatment enhances the M2a and M2c inflammatory phenotypes in APPSwDI/NOS2$^{-/-}$ transgenic mice and increases amyloid deposition in the absence of changes in total Aβ measured biochemically. Until further studies are performed that directly enhance the M2a state using agents identified in the macrophage literature the exact relationship between the M2a state and amyloid deposition will remain unknown.

The relationship between neurofibrillary tangle pathology and the inflammatory state of the brain is relatively understudied in comparison to amyloid pathology. The few studies that have been done to establish relationships of hyperphosphorylated tau and neuroinflammation would suggest an opposite relationship to that of amyloid and neuroinflammation. Where M1 inflammatory phenotypes appear to ameliorate the amyloid pathology in numerous studies, induction of M1 phenotypes in tau transgenic mice or cell culture results in the exacerbation of tau pathology. LPS injection into the brains of rTg4510 tau transgenic mice has shown that the tau hyperphosphorylation and neurofibrillary tangle pathology are increased due to the LPS. Importantly, this study used an identical protocol to

that used in APP/PS1 mice to show amyloid reductions due to LPS. Also, LPS injection in the 3XTg model of amyloid and tau pathology showed exacerbation of tau hyperphosphorylation after LPS injection [9]. Additionally, IL-1β treatment of microglia/neuron cocultures results in significant hyperphosphorylation of tau protein in the neuron [23]. Since IL-1β is an M1 cytokine, this again suggests that the M1 inflammatory state worsens the tau pathology associated with AD. Finally, my laboratory showed that biasing APPSwDI/NOS2$^{-/-}$ mice to the M2a state ameliorates tau hyperphosphorylation normally present in these mice.

Little has been studied in the human AD brain with respect to neuroinflammatory profiles, where a complete spectrum of M1 and M2 inflammatory markers has been examined. It has been shown that human AD brain is capable of expressing an array of inflammatory markers spanning the M1, M2a, M2b, and M2c inflammatory states [34]. Additionally, data from the ADAPT clinical trial that studied the preventative properties of nonsteroidal anti-inflammatory drugs (NSAIDs) in AD suggest that neuroinflammation may be complex and variable in the human population, since a subset of patients responded well to NSAIDs, while others declined more rapidly in response to the same NSAID [35]. Also, immunotherapy trials continue to show amyloid reductions in humans, while tau pathology remains relatively unchanged [36]. Future studies in humans should be focused on identifying the relationship between the pathologies of AD and the neuroinflammatory states.

5. Summary and Conclusions

In summary, this paper has described that the macrophage inflammatory states of M1, M2a, M2b, and M2c, that are extremely well characterized in the immunology field, can be applied when examining the inflammatory state of the brain. By applying these states to the body of literature on the role of neuroinflammation in AD, the field can begin to establish cause-effect relationships for each neuroinflammatory state. It can be concluded that neuroinflammation is a complex, diverse process that can be characterized by examining a profile of markers associated with distinct inflammatory states. Finally, it is essential that more attention be focused on identifying relationships between each inflammatory state and each AD pathology so that the AD field can better target in a more directed, personalized manner to therapeutically treat AD.

References

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

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

[3] E. G. McGeer and P. L. McGeer, "The importance of inflammatory mechanisms in Alzheimer disease," *Experimental Gerontology*, vol. 33, no. 5, pp. 371–378, 1998.

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

[5] G. DiCarlo, D. Wilcock, D. Henderson, M. Gordon, and D. Morgan, "Intrahippocampal LPS injections reduce Aβ load in APP+PS1 transgenic mice," *Neurobiology of Aging*, vol. 22, no. 6, pp. 1007–1012, 2001.

[6] D. L. Herber, J. L. Maloney, L. M. Roth, M. J. Freeman, D. Morgan, and M. N. Gordon, "Diverse microglial responses after intrahippocampal administration of lipopolysaccharide," *GLIA*, vol. 53, no. 4, pp. 382–391, 2006.

[7] D. L. Herber, L. M. Roth, D. Wilson et al., "Time-dependent reduction in Aβ levels after intracranial LPS administration in APP transgenic mice," *Experimental Neurology*, vol. 190, no. 1, pp. 245–253, 2004.

[8] D. C. Lee, J. Rizer, M. L. B. Selenica et al., "LPS- induced inflammation exacerbates phospho-tau pathology in rTg4510 mice," *Journal of Neuroinflammation*, vol. 7, article 56, 2010.

[9] M. Kitazawa, S. Oddo, T. R. Yamasaki, K. N. Green, and F. M. LaFerla, "Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease," *Journal of Neuroscience*, vol. 25, no. 39, pp. 8843–8853, 2005.

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

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

[12] D. M. Wilcock and C. A. Colton, "Anti-amyloid-β immunotherapy in Alzheimer's disease: relevance of transgenic mouse studies to clinical trials," *Journal of Alzheimer's Disease*, vol. 15, no. 4, pp. 555–569, 2008.

[13] D. M. Wilcock, G. DiCarlo, D. Henderson et al., "Intracranially administered anti-Aβ antibodies reduce β-amyloid deposition by mechanisms both independent of and associated with microglial activation," *Journal of Neuroscience*, vol. 23, no. 9, pp. 3745–3751, 2003.

[14] D. M. Wilcock, A. Rojiani, A. Rosenthal et al., "Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition," *Journal of Neuroscience*, vol. 24, no. 27, pp. 6144–6151, 2004.

[15] D. M. Wilcock, S. K. Munireddy, A. Rosenthal, K. E. Ugen, M. N. Gordon, and D. Morgan, "Microglial activation facilitates Aβ plaque removal following intracranial anti-Aβ antibody administration," *Neurobiology of Disease*, vol. 15, no. 1, pp. 11–20, 2004.

[16] D. M. Wilcock, J. Alamed, P. E. Gottschall et al., "Deglycosylated anti-amyloid-β antibodies eliminate cognitive deficits and reduce parenchymal amyloid with minimal vascular consequences in aged amyloid precursor protein transgenic mice," *Journal of Neuroscience*, vol. 26, no. 20, pp. 5340–5346, 2006.

[17] D. M. Wilcock, N. Gharkholonarehe, W. E. Van Nostrand, J. Davis, M. P. Vitek, and C. A. Colton, "Amyloid reduction by amyloid-β vaccination also reduces mouse tau pathology and protects from neuron loss in two mouse models of Alzheimer's

disease," *Journal of Neuroscience*, vol. 29, no. 25, pp. 7957–7965, 2009.

[18] D. M. Wilcock, Q. Zhao, D. Morgan et al., "Diverse inflammatory responses in transgenic mouse models of Alzheimer's disease and the effect of immunotherapy on these responses," *ASN Neuro*, vol. 3, no. 5, pp. 249–258, 2011.

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

[20] D. M. Wilcock, A. Rojiani, A. Rosenthal et al., "Passive immunotherapy against Aβ in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage," *Journal of Neuroinflammation*, vol. 1, article 24, 2004.

[21] S. L. Montgomery, M. A. Mastrangelo, D. Habib et al., "Ablation of TNF-RI/RII expression in Alzheimer's disease mice leads to an unexpected enhancement of pathology: implications for chronic pan-TNF-α suppressive therapeutic strategies in the brain," *American Journal of Pathology*, vol. 179, no. 4, pp. 2053–2070, 2011.

[22] S. S. Shaftel, S. Kyrkanides, J. A. Olschowka, J. N. H. Miller, R. E. Johnson, and M. K. O'Banion, "Sustained hippocampal IL-1β overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology," *Journal of Clinical Investigation*, vol. 117, no. 6, pp. 1595–1604, 2007.

[23] Y. Li, L. Liu, S. W. Barger, and W. S. T. Griffin, "Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway," *Journal of Neuroscience*, vol. 23, no. 5, pp. 1605–1611, 2003.

[24] J. G. Sheng, R. E. Mrak, R. A. Jones et al., "Neuronal DNA damage correlates with overexpression of interleukin-1β converting enzyme in APPV717F mice," *Neurobiology of Aging*, vol. 22, no. 6, pp. 895–902, 2001.

[25] D. M. Mosser and J. P. Edwards, "Exploring the full spectrum of macrophage activation," *Nature Reviews Immunology*, vol. 8, no. 12, pp. 958–969, 2008.

[26] D. C. Dale, L. Boxer, and W. Conrad Liles, "The phagocytes: neutrophils and monocytes," *Blood*, vol. 112, no. 4, pp. 935–945, 2008.

[27] Z. Szekanecz and A. E. Koch, "Macrophages and their products in rheumatoid arthritis," *Current Opinion in Rheumatology*, vol. 19, no. 3, pp. 289–295, 2007.

[28] J. P. Edwards, X. Zhang, K. A. Frauwirth, and D. M. Mosser, "Biochemical and functional characterization of three activated macrophage populations," *Journal of Leukocyte Biology*, vol. 80, no. 6, pp. 1298–1307, 2006.

[29] Z. Zhu, T. Zheng, R. J. Homer et al., "Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation," *Science*, vol. 304, no. 5677, pp. 1678–1682, 2004.

[30] D. M. Mosser, "The many faces of macrophage activation," *Journal of Leukocyte Biology*, vol. 73, no. 2, pp. 209–212, 2003.

[31] E. M. Sternberg, "Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens," *Nature Reviews Immunology*, vol. 6, no. 4, pp. 318–328, 2006.

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

[33] M. P. Vitek, C. M. Brown, and C. A. Colton, "APOE genotype-specific differences in the innate immune response," *Neurobiology of Aging*, vol. 30, no. 9, pp. 1350–1360, 2009.

[34] C. A. Colton, R. T. Mott, H. Sharpe, Q. Xu, W. E. Van Nostrand, and M. P. Vitek, "Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD," *Journal of Neuroinflammation*, vol. 3, article 27, 2006.

[35] J. M. S. Leoutsakos, B. O. Muthen, J. C. Breitner, and C. G. Lyketsos, "Effects of non-steroidal anti-inflammatory drug treatments on cognitive decline vary by phase of preclinical Alzheimer disease: findings from the randomized controlled Alzheimer's Disease Anti-inflammatory Prevention Trial," *International Journal of Geriatric Psychiatry*, vol. 27, no. 4, pp. 364–374, 2011.

[36] C. Holmes, D. Boche, D. Wilkinson et al., "Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial," *The Lancet*, vol. 372, no. 9634, pp. 216–223, 2008.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 918680, 9 pages
doi:10.1155/2012/918680

Review Article

Is There Inflammatory Synergy in Type II Diabetes Mellitus and Alzheimer's Disease?

Lih-Fen Lue,[1] Cassandra Andrade,[1] Marwan Sabbagh,[2] and Douglas Walker[3]

[1] *Laboratory of Neuroregeneration, Banner Sun Health Research Institute, 10515 West Santa Fe Drive, Sun City, AZ 85351, USA*
[2] *Cleo Roberts Center for Clinical Research, Banner Sun Health Research Institute, 10515 West Santa Fe Drive, Sun City, AZ 85351, USA*
[3] *Laboratory of Neuroinflammation, Banner Sun Health Research Institute, 10515 West Santa Fe Drive, Sun City, AZ 85351, USA*

Correspondence should be addressed to Lih-Fen Lue, lihfen.lue@bannerhealth.com

Received 31 January 2012; Accepted 19 April 2012

Academic Editor: Joseph El Khoury

Metabolic dysregulation, including abnormal glucose utilization and insulin resistance or deficiency, occurs at an early stage of AD independent of type II diabetes mellitus (T2DM). Thus, AD has been considered as type 3 diabetes. T2DM is a risk factor for AD; the coexistence of these two diseases in a society with an increasing mean age is a significant issue. Recently, research has focused on shared molecular mechanisms in these two diseases with the goal of determining whether treating T2DM can lessen the severity of AD. The progress in this field lends strong support to several mechanisms that could affect these two diseases, including insulin resistance and signaling, vascular injuries, inflammation, and the receptor for advanced glycation endproducts and their ligands. In this paper, we focus on inflammation-based mechanisms in both diseases and discuss potential synergism in these mechanisms when these two diseases coexist in the same patient.

1. Introduction

Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are diseases prevalent in the elderly population. T2DM can increase the risk for developing dementia by 1.5- to 2-fold, and it is considered an important risk factor for AD [1–8]. As the prevalence rate of T2DM is the highest in the age group 65 and older (26.8% in year 2010 according to Center for Disease Controls and Prevention; http://www.cdc.gov/diabetes/pubs/estimates07.htm), it is a serious concern how T2DM might impact the prevalence rate of AD, and how it might affect the treatment of AD patients. As the mean population age is increasing, both of these two diseases could become much more significant issues. The issue could be further compounded by the epidemic-like phenomenon of obesity that is spreading across all ages [9–11]. At the current annual increase of 0.3–0.6%, there could be 75% of adults that are overweight or obese by 2015 [11]. Obesity is a major risk factor for developing T2DM [2, 12]. Moreover, obesity in middle-age subjects is a negative modifier of T2DM [13]. It has been shown recently that insulin resistance, which is also a risk factor for AD, is associated with lower brain volume and executive function in a large, relatively healthy, middle-aged, community-based cohort [14]. A lack of comprehensive preventive and intervention strategies for these interlinked diseases could lead to a more severe crisis for the healthcare system and the health of the public [15].

There has been promising progress made in identifying links between T2DM and dementia in the last decade. Special research attention has been directed towards the mechanisms by which T2DM may affect cognitive function and pathogenesis of AD, and towards determining whether treating T2DM might be effective in reducing incidence of AD by modifying AD pathogenesis. The major mechanisms through which T2DM may influence AD include insulin resistance, impaired insulin receptor (IR), and insulin growth factor (IGF) signaling, glucose toxicity, advanced glycation endproducts (AGEs) and the receptor for advanced glycation endproducts (RAGEs), cerebrovascular

injury, vascular inflammation, and others [5, 16–20]. There are a number of comprehensive reviews available on insulin resistance and growth factor signaling as molecular mechanisms linking AD and T2DM [8, 16, 17, 21]. Additional discussion focusing on whether there is a causal relationship between AD and T2DM from the studies of epidemiology, clinical trials, and imaging can be found in a review article published in the March issue of Journal of Alzheimer's Disease [18].

The goal of this paper is to focus on a less studied topic: how inflammation-based mechanisms in T2DM might affect AD neuroinflammation and microglial activation. As T2DM and AD both have significant inflammatory components, it is important to assess whether inflammation is synergized when these two diseases coexist. As there has been little research conducted on this aspect, we will review inflammatory mechanisms with respect to each disease and discuss the possibility for these mechanisms to converge.

2. Inflammation and Diabetes

An association of inflammation with T2DM can possibly be demonstrated before clinical diagnosis. This is based on several epidemiological studies that demonstrated greater white blood cell counts or higher levels of inflammatory markers, including C-reactive protein (CRP) and interleukin-6 (IL-6) in healthy middle-aged subjects who later developed T2DM [22–24]. However, not only is chronic inflammation a risk factor for developing T2DM, but it is also an important contributor to the pathogenic mechanisms.

2.1. IL-1β and Its Receptor. The beta cells from T2DM subjects contain elevated levels of IL-1β, a potent proinflammatory cytokine, and reduced levels of IL-1 receptor antagonist (IL-1ra) [25]. IL-1ra is a naturally produced molecule that inhibits IL-1β activity on its receptor, IL-1 receptor [26]. *In vitro* studies demonstrated that IL-1β increased release of insulin by pancreatic islet cells in the presence of high glucose concentration and promoted glucose oxidation [27]. Islet beta cells can be damaged by exposure to IL-1β, in a dose- and time-dependent manner [28]. High glucose concentration induced IL-1β expression, but reduced expression of IL-1ra, resulting in an imbalance between IL-1β and IL-1ra, which impaired insulin secretion and cell proliferation and increased apoptosis [29]. A study in T2DM GK rats has shown that IL-1ra treatment at high dose improved glucose sensitivity, insulin processing, and suppressed inflammation and infiltration of immune cells [30]. The GK rats developed T2DM at a young age and the pancreatic tissues expressed elevated levels of IL-1β, and IL-1β-driven inflammatory cytokines and chemokines such as tumor necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1alpha (MIP-1α), along with abnormal infiltration of macrophages and granulocytes [30]. This study supported that an imbalance between IL-1β and IL-1ra leads to pancreatic islet inflammation and release of insulin. Clinical trials using anakinra, a recombinant human IL-1ra, or inhibition of IL-1 receptor signaling has shown effectiveness in correcting beta cells dysfunction and reduced systemic inflammation in T2DM [31, 32]. In fact, IL-1ra is the only anti-inflammatory treatment approved by Food and Drug Administration for T2DM [33].

2.2. RAGE and the Ligands. The receptor for advanced glycation endproducts (RAGE), a pattern-recognition receptor, interacts with its ligands resulting in persistent inflammatory responses at sites where the ligands concentrate. These mechanisms have been shown to play a pivotal role in propagation of vascular injuries, a major complication of diabetes [34–37]. The major RAGE ligands in diabetes are advanced glycation endproducts (AGEs), which are derivatives of lipids, proteins, and ribonucleic acids. These are modified by nonenzymatic glycosylation, followed by rearrangement, dehydration, and eventually becoming irreversible cross-linked macromolecules [38, 39]. The amount of these heterogeneous products increases with age, but is further enhanced by diabetes or hyperglycemic conditions [40–42]. Circulating neutrophils can play a role in enhancing the formation of AGE in response to inflammatory activation of the myeloperoxidase system [43]. Diabetes-associated RAGE-AGE interactions induced reactive oxygen species-mediated inflammatory responses in vascular cells (endothelial cells, smooth muscle cells, and pericytes) and mononuclear phagocytes; all of these cells are critically involved in diabetes-associated atherosclerosis, nephropathy, and retinopathy [37, 44–48].

Recent evidence also demonstrated that RAGE is involved in inflammation-based mechanisms of islet cell death. Activation of RAGE by S100B and high mobility group box 1 (HMG1) caused apoptotic death of pancreatic beta cells through an NADPH oxidase-mediated mechanism [49]. The interaction of AGE with RAGE induced apoptosis of islet beta cell and impaired the function of secreting insulin in an *in vitro* study [50]. Inhibition of AGE formation and blockade of RAGE-mediated chronic inflammatory mechanisms are currently considered to be therapeutic strategies for diabetes and diabetes-associated macro- and microvascular complications [51–54].

Human vascular cells express a novel splice variant of the RAGE gene that encodes for a soluble RAGE protein, named endogenous secretory RAGE (esRAGE). The esRAGE protein neutralizes the action of AGE on vascular cells, thus preventing AGE from activating cell-surface (or full-length) RAGE signaling [55]. There is another form of soluble RAGE (sRAGE) that is not generated by alternative splicing; instead, it is a product of catalytic cleavage of membrane bound full-length RAGE by enzymes such as a disintegrin and metalloprotease 10 [56–58]. There was a negative correlation between the expression levels of full-length membrane RAGE and sRAGE expression in monocytes from T2DM [59]. Enhancing sRAGE-associated protective mechanisms are also molecular targets in developing T2DM therapeutics [60].

2.3. Other Pattern-Recognition Receptors. Toll-like receptors (TLRs) are pattern-recognition receptors consisting of 12

family members in humans. They are crucial for innate immune functions. Evidence has emerged that some of the TLR members are involved in mediating inflammatory responses in metabolic disorders. TLR2 and TLR4 expressions were elevated in the cell surface of monocytes, derived from patients with metabolic syndrome, and released higher levels of IL-1β, IL-6, and Il-8 following lipopolysaccharide stimulation [61, 62]. High glucose increases the expression of TLR2 and TLR4, which can be accentuated by the presence of free fatty acids [63, 64]. These effects were mediated via protein kinase C (PKC)-α/PKC-δ by stimulation of NADPH oxidase [63]. The inflammatory responses induced by TLR2 and TLR4 are mediated through the activation of NF-κB [65]. TLR4 is upregulated in pancreatic islet cells and a chemokine ligand, interferon-inducible protein (IP)-10 (or CXCL10), was identified to activate this receptor leading to islet cell death [66]. IP-10 can be induced by high glucose through TLR2 and TLR4 [67].

CD36 (oxidized low-density lipoprotein receptor, oxLDL receptor, or scavenger receptor B, MSR-B) is also a pattern recognition receptor which serves as a co-receptor for TLR2 and TLR6 heterodimers, as well as TLR4 and TLR6 heterodimers [68]. High glucose, oxLDL, free fatty acids, and low high density lipoprotein receptors (HDLs) cholesterol concentrations were shown to increase the expression of CD36 in monocytes/macrophages, resulting in vascular oxidative injury, increased leukocyte adhesion, and promoting atherogenesis [69]. Deficiency of CD36 in transgenic mice improves insulin signaling, inflammation, and atherogenesis [70, 71].

3. Diabetes and Alzheimer's Disease Pathology

There have been several studies investigating whether T2DM worsens the hallmark pathology of AD, namely, neuritic plaques and neurofibrillary tangles. In a study involving 143 diabetic and 567 nondiabetic AD patients, no differences were observed between these two groups in Aβ load, neuritic plaque, and neurofibrillary tangle scores [72]. In another study, the presence of diabetes has even been shown to be negatively associated with the abundance of neuritic plaques and neurofibrillary tangles [73]. In line with this finding, Nelson and colleagues observed that although AD patients with diabetes had significantly more infarcts and vascular damage, the plaque scores, as measured by Consortium to Establish a Registry for Alzheimer Disease criteria, were significantly lower [74]. Using biochemical and histological approaches, Sonnen et al. found inconsistent results between biochemical and neuropathological results [75]. Using formic acid to extract detergent-insoluble Aβ from amyloid deposits in superior and medial temporal samples, they found that the concentrations of Aβ42 in formic-acid extract were significantly higher in AD patients without T2DM than in AD patients with T2DM. This was regardless of neuritic plaque scores and neurofibrillary tangle distribution that did not differ between AD cases with and those without T2DM. The same study also investigated whether T2DM leads to more oxidative reactivity and neuroinflammation. The results showed that AD cases without T2DM had significantly higher levels of free-radicals as measured by F_2-isoprostanes, whereas AD cases with T2DM had significantly greater IL-6 concentrations in cortical tissues than AD without T2DM. It is worth noting that IL-6 is one of three key acute phase proteins shown to be significantly elevated in temporal cortical samples of AD subjects [51]. Neurons in the brain of T2DM patients could be more vulnerable to the toxicity of Aβ due to the defective insulin receptor signaling [76]. Conversely, the defect in insulin receptor signaling could lead to increased production of Aβ and Aβ-induced oxidative damage of the mitochondria [17]. These are among the mechanisms that increase the neuronal degeneration in association with the condition of T2DM.

When determining whether T2DM affects the types and development of amyloid plaques, a significant increase in Aβ40-immunoreactive dense plaques, but not in cored plaques, was observed [77]. Dense plaques are considered to be at an earlier stage of maturation, and more toxic than core-only plaques (or burnt-out plaques). Using RAGE immunoreactivity as a marker for oxidatively stressed cells, the authors detected a significant increase in RAGE-immunoreactive cells in the hilus of dentate gyrus in AD cases with T2DM than in AD cases without T2DM [77]. It is intriguing how T2DM might affect the maturation of amyloid plaques. Could this be mediated through its effects on microglial activation? The authors noticed a looser association of activated microglia with dense plaques in AD subjects with T2DM when compared to AD subjects without T2DM. There could be several possible interpretations for this finding. It could suggest that there was an enhanced microglial phagocytic function in AD with T2DM, thus facilitating the removal of amyloid surrounding the amyloid core. This could also be due to the modification of microglia activation state by additional stimuli in AD with T2DM. Previous research has shown the association of primed, enlarged, or phagocytic microglia with amyloid plaques of different maturation stages [78]. When IL-1α used as a marker for microglial activation, a greater number of IL-1α-immunoreactive microglia were associated with diffuse neuritic amyloid plaques, but they did not associate with nonneuritic dense core plaques [78, 79]. A more detailed analysis is necessary to elucidate whether co-existence of T2DM with AD alters the development of amyloid plaques, as well as the phenotypic and functional characteristics of microglia activation. This would require utilization of various microglial activation markers along with antibodies that can detect Aβ40- or Aβ42-predominant amyloid plaques, and antibodies that can detect neuritic components within the plaques. The potential effects of T2DM on microglia activation during development of AD are proposed in Figure 1.

4. RAGE-Mediated Inflammation in AD Brain

RAGE-mediated mechanisms play crucial roles in the pathogenesis of T2DM and associated vascular complications, but RAGE is also an important cell-signaling receptor involved

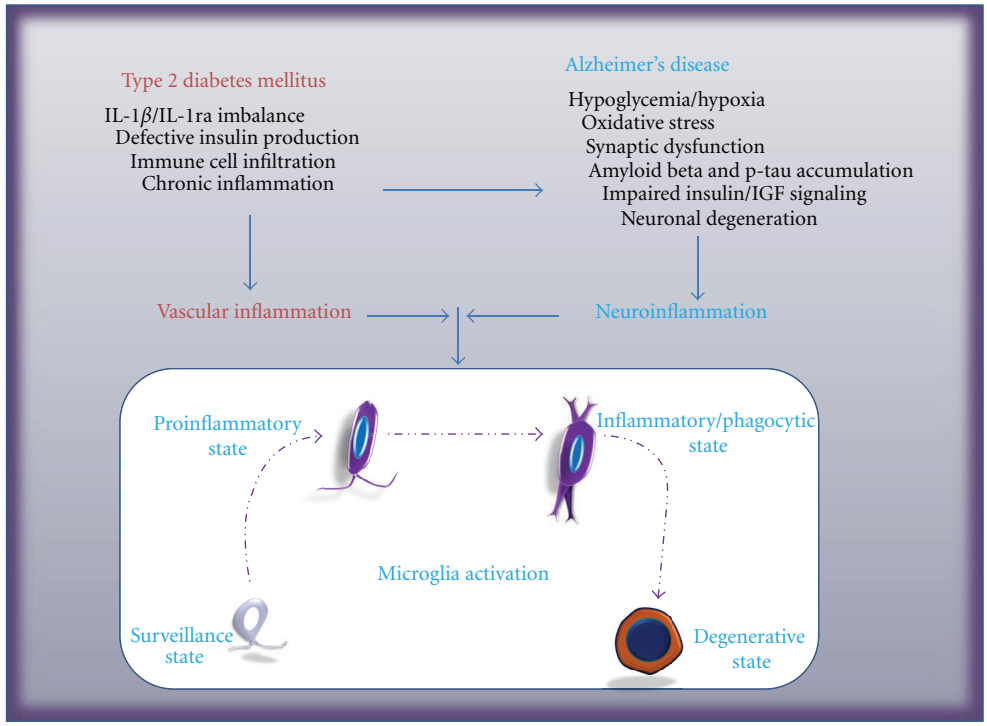


Figure 1: Potential effects of type 2 diabetes mellitus on microglial activation in Alzheimer's disease. Type 2 diabetes mellitus (T2DM) affects the brain with chronic impairment of insulin production and glycaemic control in the periphery. T2DM also causes macro- and microvascular diseases in which inflammation plays a pivotal role. Cerebral microvascular diseases developed from T2DM complications lead to compromised blood-brain barrier function and endothelial cell activation. Microglia can respond to vascular injury and inflammation. Microglial activation is a process of functional and morphological transformation. We propose here that they can be staged as surveillance, proinflammatory, inflammatory, phagocytic, and degenerative states; the transformation depends on the type, distance, potency, and duration of stimulation. We propose that T2DM might promote the activation of microglia through vascular inflammation and the effects on neuronal metabolic dysfunction.

in various aspects of AD. RAGE is expressed in the brain in neurons, microglia, and astrocytes [80–82]. Aβ is a specific ligand for RAGE, which interacts with the N-terminal domain of RAGE [83]. RAGE expression was elevated in AD pathology-enriched brain regions, including hippocampus and inferior frontal cortex, when compared to cerebellum where AD pathology is limited. RAGE expression was also increased in neurons and microglia in the hippocampus [80, 82]. The interaction of Aβ with neuronal RAGE leads to reactive oxygen species-mediated cellular stress and activation of the transcription faction NF-κB, resulting in increased inflammatory gene and protein expression. For example, elevated secretion of macrophage colony-stimulating factor (M-CSF) and tumor necrosis factor alpha (TNF-α) by microglia and BV-2 cells was observed [80, 84]. In experiments using cultures of postmortem human microglia and an *in vitro* Aβ plaque model, Aβ-induced directional migration of microglia was shown to be RAGE-dependent. This was shown by the inhibition of microglial migratory responses to Aβ when RAGE was blocked by anti-RAGE $(\text{Fab}')_2$ [80]. The involvement of RAGE-mediated microglial activation in exacerbation of synaptic degeneration, neuroinflammation, and Aβ levels has been illustrated in a study that compared human amyloid precursor protein (APP) single-transgenic mice to double-transgenic mice over expressing the human RAGE gene in microglia along with mutated APP transgene [85, 86]. Enhanced IL-1β and TNF-α production, increased infiltration of microglia and astrocytes in amyloid plaques, increased levels of Aβ40 and Aβ42, reduced acetylcholine esterase (AChE) activity, and accelerated deterioration of spatial learning/memory were observed in the double-transgenic mice when compared to single transgenic APP or RAGE mice [85]. The involvement of microglial RAGE in driving these consequences was further elucidated in the same study by using signal transduction-defective mutant RAGE [dominant negative (DN)-RAGE] to microglia. The DN-RAGE gene in APP transgenic mice prevented the loss of AChE activity, reduced plaque load, and improved spatial and memory functions [85]. These findings demonstrated that RAGE signaling in microglia played a critical role in promoting inflammatory responses that could lead to increase in Aβ levels and synaptic dysfunction.

Increased association of AGEs, a RAGE ligand, has been observed in amyloid deposits, and in astrocytes and microglia. This correlated with increased inducible nitric oxide synthase in AD pathology-rich area [87]. The nitric oxide-mediated oxidative mechanisms can mediate the cytotoxicity of AGE [88]. Other RAGE ligands upregulated in

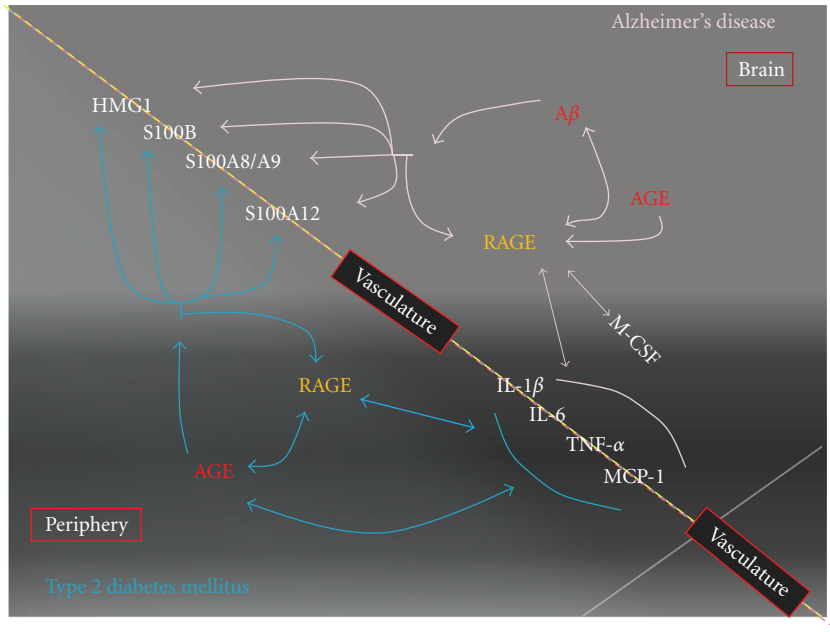


Figure 2: RAGE-driven inflammatory synergy in Alzheimer's disease with type 2 diabetes mellitus. Receptor for advanced glycation endproducts- (RAGEs) mediated inflammatory responses play an important roles in pathogenesis of Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM). In AD, Aβ is the most prominent ligand that interacts with RAGE leading to inflammatory signaling. The interaction also leads to microglia secretion of M-CSF which can further upregulate the expression of RAGE in microglia. Other inflammatory cytokines and chemokines are also produced upon the activation of RAGE, including IL-1β, IL-6, TNF-α, and MCP-1. Several of these inflammatory mediators also can modulate the expression of RAGE and its ligands. A number of other ligands are also expressed at elevated levels in the AD brain including AGE, S100A8, S100A9, S100A12, S100B, and HMG1. In T2DM, advanced glycation endproducts (AGEs) are the major ligand. Interaction with RAGE, AGE induces production of other RAGE ligands and inflammatory cytokines and chemokines, which is the major mechanism for propagation of vascular inflammatory injury in T2DM-associated vascular diseases. Thus, RAGE-mediated inflammatory responses might be accentuated when these two diseases coexist in the same patient.

AD brains include S100B, S100A9, S100A12, and HMG1 [89, 90]. Although S100B and S100A8 are known as inflammatory cytokines of myeloid phagocytes, their expression by human microglia can be induced by chronic exposure to Aβ1-42 [91].

Increases in formation of AGE could also result in upregulation of macrophage scavenger receptor CD36. Elevated expression of CD36 correlated with the presence of amyloid deposits, but not the clinical diagnosis of AD. The expression of CD36 by microglia promotes adhesion to fibrillar Aβ, increases oxidative stress and proinflammatory responses, and affects microglial uptake of Aβ [92].

5. RAGE, Ligands, and Cytokine Cascade

One feature that makes RAGE a critical inflammatory receptor is that its expression is increased by its ligands; this creates a positive feedback mechanism that can perpetuate inflammation once it sets off [37, 46, 93, 94]. The amplification of inflammatory consequences can also be further fueled by additional cytokines. For example, in monocytic lineage cells, preexposure to AGE followed by treatment of IL-6 or TNF-α can induce release of the RAGE ligands, S100A8 and S100A9 [95]. Preexposure of endothelial cells to AGE has also been shown to increase IL-6, intercellular adhesion molecule-1, vascular adhesion molecule-1, and MCP-1 upon stimulation with S100A8/A9 heterodimers [93]. These findings illustrate how RAGE and its ligands can combine with cytokine-mediated inflammation to exacerbate chronic inflammatory diseases such as AD and T2DM.

As in T2DM, there is a deficiency in the anti-inflammatory function of sRAGE in AD due to a gradual decline in the circulating levels of sRAGE [57, 96, 97]. With this protective function being compromised and with several RAGE ligands elevated, it is possible that the coexistence of AD and T2DM would result in accentuated inflammatory responses, both in the periphery and in the brain. Small molecules that can block RAGE activation or enhance the protective function of sRAGE are a strategy which may be beneficial to both AD and T2DM [98, 99].

6. Conclusion

There is strong evidence supporting inflammation as key feature in the brain of AD and in the pancreas of T2DM as summarized in Table 1. A wide range of inflammatory mediators and receptors are involved in these two diseases, although complement activation is a prominent feature in AD, but not in T2DM [100]. The presence of infiltrated lymphocytes is controversial in AD [76, 101]. Therefore,

Table 1: Inflammatory responses detected in the brain of Alzheimer's disease and the pancreas of diabetes mellitus.

	Disease-affected brain regions in Alzheimer's disease patients	Pancreas in T2DM patients
Elevated inflammatory markers	Cytokines (e.g., IL-1α, IL-1β, IL-6, TNF-α), chemokines (e.g., IL-8, MCP-1), acute phase proteins (e.g., ACT-1, Serum amyloid P), activated complement proteins (e.g., C3, C5a, C5b-9), and S100B	Cytokines (e.g., IL-1β, IL-6, TNF-α), chemokines (e.g., IL-8, IP-1, MCP-1, MIP-1α), growth factor (G-CSF), S100B, and HMGB1
Immune cell infiltration	Rare presence of lymphocytes or macrophages	Increased macrophages, T-lymphocytes, and granulocytes
Involvement of pattern recognition receptors and major ligands	MSR-A, MSR-B, RAGE, TLR2, TLR4; amyloid beta, AGE	MSR-A, MSR-B, RAGE, TLR2, TLR4: amylin, AGE, IP-10

current research findings support the inflammation-based pathogenic mechanisms in both diseases. Although research investigating that T2DM may alter brain inflammation in AD is limited, there is a great possibility that T2DM could accentuate microglial activation, neuroinflammation, and vascular inflammatory/oxidative injury in AD brains through mechanisms mediated by RAGE and other pattern-recognition receptors, and the cascade of cytokine and chemokines. Figure 2 illustrates the potential of RAGE-centric mechanisms in the central and peripheral systems when both diseases coexist. As microglia play a central role in initiation and propagation of neuroinflammation, and anti-inflammation is one of the preventive and disease modifying strategies for AD, more studies will be needed to characterize the patterns of microglial activation in AD patients with T2DM and AD patients without T2DM.

Abbreviations

AD: Alzheimer's disease
AChE: Acetylcholine esterase
(ACT-1): Anti-chymotrypsin-1
AGE: Advanced glycation endproducts
APP: Amyloid precursor protein
CRP: C-reactive protein
DN: dominant negative
esRAGE: endogenous secretory receptor for advanced glycation endproducts
G-CSF: Granulocyte-colony stimulating factor
HMG1: High mobility group box
IGF: Insulin growth factor
IR: Insulin receptor
IL: Interleukin
(IL-1ra): Interleukin-1 receptor antagonist
IP-10: Interferon-inducible protein-10
MCP-1: Monocyte chemotactic protein-1
M-CSF: Macrophage colony stimulating factor
(MIP-1): Macrophage inflammatory protein-1
MSR: macrophage scavenger receptor
HDL: High density lipoprotein
(ox-LDL): oxidized low density lipoprotein
PKD: Protein kinase
RAGE: Receptor for advanced glycation endproducts
sRAGE: Soluble RAGE
TLR: Toll-like receptor
TNF-α: Tumor necrosis factor-alpha
T2DM: Type 2 diabetes mellitus.

Acknowledgments

The authors would like to thank Alzheimer's Association (IIRG-09-91996) and Arizona Alzheimer's Research Consortium for the funding.

References

[1] S. Ahtiluoto, T. Polvikoski, M. Peltonen et al., "Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study," *Neurology*, vol. 75, no. 13, pp. 1195–1202, 2010.

[2] C. C. Lee, S. G. Glickman, D. R. Dengel, M. D. Brown, and M. A. Supiano, "Abdominal adiposity assessed by dual energy x-ray absorptiometry provides a sex-independent predictor of insulin sensitivity in older adults," *Journals of Gerontology A*, vol. 60, no. 7, pp. 872–877, 2005.

[3] J. A. Luchsinger, C. Reitz, B. Patel, M. X. Tang, J. J. Manly, and R. Mayeux, "Relation of diabetes to mild cognitive impairment," *Archives of Neurology*, vol. 64, no. 4, pp. 570–575, 2007.

[4] A. Ott, R. P. Stolk, F. van Harskamp, H. A. Pols, A. Hofman, and M. M. Breteler, "Diabetes mellitus and the risk of dementia: the Rotterdam study," *Neurology*, vol. 53, no. 9, pp. 1937–1942, 1999.

[5] G. Razay, A. Vreugdenhil, and G. Wilcock, "The metabolic syndrome and Alzheimer disease," *Archives of Neurology*, vol. 64, no. 1, pp. 93–96, 2007.

[6] E. M. Schrijvers, J. C. Witteman, E. J. Sijbrands, A. Hofman, P. J. Koudstaal, and M. M. B. Breteler, "Insulin metabolism and the risk of Alzheimer disease: the Rotterdam study," *Neurology*, vol. 75, no. 22, pp. 1982–1987, 2010.

[7] R. Stewart and D. Liolitsa, "Type 2 diabetes mellitus, cognitive impairment and dementia," *Diabetic Medicine*, vol. 16, no. 2, pp. 93–112, 1999.

[8] M. W. Strachan, R. M. Reynolds, R. E. Marioni, and J. F. Price, "Cognitive function, dementia and type 2 diabetes mellitus in the elderly," *Nature Reviews Endocrinology*, vol. 7, no. 2, pp. 108–114, 2011.

[9] M. L. Baskin, J. Ard, F. Franklin, and D. B. Allison, "Prevalence of obesity in the United States," *Obesity Reviews*, vol. 6, no. 1, pp. 5–7, 2005.

[10] E. S. Ford, C. Li, G. Zhao, and J. Tsai, "Trends in obesity and abdominal obesity among adults in the United States from 1999-2008," *International Journal of Obesity*, vol. 35, no. 5, pp. 736–743, 2011.

[11] Y. Wang and M. A. Beydoun, "The obesity epidemic in the United States—gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis," *Epidemiologic Reviews*, vol. 29, no. 1, pp. 6–28, 2007.

[12] W. T. Cefalu, Z. Q. Wang, S. Werbel et al., "Contribution of visceral fat mass to the insulin resistance of aging," *Metabolism*, vol. 44, no. 7, pp. 954–959, 1995.

[13] H. Bruehl, O. T. Wolf, V. Sweat, A. Tirsi, S. Richardson, and A. Convit, "Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus," *Brain Research*, vol. 1280, no. C, pp. 186–194, 2009.

[14] Z. S. Tan, A. S. Beiser, C. S. Fox et al., "Association of metabolic dysregulation with volumetric brain magnetic resonance imaging and cognitive markers of subclinical brain aging in middle-aged adults: the framingham offspring study," *Diabetes Care*, vol. 34, no. 8, pp. 1766–1770, 2011.

[15] J. G. Ryan, "Cost and policy implications from the increasing prevalence of obesity and diabetes mellitus," *Gender Medicine*, vol. 6, supplement 1, pp. 86–108, 2009.

[16] B. Cholerton, L. D. Baker, and S. Craft, "Insulin resistance and pathological brain ageing," *Diabetic Medicine*, vol. 28, no. 12, pp. 1463–1475, 2011.

[17] S. M. de la Monte, "Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in Alzheimer's diseas," *Drugs*, vol. 72, no. 1, pp. 49–66, 2012.

[18] J. A. Luchsinger, "Type 2 diabetes andcognitive impairment: linking mechanisms," *Journal of Alzheimer's Disease*. In press.

[19] P. A. Maher and D. R. Schubert, "Metabolic links between diabetes and Alzheimer's disease," *Expert Review of Neurotherapeutics*, vol. 9, no. 5, pp. 617–630, 2009.

[20] Å. Sjöholm and T. Nyström, "Inflammation and the etiology of type 2 diabetes," *Diabetes/Metabolism Research and Reviews*, vol. 22, no. 1, pp. 4–10, 2006.

[21] D. Bosco, A. Fava, M. Plastino, T. Montalcini, and A. Pujia, "Possible implications of insulin resistance and glucose metabolism in Alzheimer's disease pathogenesis," *Journal of Cellular and Molecular Medicine*, vol. 15, no. 9, pp. 1807–1821, 2011.

[22] A. D. Pradhan, J. E. Manson, N. Rifai, J. E. Buring, and P. M. Ridker, "C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus," *JAMA*, vol. 286, no. 3, pp. 327–334, 2001.

[23] B. Thorand, H. Löwel, A. Schneider et al., "C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg Cohort study, 1984–1998," *Archives of Internal Medicine*, vol. 163, no. 1, pp. 93–99, 2003.

[24] B. Vozarova, C. Weyer, R. S. Lindsay, R. E. Pratley, C. Bogardus, and P. A. Tataranni, "High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes," *Diabetes*, vol. 51, no. 2, pp. 455–461, 2002.

[25] M. Böni-Schnetzler, J. Thorne, G. Parnaud et al., "Increased interleukin (IL)-1β messenger ribonucleic acid expression in β-cells of individuals with type 2 diabetes and regulation of IL-1β in human islets by glucose and autostimulation," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 10, pp. 4065–4074, 2008.

[26] W. P. Arend, "Interleukin-1 receptor antagonist: discovery, structure and properties," *Cytokine and Growth Factor Reviews*, vol. 2, no. 4, pp. 193–205, 1990.

[27] D. L. Eizirik and S. Sandler, "Human interleukin-1β induced stimulation of insulin release from rat pancreatic islets is accompanied by an increase in mitochondrial oxidative events," *Diabetologia*, vol. 32, no. 11, pp. 769–773, 1989.

[28] J. P. Palmer, S. Helqvist, G. A. Spinas et al., "Interaction of β-cell activity and IL-1 concentration and exposure time in isolated rat islets of langerhans," *Diabetes*, vol. 38, no. 10, pp. 1211–1216, 1989.

[29] C. A. Dinarello, "A clinical perspective of IL-1β as the gatekeeper of inflammation," *European Journal of Immunology*, vol. 41, no. 5, pp. 1203–1217, 2011.

[30] J. A. Ehses, G. Lacraz, M. H. Giroix et al., "IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 33, pp. 13998–14003, 2009.

[31] C. A. Dinarello and R. C. Thompson, "Blocking IL-1: interleukin 1 receptor antagonist *in vivo* and *in vitro*," *Immunology Today*, vol. 12, no. 11, pp. 404–410, 1991.

[32] C. M. Larsen, M. Faulenbach, A. Vaag et al., "Interleukin-1-receptor antagonist in type 2 diabetes mellitus," *The New England Journal of Medicine*, vol. 356, no. 15, pp. 1517–1526, 2007.

[33] M. S. H. Akash, Q. Shen, K. Rehman, and S. Chen, "Interleukin-1 receptor antagonist: a new therapy for type 2 diabetes mellitus," *Journal of Pharmaceutical Sciences*, vol. 101, supplement 5, pp. 1647–1658, 2012.

[34] O. Hori, S. D. Yan, S. Ogawa et al., "The receptor for advanced glycation end-products has a central role in mediating the effects of advanced glycation end-products on the development of vascular disease in diabetes mellitus," *Nephrology Dialysis Transplantation*, vol. 11, supplement 5, pp. 13–16, 1996.

[35] A. M. Schmidt, S. D. Yan, S. F. Yan, and D. M. Stern, "The biology of the receptor for advanced glycation end products and its ligands," *Biochimica et Biophysica Acta*, vol. 1498, no. 2-3, pp. 99–111, 2000.

[36] M. Sensi, F. Pricci, D. Andreani, and U. Di Mario, "Advanced nonenzymatic glycation endproducts (age): their relevance to aging and the pathogenesis of late diabetic complications," *Diabetes Research*, vol. 16, no. 1, pp. 1–9, 1991.

[37] S. Yamagishi, "Role of advanced glycation end products (AGEs) and receptor for AGEs (RAGE) in vascular damage in diabetes," *Experimental Gerontology*, vol. 46, no. 4, pp. 217–224, 2011.

[38] P. Chellan and R. H. Nagaraj, "Protein crosslinking by the Maillard reaction: dicarbonyl-derived imidazolium crosslinks in aging and diabetes," *Archives of Biochemistry and Biophysics*, vol. 368, no. 1, pp. 98–104, 1999.

[39] S. R. Thorpe and J. W. Baynes, "Role of the maillard reaction in diabetes mellitus and diseases of aging," *Drugs and Aging*, vol. 9, no. 2, pp. 69–77, 1996.

[40] E. B. Frye, T. P. Degenhardt, S. R. Thorpe, and J. W. Baynes, "Role of the Maillard reaction in aging of tissue proteins: advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins," *Journal of Biological Chemistry*, vol. 273, no. 30, pp. 18714–18719, 1998.

[41] M. Sensi, F. Pricci, G. Pugliese et al., "Enhanced nonenzymatic glycation of eye lens proteins in experimental diabetes mellitus: an approach for the study of protein alterations

as mediators of normal aging phenomena," *Archives of Gerontology and Geriatrics*, vol. 15, supplement 1, pp. 333–337, 1992.

[42] P. Ulrich and A. Cerami, "Protein glycation, diabetes, and aging," *Recent Progress in Hormone Research*, vol. 56, pp. 1–21, 2001.

[43] M. M. Anderson, J. R. Requena, J. R. Crowley, S. R. Thorpe, and J. W. Heinecke, "The myeloperoxidase system of human phagocytes generates Nepsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation," *Journal of Clinical Investigation*, vol. 104, no. 1, pp. 103–113, 1999.

[44] G. R. Barile, S. I. Pachydaki, S. R. Tari et al., "The RAGE axis in early diabetic retinopathy," *Investigative Ophthalmology and Visual Science*, vol. 46, no. 8, pp. 2916–2924, 2005.

[45] S. Sakurai, H. Yonekura, Y. Yamamoto et al., "The AGE-RAGE system and diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 14, supplement 3, no. 8, pp. S259–S263, 2003.

[46] A. M. Schmidt, M. Hofmann, A. Taguchi, S. D. Yan, and D. M. Stern, "RAGE: a multiligand receptor contributing to the cellular response in diabetic vasculopathy and inflammation," *Seminars in Thrombosis and Hemostasis*, vol. 26, no. 5, pp. 485–493, 2000.

[47] M. T. Win, Y. Yamamoto, S. Munesue et al., "Regulation of RAGE for attenuating progression of diabetic vascular complications," *Experimental Diabetes Research*, vol. 2012, Article ID 894605, 8 pages, 2012.

[48] S. Yamagishi, M. Takeuchi, Y. Inagaki, K. Nakamura, and T. Imaizumi, "Role of advanced glycation end products (AGEs) and their receptor (RAGE) in the pathogenesis of diabetic microangiopathy," *International Journal of Clinical Pharmacology Research*, vol. 23, no. 4, pp. 129–134, 2003.

[49] B. W. Lee, H. Y. Chae, S. J. Kwon, S. Y. Park, J. Ihm, and S. H. Ihm, "RAGE ligands induce apoptotic cell death of pancreatic β-cells via oxidative stress," *International Journal of Molecular Medicine*, vol. 26, no. 6, pp. 813–818, 2010.

[50] Y. Zhu, T. Shu, Y. Lin et al., "Inhibition of the receptor for advanced glycation endproducts (RAGE) protects pancreatic β-cells," *Biochemical and Biophysical Research Communications*, vol. 404, no. 1, pp. 159–165, 2011.

[51] K. P. Chandra, A. Shiwalkar, J. Kotecha et al., "Phase I clinical studies of the advanced glycation end-product (AGE)-breaker TRC4186: safety, tolerability and pharmacokinetics in healthy subjects," *Clinical Drug Investigation*, vol. 29, no. 9, pp. 559–575, 2009.

[52] G. Li, J. Tang, Y. Du, C. Lee, and T. S. Kern, "Beneficial effects of a novel RAGE inhibitor on early diabetic retinopathy and tactile allodynia," *Molecular Vision*, vol. 17, pp. 3156–3165, 2011.

[53] J. Peyroux and M. Sternberg, "Advanced glycation end-products (AGEs): pharmacological inhibition in diabetes," *Pathologie Biologie*, vol. 54, no. 7, pp. 405–419, 2006.

[54] S. Rahbar, "Novel inhibitors of glycation and AGE formation," *Cell Biochemistry and Biophysics*, vol. 48, no. 2-3, pp. 147–157, 2007.

[55] H. Yonekura, Y. Yamamoto, S. Sakurai et al., "Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury," *Biochemical Journal*, vol. 370, no. 3, pp. 1097–1109, 2003.

[56] A. Galichet, M. Weibel, and C. W. Heizmann, "Calcium-regulated intramembrane proteolysis of the RAGE receptor," *Biochemical and Biophysical Research Communications*, vol. 370, no. 1, pp. 1–5, 2008.

[57] A. Raucci, S. Cugusi, A. Antonelli et al., "A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10)," *The FASEB Journal*, vol. 22, no. 10, pp. 3716–3727, 2008.

[58] L. Zhang, M. Bukulin, E. Kojro et al., "Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases," *Journal of Biological Chemistry*, vol. 283, no. 51, pp. 35507–35516, 2008.

[59] X. H. Tam, S. W. Shiu, L. Leng, R. Bucala, D. J. Betteridge, and K. C. Tan, "Enhanced expression of receptor for advanced glycation end-products is associated with low circulating soluble isoforms of the receptor in Type 2 diabetes," *Clinical Science*, vol. 120, no. 2, pp. 81–89, 2011.

[60] S. F. Yan, R. Ramasamy, and A. M. Schmidt, "Soluble RAGE: therapy and biomarker in unraveling the RAGE axis in chronic disease and aging," *Biochemical Pharmacology*, vol. 79, no. 10, pp. 1379–1386, 2010.

[61] M. R. Dasu, S. Devaraj, S. Park, and I. Jialal, "Increased toll-like receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects," *Diabetes Care*, vol. 33, no. 4, pp. 861–868, 2010.

[62] I. Jialal, B. A. Huet, H. Kaur, A. Chien, and S. Devaraj, "Increased toll-like receptor activity in patients with metabolic syndrome," *Diabetes Care*, vol. 35, no. 4, pp. 900–904, 2012.

[63] M. R. Dasu, S. Devaraj, L. Zhao, D. H. Hwang, and I. Jialal, "High glucose induces toll-like receptor expression in human monocytes mechanism of activation," *Diabetes*, vol. 57, no. 11, pp. 3090–3098, 2008.

[64] M. R. Dasu and I. Jialal, "Free fatty acids in the presence of high glucose amplify monocyte inflammation via toll-like receptors," *American Journal of Physiology*, vol. 300, no. 1, pp. E145–E154, 2011.

[65] G. M. Barton and R. Medzhitov, "Toll-like receptor signaling pathways," *Science*, vol. 300, no. 5625, pp. 1524–1525, 2003.

[66] F. T. Schulthess, F. Paroni, N. S. Sauter et al., "CXCL10 Impairs β cell function and viability in diabetes through TLR4 signaling," *Cell Metabolism*, vol. 9, no. 2, pp. 125–139, 2009.

[67] S. Devaraj and I. Jialal, "Increased secretion of IP-10 from monocytes under hyperglycemia is via the TLR2 and TLR4 pathway," *Cytokine*, vol. 47, no. 1, pp. 6–10, 2009.

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

[69] S. Gautam and M. Banerjee, "The macrophage Ox-LDL receptor, CD36 and its association with type II diabetes mellitus," *Molecular Genetics and Metabolism*, vol. 102, no. 4, pp. 389–398, 2011.

[70] D. J. Kennedy and S. R. Kashyap, "Pathogenic role of scavenger receptor CD36, metabolic syndrome and diabetes," *Metabolic Syndrome and Related Disorders*, vol. 9, no. 4, pp. 239–245, 2011.

[71] D. J. Kennedy, S. Kuchibhotla, K. M. Westfall, R. L. Silverstein, R. E. Morton, and M. Febbraio, "A CD36-dependent pathway enhances macrophage and adipose tissue inflammation and impairs insulin signalling," *Cardiovascular Research*, vol. 89, no. 3, pp. 604–613, 2011.

[72] I. Alafuzoff, L. Aho, S. Helisalmi, A. Mannermaa, and H. Soininen, "β-amyloid deposition in brains of subjects with diabetes," *Neuropathology and Applied Neurobiology*, vol. 35, no. 1, pp. 60–68, 2009.

[73] M. S. Beeri, J. M. Silverman, K. L. Davis et al., "Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology," *Journals of Gerontology A*, vol. 60, no. 4, pp. 471–475, 2005.

[74] P. T. Nelson, C. D. Smith, E. A. Abner et al., "Human cerebral neuropathology of Type 2 diabetes mellitus," *Biochimica et Biophysica Acta - Molecular Basis of Disease*, vol. 1792, no. 5, pp. 454–469, 2009.

[75] J. A. Sonnen, E. B. Larson, K. Brickell et al., "Different patterns of cerebral injury in dementia with or without diabetes," *Archives of Neurology*, vol. 66, no. 3, pp. 315–322, 2009.

[76] P. Picone, D. Giacomazza, V. Vetri et al., "Insulin-activated Akt rescues Aβ oxidative stress-induced cell death by orchestrating molecular trafficking," *Aging Cell*, vol. 10, pp. 832–843, 2011.

[77] T. Valente, A. Gella, X. Fernàndez-Busquets, M. Unzeta, and N. Durany, "Immunohistochemical analysis of human brain suggests pathological synergism of Alzheimer's disease and diabetes mellitus," *Neurobiology of Disease*, vol. 37, no. 1, pp. 67–76, 2010.

[78] W. S. Griffin, J. G. Sheng, G. W. Roberts, and R. E. Mrak, "Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution," *Journal of Neuropathology and Experimental Neurology*, vol. 54, no. 2, pp. 276–281, 1995.

[79] J. G. Sheng, R. E. Mrak, and W. S. Griffin, "Microglial interleukin-1α expression in brain regions in Alzheimer's disease: correlation with neuritic plaque distribution," *Neuropathology and Applied Neurobiology*, vol. 21, no. 4, pp. 290–301, 1995.

[80] L. F. Lue, D. G. Walker, L. Brachova et al., "Involvement of microglial receptor for advanced glycation endproducts (RAGE)in Alzheimer's disease: identification of a cellular activation mechanism," *Experimental Neurology*, vol. 171, no. 1, pp. 29–45, 2001.

[81] N. Sasaki, S. Toki, H. Chowei et al., "Immunohistochemical distribution of the receptor for advanced glycation end products in neurons and astrocytes in Alzheimer's disease," *Brain Research*, vol. 888, no. 2, pp. 256–262, 2001.

[82] S. D. Yan, X. Chen, J. Fu et al., "RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease," *Nature*, vol. 382, no. 6593, pp. 685–691, 1996.

[83] M. O. Chaney, W. B. Stine, T. A. Kokjohn et al., "RAGE and amyloid β interactions: atomic force microscopy and molecular modeling," *Biochimica et Biophysica Acta*, vol. 1741, no. 1-2, pp. 199–205, 2005.

[84] S. D. Yan, H. Zhu, A. Zhu et al., "Receptor-dependent cell stress and amyloid accumulation in systemic amyloidosis," *Nature Medicine*, vol. 6, no. 6, pp. 643–651, 2000.

[85] F. Fang, L. F. Lue, S. Yan et al., "RAGE-dependent signaling in microglia contributes to neuroinflammation, Aβ accumulation, and impaired learning/memory in a mouse model of Alzheimer's disease," *FASEB Journal*, vol. 24, no. 4, pp. 1043–1055, 2010.

[86] N. Origlia, C. Bonadonna, A. Rosellini et al., "Microglial receptor for advanced glycation end product-dependent signal pathway drives β-amyloid-induced synaptic depression and long-term depression impairment in entorhinal cortex," *Journal of Neuroscience*, vol. 30, no. 34, pp. 11414–11425, 2010.

[87] A. Wong, H. J. Lüth, W. Deuther-Conrad et al., "Advanced glycation endproducts co-localize with inducible nitric oxide synthase in Alzheimer's disease," *Brain Research*, vol. 920, no. 1-2, pp. 32–40, 2001.

[88] C. Loske, A. Neumann, A. M. Cunningham et al., "Cytotoxicity of advanced glycation endproducts is mediated by oxidative stress," *Journal of Neural Transmission*, vol. 105, no. 8-9, pp. 1005–1015, 1998.

[89] C. E. Shepherd, J. Goyette, V. Utter et al., "Inflammatory S100A9 and S100A12 proteins in Alzheimer's disease," *Neurobiology of Aging*, vol. 27, no. 11, pp. 1554–1563, 2006.

[90] K. Takata, Y. Kitamura, D. Tsuchiya, T. Kawasaki, T. Taniguchi, and S. Shimohama, "High mobility group box protein-1 inhibits microglial Aβ clearance and enhances Aβ neurotoxicity," *Journal of Neuroscience Research*, vol. 78, no. 6, pp. 880–891, 2004.

[91] D. G. Walker, J. Link, L.-F. Lue, J. E. Dalsing-Hernandez, and B. E. Boyes, "Gene expression changes by amyloid β peptide-stimulated human postmortem brain microglia identify activation of multiple inflammatory processes," *Journal of Leukocyte Biology*, vol. 79, no. 3, pp. 596–610, 2006.

[92] I. S. Coraci, J. Husemann, J. W. Berman et al., "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," *American Journal of Pathology*, vol. 160, no. 1, pp. 101–112, 2002.

[93] P. Ehlermann, K. Eggers, A. Bierhaus et al., "Increased proinflammatory endothelial response to S100A8/A9 after preactivation through advanced glycation end products," *Cardiovascular Diabetology*, vol. 5, article 6, 2006.

[94] S. F. Yan, R. Ramasamy, and A. M. Schmidt, "Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response," *Journal of Molecular Medicine*, vol. 87, no. 3, pp. 235–247, 2009.

[95] K. Eggers, K. Sikora, M. Lorenz et al., "RAGE-dependent regulation of calcium-binding proteins S100A8 and S100A9 in human THP-1," *Experimental and Clinical Endocrinology and Diabetes*, vol. 119, no. 6, pp. 353–357, 2011.

[96] R. Ghidoni, L. Benussi, M. Glionna et al., "Decreased plasma levels of soluble receptor for advanced glycation end products in mild cognitive impairment," *Journal of Neural Transmission*, vol. 115, no. 7, pp. 1047–1050, 2008.

[97] L. F. Lue, Y. M. Kuo, T. Beach, and D. G. Walker, "Microglia activation and anti-inflammatory regulation in alzheimer's disease," *Molecular Neurobiology*, vol. 41, no. 2-3, pp. 115–128, 2010.

[98] M. N. Sabbagh, A. Agro, J. Bell, P. S. Aisen, E. Schweizer, and D. Galasko, "PF-04494700, an oral inhibitor of receptor for advanced glycation end products (RAGE), in Alzheimer disease," *Alzheimer Disease & Associated Disorders*, vol. 25, pp. 206–212, 2010.

[99] L. Zhang, R. Postina, and Y. Wang, "Ectodomain shedding of the receptor for advanced glycation end products: a novel therapeutic target for Alzheimer's disease," *Cellular and Molecular Life Sciences*, vol. 66, no. 24, pp. 3923–3935, 2009.

[100] M. V. Kolev, M. M. Ruseva, C. L. Harris, B. P. Morgan, and R. M. Donev, "Implication of complement system and its regulators in alzheimer's disease," *Current Neuropharmacology*, vol. 7, no. 1, pp. 1–8, 2009.

[101] T. Town, J. Tan, R. A. Flavell, and M. Mullan, "T-cells in Alzheimer's disease," *NeuroMolecular Medicine*, vol. 7, no. 3, pp. 255–264, 2005.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 314185, 11 pages
doi:10.1155/2012/314185

Review Article

Microglia in Alzheimer's Disease: It's All About Context

Tara M. Weitz[1] and Terrence Town[1,2,3]

[1] *Regenerative Medicine Institute Neural Program and Department of Biomedical Sciences, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Steven Spielberg Building Room 345, Los Angeles, CA 90048, USA*
[2] *Department of Neurosurgery, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Steven Spielberg Building Room 345, Los Angeles, CA 90048, USA*
[3] *Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90048, USA*

Correspondence should be addressed to Terrence Town, terrence.town@csmc.edu

Received 13 February 2012; Accepted 9 April 2012

Academic Editor: Colin Combs

Neuroinflammation is now regarded as both an early event and prime mover in the pathobiology of Alzheimer disease (AD), a neurodegenerative disease that represents a growing public health threat. As the resident innate immune cells within the central nervous system, microglia are centrally positioned as key orchestrators of brain inflammation. It is now accepted that numerous forms of activated microglia exist. Furthermore, while some types of reactive microglia are detrimental, others can actually be beneficial. In the context of AD etiopathology, much debate surrounds whether these enigmatic cells play "good" or "bad" roles. In this article, we distill a complex clinical and experimental literature focused on the contribution of microglia to AD pathology and progression. A synthesis of the literature only seems possible when considering context– the conditions under which microglia encounter and mount immunological responses to AD pathology. In order to carry out these diverse contextual responses, a number of key receptors and signaling pathways are variously activated. It will be critically important for future studies to address molecular mediators that lead to beneficial microglial responses and therefore represent important therapeutic targets for AD.

1. Introduction

More than twenty years ago, groundbreaking studies by Wisniewski and his colleagues sparked debate over the relationship between microglia and β-amyloid plaques in Alzheimer disease (AD). In their electron microscopic observations of microglia associated with β-amyloid deposits in the brain parenchyma and cortical vessel walls of AD patients' brains, Wisniewski and his colleagues discovered that ~80% of a morphologic structure they termed the "amyloid star" was covered by microglia [1, 2]. Although they found that microglia had the ability to phagocytose Aβ *in vitro*, these innate immune cells appeared in close proximity to amyloid plaques in brains of AD patients but did not contain β-amyloid fibrils in their lysosomal compartments. These ultrastructural observations led Frackowiak and coworkers to hypothesize that microglia were unable to phagocytose and remove Aβ *in vivo* and were instead responsible for the manufacture of amyloid fibers [3]. In contrast, Wisniewski did observe β-amyloid fibrils in the lysosomes of macrophages that had infiltrated the brain from the periphery in AD patients with rare comorbid stroke [4]—findings that were later confirmed by Akiyama and McGeer [5].

Rooted in these initial landmark studies of Wisniewski, it is now recognized that there are at least two classes of phagocytic cells responsible for the innate immune responses in the brain—the endogenous microglia and the exogenous hematogenous macrophages [6–8]. Microglia are the brain-resident macrophages derived from monocyte precursors during embryogenesis and provide the initial response to injury from within the central nervous system (CNS). Activation of microglia via neural insults leads to the synthesis of an array of proinflammatory mediators, which can be beneficial for clearing infections and for tissue repair; but, if left uncontrolled, this response can go unresolved and perpetrate bystander neural insult. Thus, in addition to their role as the first responders to CNS injury, activated

microglia (and astrocytes) can participate in a form of chronic neuroinflammation termed "reactive gliosis," which has been implicated in the pathoetiology of a myriad of neurodegenerative diseases. The second class of phagocyte, peripherally-derived macrophages, arises from bone marrow precursors and will be referred to in this review as hematogenous macrophages. These cells can be recruited to the CNS in health and after CNS injury and are primarily believed to traverse the blood-brain barrier (BBB) at postcapillary venules [9]. While hematogenous macrophages typically remain in the perivascular space, they can be recruited into the brain parenchyma by chemokines and cytokines. These proteins are typically released following activation of microglia and astrocytes upon CNS insult and can cause blood-borne mononuclear cells to differentiate into cells that closely resemble brain-resident microglia. Finally, recent focus has been directed towards a third class of brain phagocyte known as the perivascular macrophage. These cells have been found to play an important role in remodeling cerebrovascular Aβ deposits in a transgenic mouse model of cerebral amyloidosis [10].

Even though the pioneering observations of Wisniewski and his colleagues were made more than two decades ago, it is only recently and with the availability of modern cellular and molecular biology techniques that the roles of brain phagocytes have been more fully interrogated in AD. Still, their significance in the pathoetiology and—even more intriguingly—as a possible treatment modality for AD, remains unclear. What is clear from the studies of the past twenty years, however, is that resident local and peripherally-infiltrating brain macrophages play complex roles in the pathobiology of AD.

2. "Bad" versus "Good" Microglia in Alzheimer's Disease

It has previously been suggested that microglial activation is not simply a single phenotype, and that a continuum exists with antigen presenting cell function (adaptive activation) at one pole and phagocytic cell function (innate activation) at the other [11]. Accordingly, expression profile studies of macrophages in AD and in mouse models of cerebral amyloid also suggest that there is functional heterogeneity in the activation states of microglia that may contribute to disease outcome [12–14]. These innovative concepts support the notion that the context of microglial activation is a key factor in determining what role (whether "bad" or "good") these enigmatic cells play in AD.

3. A Case for "Bad" Microglia

3.1. Evidence from Patients. The concept that microglia primarily play a detrimental role in AD is supported by the Wisniewski studies and by early epidemiologic findings. In general, results from these studies are interpreted as lending support to the idea that activated microglia are likely more harmful than helpful to an AD-afflicted brain. For instance, activated microglia secrete the proinflammatory innate cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β), which can directly injure neurons at superphysiologic levels [15, 16].

In addition to the well-accepted notion that microglia are closely associated with senile plaques in AD, it has also been shown that numerous inflammatory mediators are up-regulated in affected areas of the AD brain [17]. These observations have led to the hypothesis that individuals being treated on a long-term basis with anti-inflammatory medications might be afforded prophylaxis against chronic neuroinflammation and may therefore be at reduced risk for developing AD. In accord with this concept, a number of epidemiologic studies in the early 1990s found that incidence of dementia in elderly patients with arthritis was lower compared to the general population [18–20]. In addition, evidence from neuropathologic studies supports the notion that nonsteroidal anti-inflammatory drugs (NSAIDs; often prescribed for arthritis) reduce microglial activation and thereby dampen brain inflammation [21]. Specifically, Mackenzie and Munoz compared brains from elderly, nondemented arthritic patients with a history of chronic NSAID use and nondemented control subjects that were not NSAID users and found that this latter group had (on average) three-fold more activated microglia than the former. However, interpreting these results is not so straightforward, as arthritis was used as a surrogate for NSAID use in many of these early studies, raising a possible "confounding by indication" issue. Further, The Golde group has shown that a subset of NSAIDs lower $A\beta_{1-42}$, widely considered the neurotoxic Aβ species, independently of cyclooxygenase (COX) activity [22]. Nonetheless, these initial reports prompted more recent work that focused more specifically on NSAID use, and there are now over 25 epidemiologic studies that have shown an inverse risk relationship between NSAID use and AD. A systematic review of these studies uncovered an approximate 50% reduced risk of AD enjoyed by NSAID users compared to nonusers [23].

3.2. Evidence from Mouse Models. The effects of NSAIDs have also been evaluated in transgenic mouse models of cerebral amyloidosis. In the earliest study, Lim et al. tested the effects of 6-month-long treatment of Tg2576 mice with ibuprofen, beginning at 10 months of age when Aβ plaques first appear in these mice. They found that treated animals had significantly reduced amyloid deposition as well as blunted expression of the reactive astrocyte marker glial fibrillary acidic protein (GFAP) and the proinflammatory cytokine, IL-1β [24]. Other groups confirmed the ibuprofen results in Tg2576 mice, amyloid precursor protein (APP) plus presenilin-1 (PS1) double transgenics (termed APP/PS1 mice) and APPV717I transgenic mice, all of which showed reduced microglial activation and fewer amyloid deposits following treatment [25–27]. Heneka and colleagues also tested the effects of curcumin, a naturally-occurring NSAID derived from turmeric, which is considered to be a less toxic alternative to COX-2 inhibitors like ibuprofen. They found decreased IL-1β and GFAP levels as well as attenuated

plaque burden in treated mice [27]. Furthermore, treatment of PS1/APP doubly transgenic mice with the nitric oxide-donating NSAID NCX-2216 reduced amyloid load via a mechanism thought to involve phagocytic microglia [25, 28]. These studies demonstrate that early treatment with NSAIDs can reduce neuroinflammation and amyloid plaque burden in three different transgenic mouse models of cerebral amyloid deposition. However, it should be noted that studies done with the selective COX-2 inhibitor celecoxib failed to reduce inflammatory burden and, in one study, actually increased abundance of $A\beta_{1-42}$ peptide in transgenic AD model mice [25, 29]. Therefore, it seems that certain NSAIDs are capable of reducing microgliosis and amyloidosis in several different transgenic mouse models, while others are not as effective or even produce converse results. This suggests that microglia likely have different responses to such treatment depending on the mechanism of action of the compound and/or the nature of the inflammatory milieu at the initiation of, or even during the course of treatment.

Studies using mouse genetics as a powerful tool to elucidate the role of microglia in AD pathogenesis have also helped to establish the existence of "bad" microglia that promote disease progression. In early studies done by our group, Aβ-stimulated microglia treated with CD40 ligand (CD40L) showed increased TNF-α production and promoted injury of primary cortical neurons. In addition, these studies demonstrated that microglia from transgenic "Swedish" mutant APP (Tg2576) mice deficient for CD40L exhibited reduced activation compared to microglia from CD40L-sufficient transgenic Tg2576 littermates [16]. Moreover, a separate study showed that genetic ablation of CD40L in APP/PS1 mice reduced amyloid plaques and mitigated astrocytosis and microgliosis [30]. Further *in vitro* investigations showed that CD40-CD40L interaction led to decreased microglial phagocytosis of exogenous $A\beta_{1-42}$ and increased production of proinflammatory cytokines. CD40 ligation in the presence of $A\beta_{1-42}$ led to "adaptive" activation of microglia, as evidenced by increased colocalization of major histocompatibility complex class II (MHC II) with Aβ. In addition, when cultured microglia were exposed to $A\beta_{1-42}$ in the presence of CD40L and cocultured with $CD4^+$ T cells, T cell-derived interferon-gamma (IFN-γ) and interleukin-2 production were stimulated, suggesting that CD40 signaling promotes a microglial proinflammatory antigen presenting phenotype [31]. In the context of CD40-CD40L interaction, these results collectively suggest that microglia become proinflammatory and play a primarily deleterious role in progression of AD-like pathology.

Additional early evidence for detrimental effects of activated microglia in AD came from Qiao and colleagues. Those authors chronically administered the bacterial endotoxin lipopolysaccharide (LPS) intracerebroventricularly to APPV717F mice that either expressed apolipoprotein E ($apoE^{+/+}$) or were apoE deficient ($apoE^{-/-}$) [32]. While all LPS-treated mice exhibited global astrocytosis and amyloid plaque-localized microglial activation, Qiao et al. found significant acceleration of amyloid deposition in LPS-treated compared to vehicle-treated APPV717F-$apoE^{+/+}$ mice, while this effect was not observed in APPV717F-$apoE^{-/-}$ mice. These experiments suggest that experimental induction of microglial activation by chronic administration of LPS can accelerate amyloidosis in a transgenic mouse model of AD in the presence of apoE, and support the idea that activated microglia exacerbate cerebral amyloidosis.

More recent studies by Mori and colleagues showed that overexpression of the proinflammatory cytokine-like molecule human S100B (huS100B) in the Tg2576 mouse model of cerebral amyloid deposition resulted in increased parenchymal and cerebrovascular β-amyloid deposits and elevated Aβ levels [33]. These effects were accompanied by increased amyloidogenic processing of APP in addition to reactive astrocytosis and microgliosis in Tg2576-huS100B mice. The results by Mori et al. lend further support to the notion that some forms of glial activation (brought about in this case by reactive astrocyte-derived S100B) exacerbate AD-like pathology and are thus likely detrimental to the AD-afflicted brain.

In addition to proinflammatory cytokines, chemokines play critical roles in orchestrating movement (chemotaxis) of microglia toward noxious stimuli including β-amyloid deposits. Recent studies by Fuhrmann and colleagues have shown that knocking out the expression of the fractalkine receptor, Cx3cr1, can prevent neurodegeneration [34]. The expression of Cx3cr1 is exquisitely restricted within the CNS to microglia and is considered to be a critical factor in neuron-microglia communication. Fuhrmann and colleagues used intravital two-photon imaging to observe interactions between microglia and neighboring neurons in 3x Tg-AD mice, which overexpress pathogenic mutant forms of PS1 (M146V), "Swedish" mutant APP, and tau (P301L) [35]. These 3x Tg-AD mice were crossed with a transgenic mouse line expressing yellow fluorescent protein in cortical layers III and V and another line expressing green fluorescent protein in place of the endogenous murine Cx3cr1 locus. Time-lapse intravital imaging showed neuron loss in Cx3cr1-sufficient mice while neurons in Cx3cr1-deficient mice survived. In addition, they observed that Cx3cr1-sufficient microglia rapidly mobilized toward neurons destined for degeneration. However, Cx3cr1-deficient mice did not exhibit change in Aβ abundance, suggesting that the phagocytic ability of microglia in these mice was either not altered or not involved in the observed neuron loss phenotype. Similar studies by Bruce Lamb, Richard Ransohoff, and their colleagues have shown that Cx3cr1 deficiency results in a gene dose-dependent reduction in β-amyloid deposition in two different mouse models of AD: APP/PS1 and R1.40 [36]. Interestingly, in their models, Cx3cr1 deficiency also resulted in reduced numbers of microglia surrounding Aβ deposits as well as attenuated immunostaining for CD68 (but not CD45) and altered expression of inflammatory cytokines and chemokines, including reduced levels of TNF-α and CCL2 mRNAs, but elevated IL-1β mRNA levels. Moreover, the authors demonstrated *in vivo* and *in vitro* that $Cx3cr1^{-/-}$ microglia had enhanced ability to phagocytose Aβ. While the study by Fuhrmann and coworkers did not show differences in Aβ abundance between Cx3cr1-sufficient and -deficient mice, Lee et al. suggested that Aβ aggregates in 3x Tg-AD mice may be more intracellular than extracellular at the age

Table 1: "Bad" microglia in Alzheimer disease.

Publication(s)	Type of Study	Observations
Wisniewski et al., 1989 [1], 1992 [2]; Frackowiak et al., 1992 [3]	Neuropathologic	Microglia are "frustrated phagocytes" responsible for manufacture of amyloid fibrils and not for their removal.
Meda et al., 1995 [15]	*In vitro*	Activated microglia secrete proinflammatory cytokines that promote neural injury at high levels.
Tan et al., 1999 [16]	Mouse models	Aβ and CD40L-stimulated microglia release TNF-α that injures primary cortical neurons. CD40 ligand-deficient Tg2576 mice have reduced microglial activation and tau hyperphosphorylation.
McGeer et al. 1990 [18], 1992 [19], 1996 [20]	Epidemiologic	There is lower incidence of dementia in elderly patients with arthritis compared to the general population.
Mackenzie and Munoz, 1998 [21]	Neuropathologic	Chronic NSAID users with senile plaques have 3-fold less activated microglia than non-users.
Szekely et al., 2004 [23]	Epidemiologic	Systematic review of over 25 epidemiologic studies shows ~50% reduced risk of AD associated with NSAID use.
Lim et al., 2000 [24], 2001 [72]	Mouse models	NSAID-treated Tg2576 mice have significantly reduced amyloid deposition, astrogliosis, and IL-1β abundance.
Jantzen et al., 2002 [25]; Yan et al., 2003 [26]; Heneka et al., 2005 [27]	Mouse models	Ibuprofen-treated Tg2576, APP/PS1 or APPV717I transgenic mice have reduced microglial activation and amyloid deposits.
Tan et al., 2002 [30]	Mouse models	Genetic or pharmacologic ablation of CD40 ligand in Tg2576 mice reduces cerebral amyloidosis and mitigates gliosis.
Townsend et al., 2005 [31]	*In vitro*	CD40 ligand in the presence of Aβ_{1-42} promotes a microglial proinflammatory antigen presenting cell phenotype.
Qiao et al., 2001 [32]	Mouse models	Chronic intracerebroventricular injection of LPS accelerates Aβ plaque load in APPV717F transgenic mice.
Mori et al., 2010 [33]	Mouse models	Forcing expression of proinflammatory S100B accelerates glial activation and cerebral amyloid pathology in Tg2576 mice.
Fuhrmann et al., 2010 [34]	Mouse models	Cx3cr1 endorses microglial-mediated neuronal loss in 3xTg AD mice.
Lee et al., 2010 [36]	Mouse models	Cx3cr1 deficiency reduces cerebral amyloid and reactive microglia in APP/PS1 and R1.40 mice. Cx3cr1$^{-/-}$ microglia have greater Aβ uptake.
Sundaram et al., 2012 [38]	Mouse models	Neuroinflammation occurs early and promotes neurodegeneration mediated by lysophosphatidylcholine and Cdk5/p25. Inducible p25 expression *in vivo* triggers neuroinflammation and intraneuronal Aβ.

at which this study was done, therefore affecting the ability of microglia to phagocytose these deposits. Nonetheless, these studies suggest that Cx3cr1 signaling plays a role in (a) homing of microglia to neurons that are destined for death and (b) determining microglial responses to cerebral amyloidosis. In both cases, signaling through Cx3cr1 seems to direct microglia to respond in such a way that aids rather than combats the progression of AD-like pathology.

Finally, previous studies, chiefly from the group of Li-Huei Tsai, have established that activation of cyclin-dependent kinase five (Cdk5) via proteolytic cleavage of p35 to p25 is a key event in AD patient brains that can promote neurodegeneration [37]. However, the role of brain inflammation in this process was not previously clear. In a very recent study, the Kesavapany group examined the consequences of driving p25/Cdk5 activity both *in vivo* and *in vitro* on neuroinflammation and neuronal death [38]. The authors demonstrated that the Cdk5/p25 pathway triggered neuroinflammation via lysophosphatidylcholine that proceeded and led to neurodegeneration and neuronal loss *in vitro*. In addition, they showed that inducible Cdk5/p25 expression *in vivo* caused early neuroinflammation followed by later accumulation of copious intraneuronal Aβ. One interpretation of this finding is that Aβ production signifies an acute-phase stress response that can both be initiated by neuroinflammation and drive it. If this is true, then one could imagine a feed-forward loop endorsing a constellation of damaging inflammatory mediators. These findings provide direct evidence that neuroinflammation, at least as initiated by Cdk5/p25 activation, occurs early and can promote neurodegenerative changes. All of these studies suggesting detrimental actions of microglia are summarized in Table 1.

4. A Case for "Good" Microglia

4.1. Evidence from Humans and Preclinical Mouse Models. Inflammatory responses are not always deleterious, and are often even necessary for survival. Given the exquisite sensitivity of neurons to inflammation-induced bystander injury however, there is a fine line between neuroinflammation that results in tissue repair versus excessive damage to brain cells [39]. Epidemiological findings demonstrating reduced risk for AD in patients using NSAIDs led to the design of the Alzheimer disease anti-inflammatory prevention trial (ADAPT), a randomized controlled trial to test the association of NSAID use with cognitive function over time in nondemented elderly individuals. The result of this trial indicated no protection afforded by NSAIDs (naproxen or celecoxib) on cognitive scores, and weak evidence for lower cognitive scores in naproxen users [40, 41]. This latter result suggests that chronic use of naproxen might have actually been detrimental in the ADAPT study. If correct, there exists a possibility that microglial activation was actually beneficial and that treatment with naproxen could have negated this effect. While these results need to be interpreted with caution since the trial was prematurely halted due to fear of cardiotoxicity associated with certain NSAIDs, other trials testing NSAIDs for treatment of AD or as preventative agents for mild cognitive impairment also had null findings [42]. That the clinical studies did not support epidemiological findings raises the possibility that the context of microglial activation and other factors such as whether individuals were cognitively healthy or "on the verge" of conversion to mild cognitive impairment or to AD at the time of NSAID treatment are critically important outcome determinants [43]. Also, because many of the healthy elderly participants in the ADAPT trial were arthritics, these individuals' peripheral immune environment may impact their risk for later developing AD [44] and therefore could represent a confound to interpreting the risk relationship between NSAID use and development of AD.

While the above reports investigated the effects of NSAIDs in the clinic, studies in the late 1990s from Dale Schenk and colleagues at Elan pharmaceuticals yielded surprising results in preclinical mouse models. They administered peripheral injections of $A\beta_{1-42}$ plus complete Freund's adjuvant into young PDAPP transgenic mice, which overexpress mutant APP. Their intention was to worsen AD-like pathology in these mice. However, they instead found that $A\beta_{1-42}$ plus adjuvant given to young mice essentially blocked β-amyloid plaque formation, and treatment of older animals significantly mitigated the extent of cerebral amyloid deposits. In addition, Schenk and colleagues observed MHC II-positive microglia colocalized with Aβ plaques in $A\beta_{1-42}$ treated mice, which were not observed in control mice treated with PBS plus adjuvant [45]. These results were supported by independent studies that demonstrated decreased behavioral impairment in Aβ-immunized PDAPP mice [46, 47]. In a subsequent study, Bard and colleagues showed that passive transfer of Aβ antibodies from vaccinated mice to transgenic PDAPP mice also reduced cerebral amyloidosis. In that study, they observed punctate immunoreactivity for Aβ that co-localized with activated microglia, and suggested that Aβ plaque clearance was mediated by antibodies against amyloid β-peptide that crossed the BBB and triggered microglial cells through Fc receptor-mediated phagocytosis [48]. While microglia are generally regarded as inefficient Aβ phagocytes [11], Bard and colleagues suggested the passive Aβ vaccine was somehow able to lure microglia into phagocytosing Aβ antibody-opsonized plaques.

Based on encouraging results in mouse models, Elan pharmaceuticals and Wyeth partnered to develop an Aβ vaccine for use in humans. The drug, named AN-1792, consisted of synthetic $A\beta_{1-42}$ peptide in QS-21 adjuvant, and a phase I trial did not reveal significant adverse effects in a limited cohort of 80 subjects. The phase IIa trial was halted prematurely, however, when four participants (~5-6% of study subjects) developed clinical signs consistent with aseptic meningoencephalitis [49]. Shortly after the trial was halted, a case report was published of a 72-year-old woman who had a history of probable AD and had received AN-1792 and responded with elevated Aβ antibody titers. Upon histological examination, her brain showed extensive areas of the neocortex with very few plaques and regions devoid of plaques that had punctate immunoreactivity for Aβ that often colocalized with phagocytic microglia. Such results were consistent with previously published reports in AD transgenic mice suggesting that the vaccine instigated microglial clearance of Aβ [50]. A subsequent study of eight participants who received immunization and developed Aβ-specific antibodies showed clear evidence of amyloid plaque removal but no effect of immunotherapy on prevention of cognitive decline [51]. Together, these data suggest that positive signal in mouse models of cerebral amyloidosis does not always translate to human AD and, in the case of Aβ immunotherapy, that careful preclinical toxicology in non-human primates is critically important.

4.2. Evidence from Mouse Genetics Approaches. Previous studies have shown elevated CD45 expression on reactive microglia in AD brains compared with controls [52], and our group investigated the role of CD45 in responsiveness of microglia to Aβ peptides [53]. Because CD45 is a membrane-bound protein-tyrosine phosphatase, we inhibited its function in the context of Aβ stimulation by cotreating primary cultured microglia with a tyrosine phosphatase inhibitor and Aβ peptides. This resulted in secretion of TNF-α and nitric oxide that injured neurons in coculture conditions. Furthermore, treatment with an agonistic CD45 antibody markedly inhibited these detrimental effects via blocking p44/42 mitogen-activated protein kinase, suggesting that CD45 activation promotes beneficial, neuroprotective function of microglia. After stimulation with Aβ peptides, primary cultured microglia from CD45-deficient mice exhibited copious TNF-α release, nitric oxide production, and neuronal injury, and brains from Tg2576 mice deficient for CD45 had significantly increased production of TNF-α compared with CD45-sufficient Tg2576 littermates. In

more recent studies, we reported that CD45-deficient PSAPP mice had increased abundance of soluble oligomeric and insoluble Aβ (both extracellular and intracellular species), increased TNF-α and IL-1β proteins, and neuronal loss compared with CD45-sufficient PSAPP littermates [54]. These studies demonstrate that the cell surface marker CD45 promotes "good" microglial activation in the context of Aβ challenge, likely by endorsing an Aβ phagocytic phenotype that mitigates cerebral amyloidosis.

There are also studies demonstrating that proinflammatory cytokines can promote beneficial neuroinflammation that actually resolves cerebral amyloidosis in transgenic mice. In one of the earliest reports, Shaftel and colleagues studied the role of IL-1β in chronic neuroinflammation and in AD by engineering an IL-1β^{XAT} transgenic mouse [55]. This model was constructed to overexpress IL-1β in the CNS using the GFAP promoter. Following injection of the FIV-Cre construct into hippocampi of these mice, a STOP codon in the IL-1β^{XAT} transgene is excised, thus activating overexpression of IL-1β in a temporal and spatial manner. In this mouse model, IL-1β expression led to robust neuroinflammation characterized by activation of astrocytes and microglia and induction of proinflammatory cytokines. Moreover, when IL-1β^{XAT} mice were crossed with the APP/PS1 mouse model of AD and the hippocampi of the resulting compound transgenic mice were injected with FIV-Cre, those authors observed dramatically reduced amyloid plaque pathology in the injected area of the brain. Additionally, Shaftel and colleagues showed that IL-1β overexpression caused an increase in the number of microglia overlapping with amyloid plaques, an increase in Iba1 staining intensity, and high levels of MHC II expression in the same cells. These findings implicate IL-1β expression in activating a "good" form of neuroinflammation in APP/PS1 mice, which the authors suggested mediated enhanced phagocytosis of amyloid plaques by activated microglia. However, their studies do not rule out the role that other cell types might have played in this scenario.

In another study, Chakrabarty and colleagues used recombinant adeno-associated virus serotype 1 (rAAV1) to express murine IFN-γ (mIFN-γ) in brains of the TgCRND8 mouse model of cerebral amyloidosis. Those authors demonstrated the ability of this potent proinflammatory cytokine to clear amyloid plaques [56]. Specifically, neonatal TgCRND8 mice injected with rAAV1-mIFN-γ in cerebral ventricles were euthanized at 3 months of age for analysis. The results showed widespread increased immunoreactivity for both GFAP and Iba1 in their brains. In addition, brains of these mice exhibited decreased levels of soluble Aβ and Aβ plaque burden, and did not show evidence of altered APP processing. Similar results were found after rAAV1-mIFN-γ injection into hippocampi at 4 months of age and pathological analysis 6 weeks later. Furthermore, mIFN-γ expression *in vivo* resulted in significant up-regulation of several microglial markers (e.g., MHC I, MHC II, CD11b, and CD11c), suggesting a microglial phenotype reminiscent of an antigen-presenting cell. Chakrabarty and colleagues also demonstrated in *in vitro* studies that mouse microglia primed with mIFN-γ had increased uptake of fluorescently tagged Aβ_{1-42} aggregates compared to control microglia. These results were not only restricted to mIFN-γ, as similar studies from this group using IL-6 and TNF-α rAAV approaches produced consistent findings [57, 58]. In contrast to the findings of Qiao et al. outlined in the previous section, DiCarlo and colleagues found that induction of multiple proinflammatory molecules following a single intrahippocampal injection of LPS into APP/PS1 transgenic mice resulted in activated microglia but reduced Aβ plaque load compared to saline-injected mice [59]. When taken together, these results suggest that certain forms of proinflammatory microglial activation are potentially beneficial for reducing AD-like pathology in transgenic mouse models.

In a different experimental paradigm, Wilcock and colleagues deleted the microglial proinflammatory nitric oxide synthase 2 (NOS2) gene in the APPSwDI mouse model of cerebrovascular amyloidosis. Those authors were able to create a model with progressive amyloid pathology as well as tau pathology and neuronal loss [60]. The authors pointed out that a difference in nitric oxide (NO) production between human and mouse microglia may be a key factor in this result. As such, they hypothesized that lower levels of NO production by human microglia create an environment in which AD-like pathology is endorsed, whereas high levels of NO (such as those produced by mouse microglia) are neuroprotective. Therefore, the authors suggest that NOS2 deficiency creates a milieu that is conducive to AD pathology. This study provides yet another interesting example of how the context of the brain inflammatory milieu can determine whether microglia play a beneficial or detrimental role in the progression of AD-like pathology. All of the studies reviewed suggesting beneficial actions of microglia are summarized in Table 2.

5. Heterogeneous Microglial Activation States

As underscored by the studies reviewed above, it is becoming more and more appreciated that microglial activation is not simply a single phenotype. In fact, the most parsimonious interpretation of the evidence thus far points to broad heterogeneity of microglial activation states. But an open question remains as to how to best define these various forms of reactive microglia. On the one hand, an activation "continuum" likely exists that makes delimiting discrete phenotypes difficult [61]. A complementary view is that microglia exist in at least three distinct activated forms in the context of neuroinflammatory diseases: classical (proinflammatory) activation, alternative (anti-inflammatory) activation, and acquired deactivation [62, 63]. In the context of mouse models of cerebral amyloidosis, it is now clear that microglia exhibit a mixed pattern of activation, with elements of both classical and alternative activation [62]. The picture is further complicated by different populations of microglia likely coexisting, each population with its own activated phenotype. Clearly, one of the great challenges for the field will be to define robust and informative markers that predict functional outcomes of these heterogeneous microglial activation phenotypes.

Table 2: "Good" microglia in Alzheimer disease.

Publication(s)	Type of study	Observations
Martin et al., 2008 [41]	Epidemiologic	No primary prevention of AD in an NSAID clinical trial; weak evidence for lower cognitive scores in naproxen users.
Schenk et al., 1999 [45]	Mouse models	Aβ immunotherapy mitigates β-amyloid plaque pathology in PDAPP mice; phagocytic microglia colocalize with remaining Aβ deposits.
Janus et al., 2000 [46]; Morgan et al., 2000 [47]	Mouse models	Behavioral impairment is reduced in Aβ-immunized TgCRND8 or APP/PS1 transgenic mice.
Bard et al., 2000 [48]	Mouse models	Passive Aβ immunotherapy reduces cerebral amyloidosis in PDAPP mice. Remaining Aβ deposits colocalize with phagocytic microglia.
Nicoll et al., 2003 [50]	Neuropathologic	AN-1792 Aβ immunotherapy results in striking reduction of amyloid pathology; remaining plaques colocalize with phagocytic microglia.
Tan et al., 2000 [53]	Mouse models	Microglial CD45 negatively regulates microglial activation and opposes activated microglia-induced neuronal cell injury.
Zhu et al., 2011 [54]	Mouse models	Microglial CD45 endorses phagocytosis and clearance of Aβ peptides and oligomers in PSAPP mice.
Shaftel et al., 2007 [55]	Mouse models	Forcing brain IL-1β expression using IL-1β^{XAT} transgenic mice activates "good" neuroinflammation and dramatically reduces amyloid plaque pathology in APP/PS1 mice.
Chakrabarty et al., 2010 [56]	Mouse models	Forcing cerebral IFN-γ expression leads to profound neuroinflammation and clears amyloid plaques in TgCRND8 mice via microglial phagocytosis.
Chakrabarty et al., 2010 [57]	Mouse models	Overexpressing brain IL-6 leads to massive gliosis and clears amyloid plaques in TgCRND8 mice via microglial phagocytosis.
Chakrabarty et al., 2011 [58]	Mouse models	Increasing cerebral proinflammatory TNF-α expression clears amyloid pathology in TgCRND8 mice.
DiCarlo et al., 2001 [59]	Mouse models	Acute intrahippocampal injection of LPS reduces Aβ plaque load in APP/PS1 transgenic mice.
Wilcock et al., 2008 [60]	Mouse models	APPSwDI mice deficient in proinflammatory NOS2 have tau pathology and neuronal loss.

6. But Do Brain-Resident Microglia Play Any Role at All?

While many of the studies cited above suggest that microglia can be deleterious or beneficial in the context of AD-like pathology depending on contextual cues, a recent study suggests that brain-resident microglia do not play a significant role in the formation, maintenance or clearance of amyloid plaques. Mathias Jücker and colleagues used a CD11b-tyrosine kinase/ganciclovir suicide gene approach to kill microglia for two to four weeks in two different AD mouse models (APP/PS1 and APP23) [64]. Selective ablation of microglia in APP/PS1 transgenic mice did not result in differences in total Aβ burden, plaque morphology, or distribution of cerebral Aβ deposits. While it is possible that ablation of brain-resident microglia for two to four weeks is not sufficient time to observe a significant change in Aβ plaque remodeling in transgenic mouse models of cerebral amyloid deposition, this study implies that microglia are not always primary players in the complex landscape of AD pathobiology.

7. Hematogenous Macrophages

As mentioned earlier, CNS-exogenous hematogenous macrophages have been suggested to play a role in the innate immune responses in the brain. Independent studies from the laboratories of Mathias Jücker and Serge Rivest have shown limited infiltration of peripheral mononuclear cells into the CNS of cerebral amyloidosis mouse models [65, 66]. Both studies demonstrated that bone marrow-derived cells were spatially associated with amyloid plaques. While Stalder and coworkers were not able to uncover evidence of amyloid phagocytosis within these cells by electron microscopy, Simard and colleagues presented convincing evidence of amyloid deposits in GFP^+ infiltrating mononuclear phagocytes. Further, these authors crossed APP/PS1 mice with CD11b-tyrosine kinase mutant (TK^{mut30}) transgenic mice in which proliferating $CD11b^+$ cells can be specifically ablated by administering the antiviral drug ganciclovir. Using this mouse model, the authors were able to convincingly show that proliferating MHC II-positive peripheral mononuclear phagocytes played an important role in restricting β-amyloid

plaques. Another landmark study in this area was from the group of Joseph El Khoury. By crossing mice deficient in the chemokine receptor Ccr2 with the Tg2576 mouse model of cerebral amyloidosis, they were able to demonstrate that restriction of hematogenous microglia entry into the brain led to increased plaque load [67].

These observations led to studies by our group investigating whether peripheral mononuclear phagocytes (hematogenous macrophages) could be targeted to infiltrate into the brain and militate against AD-like pathology. We engineered a CD11c promoter-driven dominant-negative transforming growth factor-beta (TGF-β) type II receptor transgene in C57BL/6 mice (CD11c-DNR mice) [68], and were thus able to genetically interrupt TGF-β and downstream Smad 2/3 signaling specifically on peripheral innate immune cells. We crossed CD11c-DNR mice with the Tg2576 mouse model of cerebral amyloid and evaluated behavioral impairment and AD-like pathology [69]. Interestingly, doubly transgenic mice showed up to 90% attenuation of brain parenchymal and cerebrovascular amyloid deposits. Further, these animals exhibited partial amelioration of cognitive impairment and reduced astrocytosis, effects that were associated with increased infiltration of Aβ-containing peripheral mononuclear phagocytes in and around cerebral vessels and amyloid plaques. We were also able to observe Aβ immunoreactivity within the cytoplasm of these cells, suggesting that these peripheral macrophages were actively clearing Aβ via phagocytosis. In addition, the peripherally-derived macrophages displayed an anti-inflammatory $CD45^{+}CD11b^{+}Ly\text{-}6C^{-}$ cell surface phenotype and secreted elevated levels of the canonical anti-inflammatory cytokine, interleukin-10 [70, 71], suggesting that the beneficial effect of reduced β-amyloid did not come at the cost of increased brain inflammation. Additionally, we were able to demonstrate ~3-fold increased phagocytosis of Aβ by CD11c-DNR versus wild-type macrophages *ex vivo* and similar effects *in vitro* with pharmacologic inhibitors of TGF-β-Smad2/3 signaling, suggesting that inhibition of TGF-β-Smad 2/3 signaling allows for both peripheral mononuclear phagocyte recruitment to brains of AD model mice and for phagocytic amyloid removal.

8. Concluding Remarks

It is now becoming widely appreciated that there are numerous forms of "activated" microglia, some of which are detrimental, and others, beneficial (Figure 1). In this paper, we have attempted to survey the state of the field with respect to both "good" and "bad" forms of reactive microgliosis in terms of AD-like pathology. A synthesis of the literature only seems possible when considering context—the conditions under which microglia encounter AD-like pathological lesions. Specifically, a model emerges where microglia mount different types of activated responses depending on whether they encounter particular species of misfolded protein (Aβ or perhaps even tau) and whether this innate recognition occurs early on or after pathology is well-established. While it is not yet clear how microglial cells differentiate between these various forms of pathogenic peptides and proteins, it is likely that a varied set of immune molecules orchestrate these complex innate immune responses. A deeper understanding of these molecules, and specifically, which pathways lead to beneficial responses to clear pathogenic misfolded proteins in AD, will be key for harnessing these innate immune cells to militate against neuropathology.

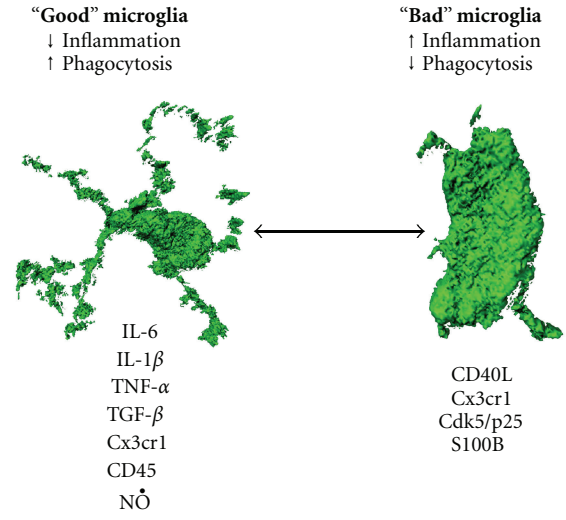


Figure 1: "Good" versus "Bad" microglia in Alzheimer disease. Illustration using 3D models of ramified "good" microglia and ovoid "bad" microglia along with the array of immune molecules that likely help to determine which activated form of microglia will respond to AD-like pathological lesions. It is postulated that microglial phenotype can interconvert between ramified and ovoid functional states. The 3D images were generated using Imaris: Bitplane 3D modeling software and are provided courtesy of David Gate.

Acknowledgments

T. Town is supported by the National Institute of Health/National Institute on Aging (5R00AG029726-04) and the National Institute of Health/National Institute on Neurologic Disorders and Stroke (1R01NS076794-01), an Alzheimer's Association Zenith Fellows Award (ZEN-10-174633), and an American Federation of Aging Research/Ellison Medical Foundation Julie Martin Mid-Career Award in Aging Research (M11472). T. M. Weitz is supported by a National Institute of Health/National Institute on Aging award (1R01NS076794-01). T. Town is the inaugural holder of the Ben Winters Endowed Chair in Regenerative Medicine.

References

[1] H. Wisniewski, J. Wegiel, K. Wang, M. Kujawa, and B. Lach, "Ultrastructural studies of the cells forming amyloid fibers in classical plaques," *Canadian Journal of Neurological Sciences*, vol. 16, no. 4, pp. 535–542, 1989.

[2] H. Wisniewski, J. Wegiel, K. C. Wang, and B. Lach, "Ultrastructural studies of the cells forming amyloid in the cortical

vessel wall in Alzheimer's disease," *Acta Neuropathologica*, vol. 84, no. 2, pp. 117–127, 1992.

[3] J. Frackowiak, H. Wisniewski, J. Wegiel, G. Merz, K. Iqbal, and K. C. Wang, "Ultrastructure of the microglia that phagocytose amyloid and the microglia that produce β-amyloid fibrils," *Acta Neuropathologica*, vol. 84, no. 3, pp. 225–233, 1992.

[4] H. Wisniewski, M. Barcikowska, and E. Kida, "Phagocytosis of β/A4 amyloid fibrils of the neuritic neocortical plaques," *Acta Neuropathologica*, vol. 81, no. 5, pp. 588–590, 1991.

[5] H. Akiyama, H. Kondo, H. Mori et al., "The amino-terminally truncated forms of amyloid β-protein in brain macrophages in the ischemic lesions of Alzheimer's disease patients," *Neuroscience Letters*, vol. 219, no. 2, pp. 115–118, 1996.

[6] V. W. Yong and S. Rivest, "Taking advantage of the systemic immune system to cure brain diseases," *Neuron*, vol. 64, no. 1, pp. 55–60, 2009.

[7] K. Rezai-Zadeh, D. Gate, and T. Town, "CNS infiltration of peripheral immune cells: D-Day for neurodegenerative disease?" *Journal of Neuroimmune Pharmacology*, vol. 4, no. 4, pp. 462–475, 2009.

[8] D. Gate, K. Rezai-Zadeh, D. Jodry, A. Rentsendorj, and T. Town, "Macrophages in Alzheimer's disease: the blood-borne identity," *Journal of Neural Transmission*, vol. 117, no. 8, pp. 961–970, 2010.

[9] R. M. Ransohoff, P. Kivisäkk, and G. Kidd, "Three or more routes for leukocyte migration into the central nervous system," *Nature Reviews Immunology*, vol. 3, no. 7, pp. 569–581, 2003.

[10] C. A. Hawkes and J. McLaurin, "Selective targeting of perivascular macrophages for clearance of β-amyloid in cerebral amyloid angiopathy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 4, pp. 1261–1266, 2009.

[11] T. Town, V. Nikolic, and J. Tan, "The microglial "activation" continuum: from innate to adaptive responses," *Journal of Neuroinflammation*, vol. 2, no. 1, article 2, 2005.

[12] C. A. Colton, R. T. Mott, H. Sharpe, Q. Xu, W. E. Van Nostrand, and M. P. Vitek, "Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD," *Journal of Neuroinflammation*, vol. 3, no. 1, article 27, 2006.

[13] C. A. Colton, "Heterogeneity of microglial activation in the innate immune response in the brain," *Journal of Neuroimmune Pharmacology*, vol. 4, no. 4, pp. 399–418, 2009.

[14] C. Colton and D. Wilcock, "Assessing activation states in microglia," *CNS and Neurological Disorders*, vol. 9, no. 2, pp. 174–191, 2010.

[15] L. Meda, M. A. Cassatella, G. I. Szendrei et al., "Activation of microglial cells by β-amyloid protein and interferon-γ," *Nature*, vol. 374, no. 6523, pp. 647–650, 1995.

[16] J. Tan, T. Town, D. Paris et al., "Microglial activation resulting from CD40-CD40l interaction after β- amyloid stimulation," *Science*, vol. 286, no. 5448, pp. 2352–2355, 1999.

[17] P. L. McGeer and E. McGeer, "Inflammation and the degenerative diseases of aging," *Annals of the New York Academy of Sciences*, vol. 1035, no. 1, pp. 104–116, 2004.

[18] P. McGeer, E. McGeer, J. Rogers, and J. Sibley, "Anti-inflammatory drugs and Alzheimer disease," *The Lancet*, vol. 335, no. 8696, p. 1037, 1990.

[19] P. McGeer and J. Rogers, "Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease," *Neurology*, vol. 42, no. 2, pp. 447–449, 1992.

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

[21] I. Mackenzie and D. Munoz, "Nonsteroidal anti-inflammatory drug use and Alzheimer-type pathology in aging," *Neurology*, vol. 50, no. 4, pp. 986–990, 1998.

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

[23] C. A. Szekely, J. E. Thorne, P. P. Zandi et al., "Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review," *Neuroepidemiology*, vol. 23, no. 4, pp. 159–169, 2004.

[24] G. Lim, F. Yang, T. Chu et al., "Ibuprofen suppresses plaque pathology and inflammation in a mouse model for alzheimer's disease," *Journal of Neuroscience*, vol. 20, no. 15, pp. 5709–5714, 2000.

[25] P. T. Jantzen, K. E. Connor, G. DiCarlo et al., "Microglial activation and β-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice," *The Journal of Neuroscience*, vol. 22, no. 6, pp. 2246–2254, 2002.

[26] Q. Yan, J. Zhang, H. Liu et al., "Anti-inflammatory drug therapy alters β-amyloid processing and deposition in an animal model of Alzheimer's disease," *Journal of Neuroscience*, vol. 23, no. 20, pp. 7504–7509, 2003.

[27] M. T. Heneka, M. Sastre, L. Dumitrescu-Ozimek et al., "Acute treatment with the PPARγ agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1-42 levels in APPV717I transgenic mice," *Brain*, vol. 128, no. 6, pp. 1442–1453, 2005.

[28] D. M. Wilcock, P. T. Jantzen, Q. Li, D. Morgan, and M. N. Gordon, "Amyloid-β vaccination, but not nitro-nonsteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid," *Neuroscience*, vol. 144, no. 3, pp. 950–960, 2007.

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

[30] J. Tan, T. Town, F. Crawford et al., "Role of CD40 ligand in amyloidosis in transgenic Alzheimer's mice," *Nature Neuroscience*, vol. 5, no. 12, pp. 1288–1293, 2002.

[31] K. P. Townsend, T. Town, T. Mori et al., "CD40 signaling regulates innate and adaptive activation of microglia in response to amyloid β-peptide," *European Journal of Immunology*, vol. 35, no. 3, pp. 901–910, 2005.

[32] X. Qiao, D. J. Cummins, and S. M. Paul, "Neuroinflammation-induced acceleration of amyloid deposition in the APPV717F transgenic mouse," *European Journal of Neuroscience*, vol. 14, no. 3, pp. 474–482, 2001.

[33] T. Mori, N. Koyama, G. W. Arendash, Y. Horikoshi-Sakuraba, J. Tan, and T. Town, "Overexpression of human S100B exacerbates cerebral amyloidosis and gliosis in the Tg2576 mouse model of Alzheimer's disease," *GLIA*, vol. 58, no. 3, pp. 300–314, 2010.

[34] M. Fuhrmann, T. Bittner, C. K. E. Jung et al., "Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease," *Nature Neuroscience*, vol. 13, no. 4, pp. 411–413, 2010.

[35] S. Oddo, A. Caccamo, J. D. Shepherd et al., "Triple-transgenic model of Alzheimer's Disease with plaques and tangles: intracellular Aβ and synaptic dysfunction," *Neuron*, vol. 39, no. 3, pp. 409–421, 2003.

[36] S. Lee, N. H. Varvel, M. E. Konerth et al., "CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models," *American Journal of Pathology*, vol. 177, no. 5, pp. 2549–2562, 2010.

[37] G. N. Patrick, L. Zukerberg, M. Nikolic, S. De La Monte, P. Dikkes, and L. H. Tsai, "Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration," *Nature*, vol. 402, no. 6762, pp. 615–622, 1999.

[38] J. R. Sundaram, E. S. Chan, C. P. Poore et al., "Cdk5/p25-induced cytosolic PLA2-mediated lysophosphatidylcholine production regulates neuroinflammation and triggers neurodegeneration," *Journal of Neuroscience*, vol. 32, no. 3, pp. 1020–1034, 2012.

[39] T. E. Golde, "Inflammation takes on Alzheimer disease," *Nature Medicine*, vol. 8, no. 9, pp. 936–938, 2002.

[40] A. R. Group, C. Lyketsos, J. Breitner et al., "Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial," *Neurology*, vol. 68, no. 21, pp. 1800–1808, 2007.

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

[42] P. L. McGeer and E. G. McGeer, "NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies," *Neurobiology of Aging*, vol. 28, no. 5, pp. 639–647, 2007.

[43] J. C. Breitner, L. D. Baker, T. J. Montine et al., "Extended results of the Alzheimer's disease anti-inflammatory prevention trial," *Alzheimer's and Dementia*, vol. 7, no. 4, pp. 402–411, 2011.

[44] C. Holmes, C. Cunningham, E. Zotova et al., "Systemic inflammation and disease progression in Alzheimer disease," *Neurology*, vol. 73, no. 10, pp. 768–774, 2009.

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

[46] C. Janus, J. Pearson, J. McLaurin et al., "Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease," *Nature*, vol. 408, no. 6815, pp. 979–982, 2000.

[47] D. Morgan, D. M. Diamond, P. E. Gottschall et al., "A β peptide vaccination prevents memory loss in an animal model of Alzheimer's disease," *Nature*, vol. 408, no. 6815, pp. 982–985, 2000.

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

[49] E. Check, "Nerve inflammation halts trial for Alzheimer's drug," *Nature*, vol. 415, no. 6871, p. 462, 2002.

[50] J. A. R. Nicolll, D. Wilkinson, C. Holmes, P. Steart, H. Markham, and R. O. Weller, "Neuropathology of human Alzheimer disease after immunization with amyloid-β peptide: a case report," *Nature Medicine*, vol. 9, no. 4, pp. 448–452, 2003.

[51] C. Holmes, D. Boche, D. Wilkinson et al., "Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial," *The Lancet*, vol. 372, no. 9634, pp. 216–223, 2008.

[52] E. Masliah, M. Mallory, L. Hansen et al., "Immunoreactivity of CD45, a protein phosphotyrosine phosphatase, in Alzheimer's disease," *Acta Neuropathologica*, vol. 83, no. 1, pp. 12–20, 1991.

[53] J. Tan, T. Town, T. Mori et al., "CD45 opposes β-amyloid peptide-induced microglial activation via inhibition of p44/42 mitogen-activated protein kinase," *Journal of Neuroscience*, vol. 20, no. 20, pp. 7587–7594, 2000.

[54] Y. Zhu, H. Hou, K. Rezai-Zadeh et al., "CD45 deficiency drives amyloid-β peptide oligomers and neuronal loss in Alzheimer's disease mice," *Journal of Neuroscience*, vol. 31, no. 4, pp. 1355–1365, 2011.

[55] S. S. Shaftel, S. Kyrkanides, J. A. Olschowka, J. N. H. Miller, R. E. Johnson, and M. K. O'Banion, "Sustained hippocampal IL-1β overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology," *Journal of Clinical Investigation*, vol. 117, no. 6, pp. 1595–1604, 2007.

[56] P. Chakrabarty, C. Ceballos-Diaz, A. Beccard et al., "IFN-γ promotes complement expression and attenuates amyloid plaque deposition in amyloid β precursor protein transgenic mice," *Journal of Immunology*, vol. 184, no. 9, pp. 5333–5343, 2010.

[57] P. Chakrabarty, K. Jansen-West, A. Beccard et al., "Massive gliosis induced by interleukin-6 suppresses Aβ deposition *in vivo*: evidence against inflammation as a driving force for amyloid deposition," *FASEB Journal*, vol. 24, no. 2, pp. 548–559, 2010.

[58] P. Chakrabarty, A. Herring, C. Ceballos-Diaz, P. Das, and T. E. Golde, "Hippocampal expression of murine TNFα results in attenuation of amyloid deposition *in vivo*," *Molecular Neurodegeneration*, vol. 6, no. 1, article 16, 2011.

[59] G. DiCarlo, D. Wilcock, D. Henderson, M. Gordon, and D. Morgan, "Intrahippocampal LPS injections reduce Aβ load in APP+PS1 transgenic mice," *Neurobiology of Aging*, vol. 22, no. 6, pp. 1007–1012, 2001.

[60] D. M. Wilcock, M. R. Lewis, W. E. van Nostrand et al., "Progression of amyloid pathology to Alzheimer's disease pathology in an amyloid precursor protein transgenic mouse model by removal of nitric oxide synthase 2," *Journal of Neuroscience*, vol. 28, no. 7, pp. 1537–1545, 2008.

[61] T. Town, V. Nikolic, and J. Tan, "The microglial "activation" continuum: from innate to adaptive responses," *Journal of Neuroinflammation*, vol. 2, article 24, 2005.

[62] C. A. Colton, R. T. Mott, H. Sharpe, Q. Xu, W. E. van Nostrand, and M. P. Vitek, "Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD," *Journal of Neuroinflammation*, vol. 3, article 27, 2006.

[63] C. A. Colton and D. M. Wilcock, "Assessing activation states in microglia," *CNS and Neurological Disorders*, vol. 9, no. 2, pp. 174–191, 2010.

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

[65] A. K. Stalder, F. Ermini, L. Bondolfi et al., "Invasion of hematopoietic cells into the brain of amyloid precursor protein transgenic mice," *Journal of Neuroscience*, vol. 25, no. 48, pp. 11125–11132, 2005.

[66] A. R. Simard, D. Soulet, G. Gowing, J. P. Julien, and S. Rivest, "Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease," *Neuron*, vol. 49, no. 4, pp. 489–502, 2006.

[67] J. El Khoury, M. Toft, S. E. Hickman et al., "Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease," *Nature Medicine*, vol. 13, no. 4, pp. 432–438, 2007.

[68] Y. Laouar, T. Town, D. Jeng et al., "TGF-β signaling in dendritic cells is a prerequisite for the control of autoimmune

encephalomyelitis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 31, pp. 10865–10870, 2008.

[69] T. Town, Y. Laouar, C. Pittenger et al., "Blocking TGF-β-Smad2/3 innate immune signaling mitigates Alzheimer-like pathology," *Nature Medicine*, vol. 14, no. 6, pp. 681–687, 2008.

[70] F. Geissmann, S. Jung, and D. R. Littman, "Blood monocytes consist of two principal subsets with distinct migratory properties," *Immunity*, vol. 19, no. 1, pp. 71–82, 2003.

[71] C. Sunderkötter, T. Nikolic, M. J. Dillon et al., "Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response," *Journal of Immunology*, vol. 172, no. 7, pp. 4410–4417, 2004.

[72] G. P. Lim, T. Chu, F. Yang, W. Beech, S. A. Frautschy, and G. M. Cole, "The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse," *Journal of Neuroscience*, vol. 21, no. 21, pp. 8370–8377, 2001.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 868972, 8 pages
doi:10.1155/2012/868972

Review Article

Microglial KCa3.1 Channels as a Potential Therapeutic Target for Alzheimer's Disease

Izumi Maezawa,[1,2] David Paul Jenkins,[3] Benjamin E. Jin,[3] and Heike Wulff[3]

[1] *Department of Pathology and Laboratory Medicine, University of California Davis, Davis, CA 95616, USA*
[2] *MIND Institute, University of California Davis Medical Center, 2805 50th Street, Sacramento, CA 95817, USA*
[3] *Department of Pharmacology, University of California Davis, Davis, CA 95616, USA*

Correspondence should be addressed to Izumi Maezawa, imaezawa@ucdavis.edu

Received 31 January 2012; Accepted 21 March 2012

Academic Editor: Lee-Way Jin

There exists an urgent need for new target discovery to treat Alzheimer's disease (AD); however, recent clinical trials based on anti-Aβ and anti-inflammatory strategies have yielded disappointing results. To expedite new drug discovery, we propose reposition targets which have been previously pursued by both industry and academia for indications other than AD. One such target is the calcium-activated potassium channel KCa3.1 (KCNN4), which in the brain is primarily expressed in microglia and is significantly upregulated when microglia are activated. We here review the existing evidence supporting that KCa3.1 inhibition could block microglial neurotoxicity without affecting their neuroprotective phagocytosis activity and without being broadly immunosuppressive. The anti-inflammatory and neuroprotective effects of KCa3.1 blockade would be suitable for treating AD as well as cerebrovascular and traumatic brain injuries, two well-known risk factors contributing to the dementia in AD patients presenting with mixed pathologies. Importantly, the pharmacokinetics and pharmacodynamics of several KCa3.1 blockers are well known, and a KCa3.1 blocker has been proven safe in clinical trials. It is therefore promising to reposition old or new KCa3.1 blockers for AD preclinical and clinical trials.

1. Repositioning an "Old," Non-AD Specific Target for AD Therapy

All currently FDA-approved drugs for Alzheimer's disease (AD), the three acetylcholinesterase inhibitors Aricept, Razadyne, and Exelon, and the *N*-methyl-D-aspartate receptor antagonist, Namenda, only treat the symptoms of AD and cannot hold its progression. There therefore exists an urgent need for new target discovery to treat AD. The main approaches for AD drug discovery tend to focus on AD-specific molecular targets, such as those involved in the generation and aggregation of amyloid-β protein (Aβ). Several such targets have been investigated and have driven developments of therapeutic reagents showing impressive preclinical efficacy. However, very few of these developments have resulted in target validation in humans or successful translation to disease-modifying therapies [1].

These setbacks could still be overcome [2], but, in our view, should prompt pursuit of alternative approaches to target molecules that are not AD specific, which could provide additional chance of success. There are two major approaches under this category: either one could devise a broadly neuroprotective compound that is useful for many CNS indications, or one could "reposition" an existing target which has been previously pursued by both industry and academia for other indications and therefore is better understood. The former approach makes sense because it is increasingly obvious that AD is caused by multiple converging insults related but not specific to AD rather than by a single cascade pathway [3, 4]. This, in our view, is perhaps one of the reasons for failures of clinical trials targeting AD-specific pathways. We need to recall that the overwhelmingly major risk factor for AD is aging, and aging is certainly a multifactorial process. In addition, in

reality, "pure AD" is relatively uncommon; most demented individuals show multiple pathologies including Lewy body pathology, TDP-43 pathology, and cerebrovascular diseases (CVDs), in addition to the traditional AD-type amyloid plaques and neurofibrillary tangles [5, 6]. In particular, the combination of CVD and AD commonly called "mixed dementia," accounts for most dementia cases in community-dwelling older persons [7]. Approximately 60 to 90% of individuals with AD also have vascular brain pathologies [8]. Drugs specifically targeting pure AD pathologies may not address these common comorbidities, while broadly neuroprotective compounds could perhaps better address the downstream common pathways leading to synaptic and neuronal dysfunction. As an example, recently the Schubert group developed a drug screening procedure that is based upon old age-associated pathologies without requiring preselected molecular targets. The panel of screening assays was able to identify compounds that protect neurons from loss of trophic support, oxidative stress, aberrant energy metabolism, and amyloid toxicity. They subsequently identified a lead compound that showed promising effects in enhancing the memory performance of a transgenic AD mouse model [9]. Because the targets of this compound, although unknown, are not restricted to the AD-related amyloid toxicity pathway, this compound also facilitated memory in normal rodents. Viewed as a "memory enhancer," this compound is predicted to be useful in other CNS indications affecting memory.

Our own approach, being reviewed here, is to reposition a target, KCa3.1, which has been pursued for both non-CNS and CNS indications for years, for AD therapy. We have to keep in mind that on average it currently takes at least 15 years and $1.5 billion to bring a drug for a major indication like AD to market. As an example, for more than a decade, remarkable efforts and resources have been devoted to anti-Aβ strategies based on the widely accepted amyloid cascade hypothesis [10]. However, results from several clinical trials are disappointing, for a multitude of reasons. In essence, Golde et al. pointed out that none of the putative anti-Aβ agents that have failed in pivotal phase 3 trials were optimal or even optimized agents within their class of anti-Aβ therapeutics [2]. They were hampered by low potency, poor brain penetration, and significant mechanism-based toxicity (such as from the nonselective action of γ-secretase inhibitors to block physiological functions), illustrating the practical difficulties of translating a brand new target to a new clinically useful drug. In addition, toxicity or poor tolerance during clinical trials is a common reason leading to failure of a new compound. It is therefore advantageous to reposition known targets, for which a wealth of pharmacological knowledge has been accumulated, and safety has been demonstrated in clinical trials. For these old targets, there typically exist useful pharmacological tool compounds that can be quickly resynthesized, evaluated in animal models to obtain proof-of-concept, and then optimized for specific properties such as brain penetration. This approach should expedite new drug development which is currently urgently needed for AD.

2. Three Criteria for Developing a "Pathway-Selective" Inhibitor of Microglial Activation for Anti-Inflammatory Therapy

Neuroinflammation and associated neuronal dysfunction mediated by activated microglia play an important role in the pathogenesis of AD [14]. Microglia, the resident macrophages and major mediator of neuroinflammation in the brain, can be activated by a variety of pathologic stimuli including the amyloid aggregates formed by amyloid-β protein (Aβ) [15–17]. Although microglia were initially noted to be abundantly present around amyloid plaques [18] and thought to be involved in plaque formation, recent positron emission tomography studies of patients with mild cognitive impairment (MCI) concluded that microglia activation occurs even before plaque and tangle formation [19] and is correlated with early cognitive deficits [20]. Although the exact stimuli that induce pathologic activation of microglia await further study, our recent results suggest that soluble Aβ oligomers (AβOs), the small and early-stage amyloid aggregates could be a potent stimulus [17]. A reasonable assumption is that multiple stimuli converge to cause microglial dysfunction and aberrant activation, thus aggravating microglia-mediated neurotoxicity and reducing their neuroprotective capacity. Indeed, a variety of life events, such as trauma, infection, stroke, metabolic disorders, and network hyperexcitability (epileptic seizures), have been implicated in contributing to the development of AD. Notably, all these conditions invariably activate microglia. Activated microglia release cytotoxic substances and proinflammatory cytokines to cause neuronal damage and age-associated microglial dysfunction [17, 21–23]. Above all, aging is perhaps the major risk and a prerequisite for "pathologic activation" that prevents microglia from performing the intended neuroprotective and repair functions [24, 25].

Irrespective of the events causing neuroinflammation in AD, curbing the harmful proinflammatory response of microglia activation is a reasonable approach toward prevention or therapy of AD. However, despite abundant preclinical evidence of their benefit, various anti-inflammatory approaches have not proven successful in clinical trials for a multitude of reasons. The most widely tested anti-inflammatory agents are the nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs show multiple beneficial effects on preclinical cell culture and animal models of AD, although the exact molecular targets mediating these effects are not known. Unfortunately, results from several clinical trials are disappointing [26], partly due to inadequate CNS drug penetration of existing NSAIDs, suboptimal doses, unknown molecular targets (therefore unknown pharmacodynamics), and toxicities. For example, a recent large-scale AD prevention trial with NSAIDs, including naproxen and celecoxib, was stopped early because of drug safety concerns. Despite this, these setbacks should prompt investigations to develop novel anti-inflammatory agents with known specific targets, satisfactory CNS penetrance, and low toxicities.

We also have to consider that microglial activation can be neuroprotective through the release of neurotrophic factors

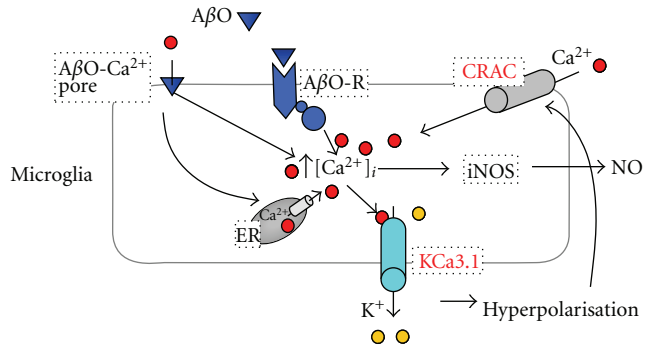


Figure 1: KCa3.1 regulates microglial activation by modulating Ca^{2+} influx. AβO initiates an increase of intracellular Ca^{2+} either directly by forming a Ca^{2+}-permeable membrane pore (AβO-Ca^{2+} pore) [11, 12] or indirectly through interaction with a receptor (tentatively termed AβO-R). Intracellular Ca^{2+} activates KCa3.1 to induce K^+ efflux. The resulting hyperpolarisation provides the driving force for Ca^{2+} entry through store-operated inward-rectifier calcium channels like CRAC, thus sustaining the Ca^{2+} signal necessary for selective Ca^{2+} activated pathways. One example illustrated here is iNOS activation and nitric oxide (NO) production to cause microglia-mediated neurotoxicity.

and by phagocytosing Aβ and debris from degenerated neurons [14, 23]. Any anti-inflammatory therapies for AD should take these dichotomous microglial functions into consideration [23]. This constitutes our *criteria no.1* for microglia-targeted therapy in AD: the therapy should maintain microglial ability to migrate and clear Aβ, while inhibiting their release of neurotoxic mediators. Recent evidence suggests that this could be achieved by controlling the activities of specific pathways that can modulate certain aspects of microglia activation. Proposed approaches include modulation of the peroxisome proliferator-activated receptor-γ (PPAR-γ) [14] and the E prostanoid receptor subtype 2 [27]. Evidence obtained from our laboratory and from other groups strongly suggests blockade of the calcium-activated K^+ channel KCa3.1 as another promising approach that could curb inflammatory brain pathologies while preserving microglial migration and phagocytosis [13, 17, 28–30].

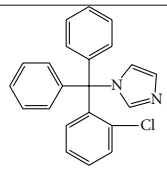
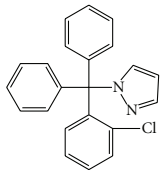
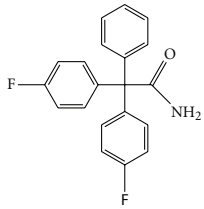
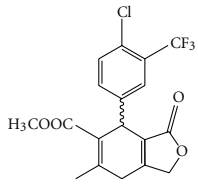
Two additional criteria for an anti-inflammatory drug for AD therapy are: *criteria no.2*, it should be relatively specific to microglia to avoid adverse neuronal effects and *criteria no.3*, it should not be broadly immunosuppressive. We will review evidence supporting that KCa3.1 is a suitable therapeutic target for AD using the above three criteria.

3. KCa3.1 as a Microglia-Selective Target in CNS

K^+ channels are encoded by a super-family of 78 genes [31] and are involved in diverse physiological and pathological processes [32]. K^+ channels accordingly already serve as drug targets for cardiac arrhythmia, type-2 diabetes, and epilepsy and have been proposed as potential targets for various neurological diseases such as multiple sclerosis, Parkinson's disease, stroke, pain, schizophrenia, and migraine. Two K^+ channels, the calcium-activated KCa3.1 (also known as IK1, SK4 or KCNN4) and the voltage-gated Kv1.3, play important roles in microglia activation by modulating Ca^{2+} signaling and membrane potential. Similar to T cells, where their roles have been studied in much more detail [33], K^+ efflux through microglial KCa3.1 and Kv1.3 helps maintain a negative membrane potential for Ca^{2+} influx through the store-operated inward rectifier calcium channel CRAC (Ca^{2+} release activated Ca^{2+} channel) (Figure 1). However, the two K^+ channels appear to be differentially expressed following immune cell activation and differentially modulate cytokine production and cellular proliferation in different T and B cell subsets. While Kv1.3 is primarily important in $CCR7^-$ effector memory T cells and class-switched IgD^-CD27^+ B cells, $CCR7^+$ T cells and IgD^+ B cells rely on KCa3.1 for part of their calcium-signaling and activation events [34–37]. In microglia, KCa3.1-mediated control of Ca^{2+} entry has been shown to be involved in oxidative burst, nitric oxide production, and microglia-mediated neuronal killing, including that induced by Aβ oligomer (AβO) [17, 28, 38, 39].

Although no human diseases involving KCa3.1 mutations have been described so far, KCa3.1 constitutes a very attractive and (in some cases) relatively well-validated drug target for diseases or conditions ranging from sickle cell disease, restenosis, and atherosclerosis to asthma and traumatic brain injury (see [40] for a recent review). KCa3.1 channels are widely expressed throughout the body and primarily found in hematopoietic-derived cells including macrophages/microglia. A significant advantage of KCa3.1 channels as a therapeutic target for CNS indications is that expression seems to be restricted to hematopoietic-derived cells and peripheral tissues such as secretory epithelial, fibroblasts, and proliferating neointimal smooth muscle cells [40], but the channels have been found to be absent from excitable tissues such as neurons and cardiomyocytes [41–43]. KCa3.1 channels appear also not expressed in astrocytes. However, evidence shown in a recent article by Bouhy et al. suggests expression of KCa3.1 in reactive astrocytes in the spinal cord of a mouse spinal cord injury model, although only one anti-KCa3.1 antibody was used in immunohistochemistry [30]. We feel that this is clearly not the case for the forebrain, based on previously published gene expression data [41, 43]. Our own experiments using several polyclonal

Table 1

Structure	Pharmacokinetics/pharmacodynamics/safety	Development status
	*Clotrimazole* (1) KCa3.1 IC_{50} 70–250 nM (2) Acute inhibition and chronic induction of cytochrome P450-dependent enzymes (3)Liver toxic	Topical antifungal generally regarded as too toxic for internal use
	*TRAM-34* (1) KCa3.1 IC_{50} 20 nM (2) No toxicity in 28-day and 6-month tox studies in rodents (3) $t_{1/2}$= 2 hours (rats, primates) (4) C_{brain}/C_{plasma} 1.2 (5) Not orally available	Patented by the University of California WO 01/49663 (2001)
	*ICA-17043 (senicapoc)* (1) KCa3.1 IC_{50} 11 nM (2) Orally available in humans (3) $t_{1/2}$= 12.8 days (humans) (4) IND enabling preclinical toxicity studies in two species	Failed to reduce number of sickling crisis in Phase-3 clinical trial for sickle cell anemia after having been found safe and effective in Phase-1 and Phase-2 Developed at Icagen
	*Cyclohexadiene lactone* (1) KCa3.1 IC_{50} 8 nM (2) C_{brain}/C_{plasma} 10 (3) Used for traumatic brain injury studies [13]	Compounds seem to have been abandoned when Bayer pulled out of stroke research Patented by Bayer AG Germany, DE-9619612645 (1997)

and monoclonal anti-KCa3.1 antibodies to stain sections from models of AD (unpublished results) and stroke [29] show that KCa3.1 is substantially upregulated in activated microglia, but not in astrocytes. We therefore feel that we can perhaps conclude that the major parenchymal cells in the cerebrum in which KCa3.1 channels play a significant role are microglia, and in some pathological conditions, invading macrophages. Therefore, it is reasonable to assume that a CNS-permeating KCa3.1 blocker would have relatively selective actions on microglia and would avoid adversely affecting neuronal functions.

4. KCa3.1 Blockers Are Neuroprotective

Recognizing the important role of KCa3.1 in regulating immune cell functions, Wulff et al. synthesized a specific KCa3.1 blocker called TRAM-34 using as a template the antimycotic clotrimazole, which is a potent but poorly tolerated KCa3.1 inhibitor [44]. TRAM-34 (IC_{50}20 nM) is currently the most widely used pharmacological tool compound for studying the pathophysiology of KCa3.1 because of its high selectivity over other K^+ channels and its availability to academic researchers. Table 1 shows the structures of TRAM-34 and several other KCa3.1 blockers developed by pharmaceutical companies and summarizes pharmacokinetic, safety, and development information. For more extensive reviews on KCa3.1 pharmacology, interested readers are referred to two review articles [40, 45].

TRAM-34 has been tested in various animal models, including optic nerve transaction [28], middle cerebral artery occlusion [29], traumatic brain injury [13] and restenosis [46] in rats; traumatic spinal cord injury [30], atherosclerosis [47], and inflammatory bowel disease [37] in mice; and angioplasty in pigs [48]. In particular, the following *in vivo* observations taken together provide strong evidence that KCa3.1 inhibitors can curb brain inflammation and provide neuroprotection.

(1) Our own group recently demonstrated that TRAM-34 inhibits AβO-induced microglia activation and microglia-mediated neuronal toxicity [17].

(2) Our group further showed that TRAM-34 inhibits microglia activation and reduces infarct area and neurological deficit scores in a rat model of ischemic stroke even if treatment is commenced 12 h after reperfusion [29].

(3) The Schlichter group showed that TRAM-34 reduces retinal ganglion cell degeneration after optic nerve transection in rats [28]. Interestingly, KCa3.1 blockade did not prevent microglia from aligning with damaged axons or from phagocytosing damaged neurons, but increased the number of surviving retinal ganglion cells presumably by reducing the production and/or secretion of neurotoxic molecules in the retina [28]. This could possibly be explained by

the observation that the Ca^{2+} influx during phagocytosis appears to be mediated through reverse mode Na^+/Ca^{2+} exchange [49] and not through KCa3.1-regulated CRAC channels, supporting the "pathway-selective" nature of KCa3.1 inhibition.

(4) The David group showed that TRAM-34 reduces the secondary damage and improves locomotor function in a mouse model of spinal cord injury in a dose-dependent manner [30].

(5) Scientists at Bayer demonstrated that two structurally different KCa3.1 inhibitors, a triarylmethane and a cyclohexadiene (Table 1), reduced infarct volume and brain edema following traumatic brain injury caused by acute subdural haematoma in rats [13].

(6) Scientists at Schering resynthesized TRAM-34 and showed that it treats MOG-induced experimental autoimmune encephalomyelitis in mice by reducing the production of the inflammatory cytokines INF-γ and TNF-α in the brain and spinal cord [50].

5. Targeting KCa3.1 Could Ameliorate AβO-Induced Neuronal Damage

AβOs, the small soluble and diffusible aggregates of Aβ peptides, were initially considered transient or metastable intermediates in fibril formation [51]. However, some of them may not be obligate intermediates in the fibril formation pathway and can be stable [52, 53]. Importantly, recent *in vitro* and *in vivo* studies have revealed that the buildup of soluble AβO may be an early and central event in the pathogenesis of AD [54–57]. The strong and rapidly disruptive effect of AβO on synaptic plasticity and neuronal integrity is hypothesized to cause memory problems in AD and is generally attributed to their direct neuro- or synaptotoxicity [10]. However, one plausible but less studied possibility is that AβO activates microglia and causes indirect, microglia-mediated neuro- and synaptotoxicity. Recently we found that AβO, either assembled *in vitro* from synthetic Aβ1-42 peptide or isolated from AD brains, is a highly potent activator of microglia [17]. Although the mechanism mediating AβO-induced microglia activation and the exact pattern of activation are still under investigation, a particularly interesting observation is that this mode of microglia activation and related neurotoxicity are dependent on microglial KCa3.1. We found that TRAM-34 blocked AβO-induced microglia proliferation, p38MAPK phosphorylation, NFκB activation, and nitric oxide generation. We further showed that the neurotoxic effects of low concentrations of AβO (10–50 nM) applied to mixed microglia-neuron cultures or organotypic hippocampal slices were almost completely blocked by cotreatment with TRAM-34, another microglial activation inhibitor doxycycline, and inhibitors of iNOS. This set of results suggests that AβO, although generally considered a neurotoxin, may more potently cause indirect neuronal damage by activating microglia in AD. Consistent with this notion, a previous study showed that the inhibition of NMDA receptor-dependent long-term potentiation by soluble Aβ can be prevented by minocycline, a microglia activation inhibitor in the same class as doxycycline, and iNOS inhibition to reduce nitric oxide production from microglia [58]. Taken together, these results suggest that KCa3.1 blockers could potentially also inhibit microglial neurotoxicity and thus preserve memory in AD.

6. Targeting KCa3.1 Could Also Effectively Address Cerebrovascular and Traumatic Comorbidities in AD

As discussed above, cerebrovascular insults and traumatic brain injuries are significant comorbidities in AD. In addition to clinically apparent strokes, carotid, vertebral, and intracranial vascular stenosis can cause chronic cerebral hypoperfusion, microinfarcts, and lacunar infarcts, contributing to dementia. These vascular and traumatic pathologies cannot possibly be addressed by AD-specific therapies, such as antiamyloid drugs or vaccines. The well-documented beneficial effects of KCa3.1 blockers in models of ischemic stroke [29], traumatic brain injury [13], and atherosclerosis [47], which primarily seem to be mediated through inhibition of detrimental microglia/macrophage function, considerably add to KCa3.1′s attractiveness as a novel target for treating the dominant group of AD patients presenting with both degenerative and vascular pathologies. KCa3.1 is further expressed in dedifferentiated, proliferative vascular smooth muscles cells, which, as Köhler et al. showed, switch from their normal KCa1.1 (BK) channel expression to KCa3.1 expression following balloon catheter injury. In keeping with a role of KCa3.1 in driving aberrant smooth muscle cell proliferation, TRAM-34 prevents vascular restenosis in a rat model [46]. These findings were more recently confirmed by a study in which coating of TRAM-34 onto balloon catheters significantly reduced restenosis in pigs, which very closely resemble humans with respect to postangioplasty restenosis [48]. A similar increase in KCa3.1 expression was found in coronary vessels from patients with coronary artery disease and in aortas from $ApoE^{-/-}$ mice, suggesting that KCa3.1 is involved in atherogenesis. KCa3.1 blockade with TRAM-34 prevented atherosclerosis development in $ApoE^{-/-}$ mice by reducing smooth muscle cell proliferation and macrophage infiltration into atherosclerotic plaques [47]. Furthermore, TRAM-34 administration reduced the inflammatory neurotoxicity and infarct areas in the wake of ischemic stroke, even when the first dose was applied at 12 hours after reperfusion. This was accompanied by a dose-dependent improvement in neurological deficit score, a reduction in the number of $ED1^+$ activated microglia and an increase in $NeuN^+$ surviving neurons [29].

7. KCa3.1 Blockers Are Mild Immunosuppressants and Are Relatively Safe

The promise of KCa3.1 as a therapeutic target for AD is further strengthened by the observations that KCa3.1 blockers are very mild immunosuppressants that do not

reduce the ability of rodents to clear viral infections like flu [47]. In addition, genetic or pharmacological blockade of KCa3.1 seems relatively safe and well tolerated. Two independently generated KCa3.1$^{-/-}$ mice were both viable, of normal appearance, produced normal litter sizes, did not show any gross abnormalities in any of their major organs, and exhibited rather mild phenotypes: impaired volume regulation in erythrocytes and lymphocytes [59], a reduced EDHF (endothelium derived hyperpolarizing factor) response together with a mild ~7 mmHg increase in blood pressure [60], and subtle erythrocyte macrocytosis and progressive splenomegaly [61]. A 28-day toxicity study with TRAM-34 in mice resulted in no observable changes in blood chemistry, hematology or necropsy of any of the major organs [47]. A subsequent 6-month toxicity study with TRAM-34 in rats also did not find any changes in the same parameters and also did not report any increases in susceptibility to viral or bacterial infections [29]. Senicapoc, a KCa3.1 blocker structurally similar to TRAM-34 (see Table 1), was safe and well tolerate, in a Phase-1 clinical trial in healthy volunteers [62] and was afterwards found to significantly reduce hemolysis and increase hemoglobin levels in a 12-week, multicenter, randomized double-blind Phase-2 study in sickle cell disease patients [63]. However, in a subsequent Phase-3 study, which was designed to compare the rate of acute vasoocclusive pain crisis occurring in sickle cell disease patients, Senicapoc failed to reduce this desired clinical endpoints despite again reducing hemolysis and increasing hemoglobin levels and not inducing any significant adverse events (see [40] for a more extensive discussion of the clinical experiences with KCa3.1 blockers).

8. Conclusion: Microglial KCa3.1 Is a Promising Target for AD

Concluding the above discussion, we here propose that microglial KCa3.1 is a promising therapeutic target for AD because KCa3.1 blockade comes close to fulfilling three criteria we set for anti-inflammatory therapy. Using the specific KCa3.1 inhibitor TRAM-34 as a pharmacological tool compound, proof-of-concept studies have shown that KCa3.1 inhibition can reduce AβO-induced microglial neurotoxicity and protect neurons in other non-Aβ neuronal injury models by reducing the production of neurotoxic proinflammatory mediators while preserving the neuroprotective functions of microglia, such as migration and phagocytosis. This "pathway-selectivity" is likely due to the ability of KCa3.1 to "fine-tune" the pattern of microglial activation by selectively regulating various Ca^{2+}-activated signaling pathways. Due to its demonstrated effects on models of CVD and traumatic brain injuries, two well-known risk factors for AD, KCa3.1 inhibition could offer additional therapeutic benefits for mixed pathologies commonly seen in AD patients. KCa3.1 blockade by either pharmacological inhibition or genetic knockout only resulted in minimal immunosuppression. Importantly, a KCa3.1 blocker has been proven safe in clinical trials. Therefore, it is promising to either directly reposition existing KCa3.1 blockers for AD preclinical proof-of-concept studies and subsequent clinical trials and/or make efforts to optimize existing or newly-discovered compounds for oral availability and brain penetration in order to expedite drug development for AD.

Acknowledgment

This work is supported in part by Grants GM076063 and AG10129 from the National Institute of Health.

References

[1] D. W. Shineman, G. S. Basi, J. L. Bizon et al., "Accelerating drug discovery for Alzheimer's disease: best practices for preclinical animal studies," *Alzheimer's Research & Therapy*, vol. 3, no. 5, article 28, 2011.

[2] T. E. Golde, L. S. Schneider, and E. H. Koo, "Anti-Aβ therapeutics in alzheimer's disease: the need for a paradigm shift," *Neuron*, vol. 69, no. 2, pp. 203–213, 2011.

[3] C. Reitz, C. Brayne, and R. Mayeux, "Epidemiology of Alzheimer disease," *Nature Reviews Neurology*, vol. 7, no. 3, pp. 137–152, 2011.

[4] S. Mondragón-Rodríguez, G. Basurto-Islas, H. G. Lee et al., "Causes versus effects: the increasing complexities of Alzheimer's disease pathogenesis," *Expert Review of Neurotherapeutics*, vol. 10, no. 5, pp. 683–691, 2010.

[5] K. A. Jellinger, "Neuropathological aspects of Alzheimer disease, Parkinson disease and frontotemporal dementia," *Neurodegenerative Diseases*, vol. 5, no. 3-4, pp. 118–121, 2008.

[6] H. C. Chui, L. Zheng, B. R. Reed, H. V. Vinters, and W. J. Mack, "Vascular risk factors and Alzheimer's disease: are these risk factors for plaques and tangles or for concomitant vascular pathology that increases the likelihood of dementia? An evidence-based review," *Alzheimer's Research & Therapy*, vol. 3, no. 6, article 36, 2012.

[7] J. A. Schneider, Z. Arvanitakis, W. Bang, and D. A. Bennett, "Mixed brain pathologies account for most dementia cases in community-dwelling older persons," *Neurology*, vol. 69, no. 24, pp. 2197–2204, 2007.

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

[9] Q. Chen, M. Prior, R. Dargusch et al., "A novel neurotrophic drug for cognitive enhancement and Alzheimer's disease," *PLoS ONE*, vol. 6, article e27865, 2011.

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

[11] A. Demuro, E. Mina, R. Kayed, S. C. Milton, I. Parker, and C. G. Glabe, "Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers," *The Journal of Biological Chemistry*, vol. 280, no. 17, pp. 17294–17300, 2005.

[12] A. Demuro, M. Smith, and I. Parker, "Single-channel Ca^{2+} imaging implicates Aβ1–42 amyloid pores in Alzheimer's disease pathology," *Journal of Cell Biology*, vol. 195, pp. 515–524, 2011.

[13] F. Mauler, V. Hinz, E. Horváth et al., "Selective intermediate-/small-conductance calcium-activated potassium channel (KCNN4) blockers are potent and effective therapeutics in experimental brain oedema and traumatic brain injury caused by acute subdural haematoma," *European Journal of Neuroscience*, vol. 20, no. 7, pp. 1761–1768, 2004.

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

[15] L. Meda, M. A. Cassatella, G. I. Szendrei et al., "Activation of microglial cells by β-amyloid protein and interferon-γ," *Nature*, vol. 374, no. 6523, pp. 647–650, 1995.

[16] S. Jimenez, D. Baglietto-Vargas, C. Caballero et al., "Inflammatory response in the hippocampus of PS1M146L/APP 751SL mouse model of Alzheimer's disease: age-dependent switch in the microglial phenotype from alternative to classic," *Journal of Neuroscience*, vol. 28, no. 45, pp. 11650–11661, 2008.

[17] I. Maezawa, P. I. Zimin, H. Wulff, and L. W. Jin, "Amyloid-β protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity," *The Journal of Biological Chemistry*, vol. 286, no. 5, pp. 3693–3706, 2011.

[18] J. Rogers, "The inflammatory response in Alzheimer's disease," *Journal of Periodontology*, vol. 79, no. 8, pp. 1535–1543, 2008.

[19] A. Okello, P. Edison, H. A. Archer et al., "Microglial activation and amyloid deposition in mild cognitive impairment: a PET study," *Neurology*, vol. 72, no. 1, pp. 56–62, 2009.

[20] M. Sastre, J. C. Richardson, S. M. Gentleman, and D. J. Brooks, "Inflammatory risk factors and pathologies associated with Alzheimer's disease," *Current Alzheimer Research*, vol. 8, no. 2, pp. 132–141, 2011.

[21] J. El Khoury and A. D. Luster, "Mechanisms of microglia accumulation in Alzheimer's disease: therapeutic implications," *Trends in Pharmacological Sciences*, vol. 29, no. 12, pp. 626–632, 2008.

[22] B. Cameron and G. E. Landreth, "Inflammation, microglia, and alzheimer's disease," *Neurobiology of Disease*, vol. 37, no. 3, pp. 503–509, 2010.

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

[24] W. J. Streit, K. R. Miller, K. O. Lopes, and E. Njie, "Microglial degeneration in the aging brain—bad news for neurons?" *Frontiers in Bioscience*, vol. 13, no. 9, pp. 3423–3438, 2008.

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

[26] S. A. Frautschy, F. Yang, M. Irrizarry et al., "Microglial response to amyloid plaques in APPsw transgenic mice," *American Journal of Pathology*, vol. 152, no. 1, pp. 307–317, 1998.

[27] P. J. Cimino, C. D. Keene, R. M. Breyer, K. S. Montine, and T. J. Montine, "Therapeutic targets in prostaglandin E2 signaling for neurologic disease," *Current Medicinal Chemistry*, vol. 15, no. 19, pp. 1863–1869, 2008.

[28] V. Kaushal, P. D. Koeberle, Y. Wang, and L. C. Schlichter, "The Ca^{2+}-activated K^+ channel KCNN4/KCa3.1 contributes to microglia activation and nitric oxide-dependent neurodegeneration," *Journal of Neuroscience*, vol. 27, no. 1, pp. 234–244, 2007.

[29] Y. J. Chen, G. Raman, S. Bodendiek, M. E. O'Donnell, and H. Wulff, "The KCa3.1 blocker TRAM-34 reduces infarction and neurological deficit in a rat model of ischemia/reperfusion stroke," *Journal of Cerebral Blood Flow and Metabolism*, vol. 31, no. 12, pp. 2363–2374, 2011.

[30] D. Bouhy, N. Ghasemlou, S. Lively et al., "Inhibition of the Ca^{2+}-dependent K^+ channel, KCNN4/KCa3.1, improves tissue protection and locomotor recovery after spinal cord injury," *The Journal of Neuroscience*, vol. 31, pp. 16298–16308, 2011.

[31] A. J. Harmar, R. A. Hills, E. M. Rosser et al., "IUPHAR-DB: the IUPHAR database of G protein-coupled receptors and ion channels," *Nucleic Acids Research*, vol. 37, no. 1, pp. D680–D685, 2009.

[32] H. Wulff, N. A. Castle, and L. A. Pardo, "Voltage-gated potassium channels as therapeutic targets," *Nature Reviews Drug Discovery*, vol. 8, no. 12, pp. 982–1001, 2009.

[33] M. D. Cahalan and K. G. Chandy, "The functional network of ion channels in T lymphocytes," *Immunological Reviews*, vol. 231, no. 1, pp. 59–87, 2009.

[34] S. Ghanshani, H. Wulff, M. J. Miller et al., "Up-regulation of the IKCa1 potassium channel during T-cell activation: molecular mechanism and functional consequences," *The Journal of Biological Chemistry*, vol. 275, no. 47, pp. 37137–37149, 2000.

[35] H. Wulff, P. A. Calabresi, R. Allie et al., "The voltage-gated Kv1.3 K^+ channel in effector memory T cells as new target for MS," *Journal of Clinical Investigation*, vol. 111, no. 11, pp. 1703–1713, 2003.

[36] H. Wulff, H. G. Knaus, M. Pennington, and K. G. Chandy, "K^+ channel expression during B cell differentiation: implications for immunomodulation and autoimmunity," *Journal of Immunology*, vol. 173, no. 2, pp. 776–786, 2004.

[37] L. Di, S. Srivastava, O. Zhdanova et al., "Inhibition of the K^+ channel KCa3.1 ameliorates T cell-mediated colitis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 4, pp. 1541–1546, 2010.

[38] T. Schilling, C. Stock, A. Schwab, and C. Eder, "Functional importance of Ca^{2+}-activated K^+ channels for lysophosphatidic acid-induced microglial migration," *European Journal of Neuroscience*, vol. 19, no. 6, pp. 1469–1474, 2004.

[39] R. Khanna, L. Roy, X. Zhu, and L. C. Schlichter, "K^+ channels and the microglial respiratory burst," *American Journal of Physiology*, vol. 280, no. 4, pp. C796–C806, 2001.

[40] H. Wulff and N. A. Castle, "Therapeutic potential of KCa3.1 blockers: recent advances and promising trends," *Expert Review of Clinical Pharmacology*, vol. 3, no. 3, pp. 385–396, 2010.

[41] W. J. Joiner, L. U. Y. Wang, M. D. Tang, and L. K. Kaczmarek, "hSK4, a member of a novel subfamily of calcium-activated potassium channels," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 20, pp. 11013–11018, 1997.

[42] T. M. Ishii, C. Silvia, B. Hirschberg, C. T. Bond, J. P. Adelman, and J. Maylie, "A human intermediate conductance calcium-activated potassium channel," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 21, pp. 11651–11656, 1997.

[43] N. J. Logsdon, J. Kang, J. A. Togo, E. P. Christian, and J. Aiyar, "A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes," *The Journal of Biological Chemistry*, vol. 272, no. 52, pp. 32723–32726, 1997.

[44] H. Wulff, M. J. Miller, W. Hänsel, S. Grissmer, M. D. Cahalan, and K. G. Chandy, "Design of a potent and selective inhibitor of the intermediate- conductance Ca^{2+}-activated K^+ channel, IKCa1: a potential immunosuppressant," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 14, pp. 8151–8156, 2000.

[45] H. Wulff, A. Kolski-Andreaco, A. Sankaranarayanan, J. M. Sabatier, and V. Shakkottai, "Modulators of small- and intermediate-conductance calcium-activated potassium channels and their therapeutic indications," *Current Medicinal Chemistry*, vol. 14, no. 13, pp. 1437–1457, 2007.

[46] R. Köhler, H. Wulff, I. Eichler et al., "Blockade of the intermediate-conductance calcium-activated potassium channel as a new therapeutic strategy for restenosis," *Circulation*, vol. 108, no. 9, pp. 1119–1125, 2003.

[47] K. Toyama, H. Wulff, K. G. Chandy et al., "The intermediate-conductance calcium-activated potassium channel KCa3.1 contributes to atherogenesis in mice and humans," *Journal of Clinical Investigation*, vol. 118, no. 9, pp. 3025–3037, 2008.

[48] D. L. Tharp, B. R. Wamhoff, H. Wulff, G. Raman, A. Cheong, and D. K. Bowles, "Local delivery of the KCa3.1 blocker, TRAM-34, prevents acute angioplasty-induced coronary smooth muscle phenotypic modulation and limits stenosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 6, pp. 1084–1089, 2008.

[49] E. W. Newell, E. F. Stanley, and L. C. Schlichter, "Reversed Na+/Ca^{2+} exchange contributes to Ca^{2+} influx and respiratory burst in microglia.," *Channels (Austin, Tex.)*, vol. 1, no. 5, pp. 366–376, 2007.

[50] E. P. Reich, L. Cui, L. Yang et al., "Blocking ion channel KCNN4 alleviates the symptoms of experimental autoimmune encephalomyelitis in mice," *European Journal of Immunology*, vol. 35, no. 4, pp. 1027–1036, 2005.

[51] B. Caughey and P. T. Lansbury, "Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders," *Annual Review of Neuroscience*, vol. 26, pp. 267–298, 2003.

[52] M. Necula, R. Kayed, S. Milton, and C. G. Glabe, "Small molecule inhibitors of aggregation indicate that amyloid β oligomerization and fibrillization pathways are independent and distinct," *The Journal of Biological Chemistry*, vol. 282, no. 14, pp. 10311–10324, 2007.

[53] C. G. Glabe, "Structural classification of toxic amyloid oligomers," *The Journal of Biological Chemistry*, vol. 283, no. 44, pp. 29639–29643, 2008.

[54] M. P. Lambert, A. K. Barlow, B. A. Chromy et al., "Diffusible, nonfibrillar ligands derived from Aβ1-42 are potent central nervous system neurotoxins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 11, pp. 6448–6453, 1998.

[55] S. Lesné, T. K. Ming, L. Kotilinek et al., "A specific amyloid-β protein assembly in the brain impairs memory," *Nature*, vol. 440, no. 7082, pp. 352–357, 2006.

[56] G. M. Shankar, S. Li, T. H. Mehta et al., "Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory," *Nature Medicine*, vol. 14, no. 8, pp. 837–842, 2008.

[57] R. Roychaudhuri, M. Yang, M. M. Hoshi, and D. B. Teplow, "Amyloid β-protein assembly and Alzheimer disease," *The Journal of Biological Chemistry*, vol. 284, no. 8, pp. 4749–4753, 2009.

[58] Q. Wang, M. J. Rowan, and R. Anwyl, "β-amyloid-mediated inhibition of NMDA receptor-dependent long-term potentiation induction involves activation of microglia and stimulation of inducible nitric oxide synthase and superoxide," *Journal of Neuroscience*, vol. 24, no. 27, pp. 6049–6056, 2004.

[59] T. Begenisich, T. Nakamoto, C. E. Ovitt et al., "Physiological roles of the intermediate conductance, Ca^{2+}- activated potassium channel Kcnn4," *The Journal of Biological Chemistry*, vol. 279, no. 46, pp. 47681–47687, 2004.

[60] H. Si, W. T. Heyken, S. E. Wölfle et al., "Impaired endothelium-derived hyperpolarizing factor-mediated dilations and increased blood pressure in mice deficient of the intermediate-conductance Ca^{2+}-activated K^+ channel," *Circulation Research*, vol. 99, no. 5, pp. 537–544, 2006.

[61] I. Grgic, B. P. Kaistha, S. Paschen et al., "Disruption of the Gardos channel (KCa3.1) in mice causes subtle erythrocyte macrocytosis and progressive splenomegaly," *Pflugers Archiv European Journal of Physiology*, vol. 458, no. 2, pp. 291–302, 2009.

[62] K. I. Ataga, E. P. Orringer, L. Styles et al., "Dose-escalation study of ICA-17043 in patients with sickle cell disease," *Pharmacotherapy*, vol. 26, no. 11, pp. 1557–1564, 2006.

[63] K. I. Ataga, W. R. Smith, L. M. De Castro et al., "Efficacy and safety of the Gardos channel blocker, senicapoc (ICA-17043), in patients with sickle cell anemia," *Blood*, vol. 111, no. 8, pp. 3991–3997, 2008.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 489456, 10 pages
doi:10.1155/2012/489456

Review Article

Microglial Scavenger Receptors and Their Roles in the Pathogenesis of Alzheimer's Disease

Kim Wilkinson and Joseph El Khoury

Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, 02129, USA

Correspondence should be addressed to Kim Wilkinson, kwilkinson1@partners.org

Received 29 December 2011; Accepted 19 February 2012

Academic Editor: Lee-Way Jin

Alzheimer's disease (AD) is increasing in prevalence with the aging population. Deposition of amyloid-β (Aβ) in the brain of AD patients is a hallmark of the disease and is associated with increased microglial numbers and activation state. The interaction of microglia with Aβ appears to play a dichotomous role in AD pathogenesis. On one hand, microglia can phagocytose and clear Aβ, but binding of microglia to Aβ also increases their ability to produce inflammatory cytokines, chemokines, and neurotoxic reactive oxygen species (ROS). Scavenger receptors, a group of evolutionally conserved proteins expressed on the surface of microglia act as receptors for Aβ. Of particular interest are SCARA-1 (scavenger receptor A-1), CD36, and RAGE (receptor for advanced glycation end products). SCARA-1 appears to be involved in the clearance of Aβ, while CD36 and RAGE are involved in activation of microglia by Aβ. In this review, we discuss the roles of various scavenger receptors in the interaction of microglia with Aβ and propose that these receptors play complementary, nonredundant functions in the development of AD pathology. We also discuss potential therapeutic applications for these receptors in AD.

1. Microglia and Alzheimer's Disease Pathology

Alzheimer's disease (AD) is a devastating neurological condition characterized by increasing memory loss, inability to perform daily tasks, and eventually dementia. This fatal condition currently has no cure, and by 2050, 13 million people in the United States are projected to be affected by the disease [1]. AD brains show deposition of the protein amyloid-β (Aβ) in senile plaques [2], this protein is produced by cleavage of APP (amyloid precursor protein), by the enzymes β-secretase and γ-secretase [3]. Aβ accumulates in soluble form and also undergoes conformational changes to become fibrillar, microglia interact with both soluble and fibrillar forms of Aβ [4–6].

In addition to Aβ accumulation during the development of AD, tau protein also accumulates in neurofibrillary tangles (NFT) in cell bodies of neurons [7] and apical dendrites [8]. Tau is a microtubule-associated protein that segregates into axons and stabilizes their microtubules. In AD, tau dissociates from the microtubules and begins to accumulate in the somatodendritic compartment of the axon, a process which is not fully understood [9]. Tau contains a high number of phosphorylation sites and upon phosphorylation dissociates from the microtubules, as observed in AD [10]. Tau protein then undergoes conformational changes which form fibrils [11]. NFT could possibly activate microglia which may prove deleterious for the surrounding neurons and contribute to disease progression, however, little is known about the NFT mechanism of action [12].

Microglia are the major phagocytic cell of the brain and become activated upon encountering Aβ [6] and can release chemokines and cytokines into their environment [13]. Microglia are believed to initially clear Aβ deposits, but as the disease progresses, they produce proinflammatory cytokines, chemokines, and reactive oxygen species (ROS) and lose their ability to clear Aβ [14], and despite increased numbers of microglia in the AD brain, the plaques continue to increase in size and number [15]. This inflammatory environment ultimately becomes toxic to the surrounding neurons, resulting in neuronal degeneration and disease progression [16].

Scavenger receptors bind many ligands with high affinity, including Aβ [4, 17], and have been shown to be expressed on microglia surrounding Aβ plaques in the brain [18]. This review will focus on the role of scavenger receptors in AD development and discuss efforts to examine scavenger receptors as targets for therapy.

2. What Are Scavenger Receptors (SRs)?

Several families of pattern recognition receptors (PRR) have been identified including the well-defined and extensively studied Toll-like and Nod-like receptors (TLR and NLRs). In addition to these proteins, the scavenger receptor (SR) family represents another major class of PRR. SRs were first described in 1979 by Brown and Goldstein as macrophage receptors that mediate endocytosis of modified low-density lipoprotein (LDL) leading to foam cell formation and were shown to play a role in the pathogenesis of atherosclerosis [19, 20]. Since then, the definition of SRs has been broadened: SRs are defined as a family of molecules that share the ability to bind polyanionic ligands. This simple definition belies the importance of SRs as PRRs-SRs are archetypal multifunctional receptors, often able to bind ligands of both pathogen and self-origin. These receptors are teleologically ancient pathogen receptors, emphasizing their important role in host defense. However, while the critical roles SRs play in atherosclerosis and host defense against a variety of pathogens is well characterized, the exact role of these receptors in the pathogenesis of neurodegenerative disorders including AD is not clearly understood.

3. Classification of SRs

SRs are structurally unrelated membrane receptors that are highly expressed by phagocytes such as macrophages, dendritic cells, and microglia, and also found on selected endothelial cells (Figure 1). To date, the SRs family has been classified into 6 classes but additional members of this family, like CD163, RAGE, and SR-PSOX (scavenger receptor that binds phosphatidylserine and oxidized lipoprotein) remain unclassified. SRs are defined by their common ability to bind polyanionic ligands with high affinity and have broad specificity. SRs cooperate with the other innate immune PRRs like the TLRs to define pathogen-specific responses [21]. However, unlike the TLRs and NLRs, SRs facilitate ligand uptake by phagocytosis and endocytosis [22].

4. Class A Scavenger Receptors (SCARA)

SCARA are required for host defense against several bacterial and viral pathogens: SCARA-1 is an arrangement of three coiled extracellular regions with cysteine-rich domains connected to the plasma membrane by a long fibrous stalk composed of an alpha-helical coiled coil and a collagen rich triple helix which is believed to bind ligands through the collagen-like domain [23–25] (Figure 1). SCARA-1 exemplify this family of multiligand receptors, in addition to binding to modified lipoproteins, lipopolysaccharide (LPS),

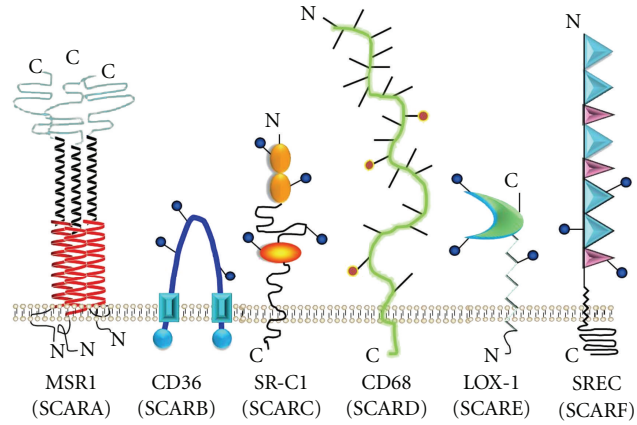


Figure 1: Structural diversity of the scavenger receptors. SCARA have a collagen-like domain believed to be their ligand-binding domain. CD68 has a mucin-like domain; LOX-1(oxidized-low density lipoprotein (lectin-like) receptor-1) (SCARE) has a C-type lectin domain and binds oxidized LDL. SCARF (scavenger receptor class F) has multiple extracellular EGF-like repeats.

[26] and lipoteichoic acid (LTA) [27, 28], we and others have shown that SCARA-1/2 also bind fibrillar β-amyloid and advanced glycosylation end products (AGEs) [4, 25]. SCARA-1/2 mediate phagocytosis of infectious organisms like *Staphylococcus aureus* [28] and *Neisseria meningitides* [29] and also mediate clearance of apoptotic thymocytes [30]. Because of their broad ligand specificity, SCARA-1/2 contribute to resistance to gram-positive and gram-negative microbial infections and some viral infections *in vivo,* and SCARA-1/2 null mice succumb to *S. aureus,* and *Herpes simplex* infections faster than normal mice [28, 31]. In addition, SCARA-1/2 null mice also demonstrate increased susceptibility to LPS-induced shock, [32] suggesting a potential role in regulating the endotoxin response.

4.1. SCARA Role in Alzheimer's Disease. Murine microglia bind and phagocytose Aβ via SCARA1. Studies using a transgenic mouse model of AD expressing the human form of Aβ, and developing Aβ plaques in the brain over time [33] showed an increased level of SCARA-1 on microglia around Aβ plaques [34]. Microglia isolated from human brains were also able to bind to and ingest Aβ via SCARA-1 [35]. Additional evidence that SCARA-1 is an important phagocytic receptor for Aβ comes from studies using microglia from SCARA-1 knock-out mice. SCARA-1 knock-out microglia isolated from these animals showed a 60% decrease in the ability to take up amyloid-β compared with wild-type cells [36]. These results suggest that in addition to SCARA-1, other receptors may be involved in clearance of Aβ by microglia. Since SCARA-1 is involved in clearance of Aβ, from a therapeutic standpoint, it may be beneficial to upregulate expression of this receptor on microglia thereby increasing the ability of these cells to clear Aβ.

Another SCARA that has been shown to interact with Aβ is the macrophage scavenger receptor with collagenous structure (MARCO). MARCO is a SCARA family member

but is encoded by a distinct gene from SCARA-1/2. Importantly, MARCO and SCARA1/2 have both common and distinct ligands [37, 38]. MARCO is expressed on cultured neonatal rat microglia and is also a receptor for Aβ [39]. It is not known if MARCO is expressed in microglia in the AD brain. By coimmunoprecipitation, MARCO has been shown to form a complex with formyl peptide-receptor-like 1 (FPRL1) upon encountering Aβ. Intracellular signaling via ERK 1/2 and inhibition of cAMP is then initiated through FPRL1 which may play a part in decreasing the inflammatory response mediated through MARCO in microglia [40].

5. Class B Scavenger Receptors (SCARB)

SCARB are also important in the innate host response to bacterial and fungal pathogens. The B class receptors are characterized by the presence of membrane-spanning N and C termini and a large extracellular loop [41] (Figure 1). CD36 (SCARB-2), the first classified SCARB was initially identified as a receptor for thrombospondin [42] and for malaria parasitized erythrocytes [43]. Endemann and colleagues subsequently identified CD36 as the "second" modified lipoprotein receptor [44], the first receptor being SCARA-1/2. By virtue of their ability to bind HDL and act as fatty acid transporters, CD36 and SCARB-1 play major roles in cholesterol metabolism [45–47]. CD36 also binds and internalizes *S. aureus* and is required for protection against this pathogen [21]. Recently, we have uncovered a novel role for CD36 as a receptor for β-glucan [48]. We also found that CD36 and its *C. elegans* ortholog play a major role in host defense to opportunistic infections with pathogenic yeast such as *Candida albicans and Cryptococcus neoformans*. Interestingly, SCARB-1 act as a coreceptor for the hepatitis C virus and may play a role in viral pathogenesis of hepatitis C [49, 50]. In addition to binding to microbial ligands, CD36 binds several modified "self" antigens including AGE-modified proteins involved in the pathogenesis of vascular complications of diabetes [51].

5.1. SCARB Role in Alzheimer's Disease. Scavenger receptor B1 (SCARB-1) is a receptor for Aβ on microglia. A study by Husemann et al. [52] proposed that SCARB-1 is developmentally regulated and was not found on microglia in adult human brain and was only expressed on astrocytes. In a mouse model of AD, a reduction in SCARB-1 protein expression increased Aβ plaque deposition had no effect on microglial accumulation around Aβ plaques and in fact worsened cognitive defects in learning and memory [53].

CD36/SCARB-2 is also expressed on microglia and a receptor for amyloid-β [54]. Intracellular signaling through CD36 is activated upon Aβ binding and activates microglia to produce cytokines and chemokines that induce microglial migration [55]. CD36 knock-out microglia have less Aβ-mediated activation and reduced chemokines and cytokine production compared with wild-type cells. Injection of Aβ into CD36 knock-out mouse brains induced less accumulation of microglia compared with wild-type brains, demonstrating an important role for CD36 in the inflammatory response to Aβ [54]. Microglial CD36 signals upon Aβ engagement via the Src family members Fyn and Lyn and by activation of mitogen-activated protein kinase (MAPK) and subsequent chemokine and ROS production [56].

Further investigation of the signaling properties of CD36 has recently been shown to be intimately involved with two toll-like receptors TLR-4 and TLR-6. Upon Aβ engagement, CD36 forms a heterodimeric complex with TLR-4 and TLR-6 on microglia resulting in ROS production, and an increase in IL-β mRNA, indicative of inflammasome activation [5].

From a therapeutic standpoint, it may be advantageous to inhibit the ability of CD36 to signal when bound to Aβ, and thus prevent the release of chemokines and ROS by microglia that are deleterious to the surrounding neurons. We recently undertook a high-content screen to identify small molecule inhibitors of CD36. Screening of an FDA-approved compound library discovered ursolic acid to be an inhibitor of CD36 binding to Aβ. In addition, ursolic acid also inhibited Aβ-mediated ROS production in microglia without effecting microglial ability to phagocytose Aβ [57].

6. Class C Scavenger Receptors (SCARC)

SCARC-1 was identified in *Drosophila* but no mammalian ortholog for this receptor has yet been discovered. SCARC-1 is involved in phagocytosis of Gram-negative and Gram-positive bacteria but not yeast [58, 59]. Intriguingly, SCARC-1 also mediates uptake of dsRNA [59]. Since no mammalian ortholog for SCARC has been identified, it remains to be determined if this class of SR plays any role(s) in mammalian physiology or disease pathogenesis.

7. Class D Scavenger Receptors (SCARD)

The class D SRs are characterized by the presence of a mucin-like extracellular domain (Figure 1). The best characterized SCARD is CD68 (also known as macrosialin), which is expressed by macrophages, dendritic cells microglia, and osteoclasts [60, 61] For this reason, CD68 has been used for many years as a histological marker for these cells. It is found mainly intracellularly in late endosomes but cell surface expression increases following activation [62]. CD68/macrosialin plays a minor role in the binding and uptake of oxidized lipoproteins and apoptotic cells by macrophages [63].

7.1. Role of SCARD in Alzheimer's Disease. Studies investigating a role of SCARD in AD have been mostly descriptive. Immunizing human subjects with AD with Aβ 1–42 caused a reduction in plaques and an upregulation of CD68 expressed on microglia [64]. However in two longer surviving subjects, even though Aβ plaques were cleared, the expression of CD68 was found to be lower than in nonimmunized AD brains [65]. No studies to date investigated whether this class of SRs is mechanistically involved in CNS disorders including AD.

8. Class E Scavenger Receptors (SCARE)

Lectin-like oxidized LDL receptor (LOX-1) was the first scavenger receptor with a C-type lectin-like domain (Figure 1) to be identified. LOX-1 was initially cloned from endothelial cells and appears to play a role in atherosclerosis [66]. LOX-1 is expressed on freshly isolated human monocytes [67], LOX-1 decreases with monocyte differentiation into macrophages. LOX-1 binds oxidized LDL [66] and has also been implicated in the transport of β-amyloid across the blood brain barrier [68, 69]. In addition, LOX-1 binds gram positive and gram negative bacteria, although its exact role in the innate immune response to these pathogens is unknown [70]. It is not known if LOX-1 is expressed on microglia.

9. Class F Scavenger Receptors-SCARF

SCARFs are characterized by the presence of multiple extracellular epidermal growth factor-like repeats (Figure 1). The first SCARF was identified as an endothelial receptor for modified LDL, termed SREC [71]. In addition to binding modified LDL, SCARFs are receptors for heat shock proteins [72] and calreticulin which is involved in trafficking associated peptides into the major histocompatibility complex class I cross-presentation pathway of antigen-presenting cells [73]. We found that SCARF-1 is expressed on macrophages, and that SCARF-1 plays an important role in binding of the pathogenic yeasts *Candida albicans* and *Cryptococcus neoformans.* SCARF appears to be conserved through evolution. CED-1, a *C. elegans* ortholog of SCARF also appears to play a major role in the innate worm immune response to pathogenic yeast, and CED-1-deficient mutant worms have a dramatic increase in their susceptibility to these infections [48]. Interestingly, CED-1 also binds to cell corpses [74]. Similarly, related families of molecules have been identified in *Drosophila melanogaster* where they also contribute to host defense [75].

9.1. Role of SCARF in Alzheimer's Disease. It is not known if SCARF1 is expressed in the brain and/or involved in AD pathogenesis. MEGF10 (multiple EGF-like domains-10), also a member of the SCARF family, has recently been shown to be a receptor for Aβ [76]. MEGF10 is a type 1 transmembrane protein containing 17 EGF-like domains in the extracellular portion (Figure 1) [77]. MEGF10 is expressed in the brain of a transgenic mouse model of AD in the hippocampus and cortex region of the brain where Aβ plaques are also found, but it is unknown whether MEGF10 is expressed on microglia [76].

10. Unclassified Scavenger Receptors

10.1. RAGE. RAGE (receptor for advanced glycation end products) is a member of the immunoglobulin superfamily of receptors [78] expressed on endothelial cells [79] and microglia which is capable of binding many ligands including Aβ [80], AGEs (advanced glycation end products) and S100 protein [81]. Ligand binding to RAGE induces many intracellular signaling pathways such as Ras-extracellular signal-regulated kinase 1/2 (ERK1/2) [82], Cdc42/Race [83] stress-activated protein kinase/c-Jun-NH2-terminal kinase (SAPK/JNK), and p38 mitogen-activated protein (MAP) kinase pathways [84] that activate transcription factors, for example, NFκB [85], cAMP response element-binding (CREB) protein [82], or (STAT3), a member of the signal transducers and activators of transcription family [86]. RAGE activation through ligand binding induces a positive signaling feedback loop causing sustained activation of NFκB and a chronic state of inflammation [87].

10.1.1. Role of RAGE in Alzheimer's Disease. RAGE expressed on endothelial cells has previously been shown to play a role transporting Aβ into the brain [88], and also increasing the diapedesis of monocytes across the blood- brain barrier through RAGE-mediated signaling [89].

RAGE is expressed in higher levels on neurons and vasculature in AD brains compared with undiseased brains [90]. Soluble Aβ bound to RAGE induces microglial activation and chemotaxis along a concentration gradient, which may lead to microglial accumulation around Aβ plaques. [80].

In a double-transgenic mouse model of AD (both expressing mutated human APP and increased levels of RAGE), Aβ deposition, and microglial activation through NFκB were observed, providing evidence that RAGE also functions as a signaling receptor [91]. Studies using transgenic mice expressing human Aβ and a dominant negative form of RAGE showed microglial RAGE to be an essential signaling receptor, signaling through p38 MAPK and JNK, which leads to synaptic dysfunction through JNK-mediated IL-β release [92].

However, more recent evidence shows that transgenic mice expressing both the Swedish and Arctic forms of human APP, and deficient in RAGE have a decrease in Aβ deposition and an increase in insulin degrading enzyme (IDE), an enzyme known to cleave Aβ when compared to mice expressing RAGE [93]. Such decrease in Aβ was found at 6 months of age. No improvement in cognitive function or difference in microglial recruitment to plaques was seen in 12 months old mice. This suggests RAGE may not be essential for microglial recruitment but could be involved in Aβ processing in the early disease state [94].

10.2. CD163. CD163 contains nine scavenger receptor cysteine-rich (SRCR) domains, an ancient and highly conserved protein motif, belonging to the SRCR superfamily. CD163 is expressed on mature tissue macrophages and has previously been shown to be involved in the clearance of hemoglobin-haptoglobin from the circulation [95]. Engagement of macrophage CD163-induced nitric oxide, IL-1β, and TNFα production, suggesting CD163 may be involved in activation of macrophages at sites of inflammation [96]. CD163 is also involved in host defense against both gram-positive and gram-negative bacteria, acting as an immune sensor and mediator of inflammation [97]. In the brains of patients with HIV-associated dementia, CD163 was found to be expressed on microglia [98], and on perivascular

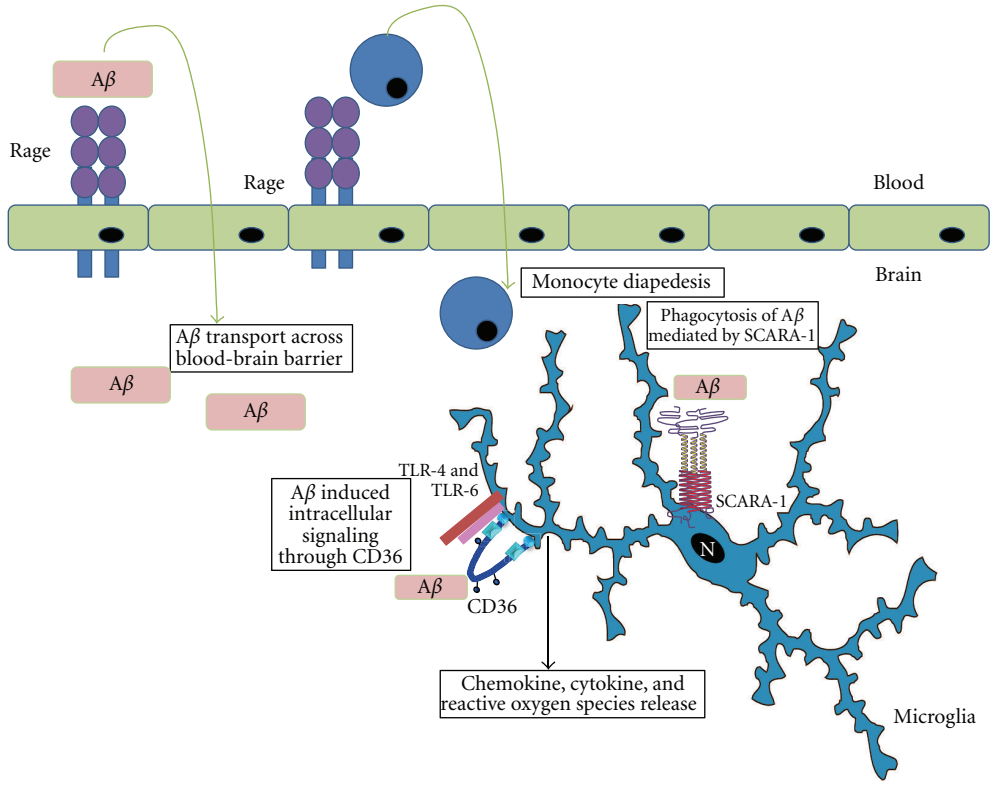


Figure 2: Diverse functions of SRs in development of Alzheimer's disease. RAGE facilitates transport of monocytes and Aβ across the blood-brain-barrier, whereas SCARA-1 mediates internalization of Aβ by microglia. CD36 and TLR-4 and TLR-6 ligation with Aβ induces intracellular signaling and release of ROS, chemokines and cytokines.

macrophages near the blood-brain barrier, [99] and CD163 is considered a marker for perivascular macrophages. However it is unknown whether CD163 is involved in the pathogenesis of AD.

10.3. SR-PSOX. Scavenger receptor that binds phosphatidylserine and oxidized lipids (SR-PSOX), also known as CXCL16, is a class H SR that can be both a membrane-bound and soluble protein. SR-PSOX is expressed on dendritic cells, and on monocyte-derived macrophages surrounding atherosclerotic plaques but not on smooth muscle cells or endothelial cells [100]. The membrane-bound form is composed of type I transmembrane glycoprotein, consisting of CXC chemokine, mucin stalk, transmembrane, and cytoplasmic domains [101]. Treatment of human peripheral blood mononuclear cells (PBMCs) with IFNγ induced increased uptake of oxLDL and increased levels of SR-PSOX [102]. The soluble form of SR-PSOX has been shown to ligate the T-cell receptor CXCR6 and attracts IFNγ-producing T cells to lymph nodes [103]. It is not known if this receptor binds Aβ or whether it plays a role in AD pathogenesis.

11. SR and Innate Immune Signaling

A common emerging theme is that SRs are not only innate immune recognition and phagocytic receptors but also act as critical regulators of inflammatory signaling and function as sensors for the innate immune response. The mechanism(s) by which SRs regulate the outcome of ligand engagement is currently unknown.

Two potential and not mutually exclusive possibilities exist. First, SRs may facilitate ligand delivery to other PRRs, particularly to the TLRs, which have not been shown to be sufficient for cellular binding to their targets. Pertinent to this, recent work has demonstrated that many TLRs do not interact with their ligands on the cell surface but within intracellular compartments such as endosomes or phagosomes. However, the mechanisms that deliver TLR ligands to the appropriate compartments are poorly defined. One possibility is that ligand delivery is facilitated by coreceptors such as Dectins [104–107] and SRs. The second scenario is that signals triggered directly from the SRs combine with those from other PRRs to define the ligand-specific response.

Such dichotomous cooperative role of SRs with other PRRs is nicely illustrated by CD36. We have observed that CD36 knockout macrophages and microglia show a decreased response to bacterial and self-ligands including Aβ but expression of CD36 is not by itself sufficient to initiate such responses. CD36 requires the presence of TLRs to mediate the responses to its ligands [5]. This reflects two roles for CD36 which contributes to response by both binding the ligand and also by synergistic and cooperative signaling with other PRRS such as TLRs. This cooperation may define the specificity of the response to a particular ligand. Indeed, we found that CD36/TLR2/6 respond to pathogens [108]

whereas CD36/TLR4/6 respond to endogenous ligands such as β-amyloid and oxLDL. These roles for CD36 have been mostly described *in vitro*. It remains to be determined if such sophisticated ligand-receptor specificity of interaction also occurs *in vivo* and whether it affects diseases processes such as AD.

12. Diverse Roles of SRs in AD

Promising evidence has shown the diversity of SRs with regards to their functions during the development of AD. SCARA-1 and CD36 play complementary nonredundant roles in the interactions of microglia with Aβ. SCARA-1-Aβ interactions are beneficial and promote phagocytosis and clearance of Aβ, whereas CD36-Aβ interactions are harmful and together with TLR-4 and TLR-6 lead to production of neurotoxins and proinflammatory molecules. This is reminiscent of the role CD36 plays in the interaction between macrophages and oxidized low-density lipoproteins in atherosclerosis. Indeed, we have shown in the past that while SCARA-1 mediates adhesion to oxidized LDL coated surfaces, CD36 mediates macrophage activation by oxidized LDL to produce reactive oxygen species [109]. Such differential roles of SCARA-1 and CD36 may have therapeutic implications for AD. Indeed, because of the role of SCARA-1 in Aβ clearance, drugs that upregulate SCARA-1 expression or functions may be helpful for treatment of AD. In contrast, drugs that block CD36 interactions with Aβ or reduce its surface expression may be helpful to stop or delay progression of AD. Similarly, since RAGE expressed on endothelium facilitates the transport of circulating Aβ from the blood across the blood-brain barrier into the brain, compounds that block Aβ-RAGE interactions may be beneficial. In addition to SCARA-1, CD36 and RAGE, and other SRs such as MARCO, LOX-1, and MEGF-10 are also emerging as receptors that bind Aβ and/or may have a role in the pathogenesis of disease (Figure 2). Dissecting the complex roles of various scavenger receptors in microglia-Aβ interactions, is therefore, important to understand the role of microglia in this disease and has therapeutic implications for treatment of this devastating disorder.

Acknowledgments

The work summarized in this manuscript was supported by NIH Grants NS059005, AG032349, AI082660 and a grant from the Dana Foundation Neuroimmunology Program to J. El Khoury

References

[1] J. J. Jalbert, L. A. Daiello, and K. L. Lapane, "Dementia of the Alzheimer type," *Epidemiologic Reviews*, vol. 30, no. 1, pp. 15–34, 2008.

[2] J. Näslund, V. Haroutunian, R. Mohs et al., "Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline," *Journal of the American Medical Association*, vol. 283, no. 12, pp. 1571–1577, 2000.

[3] G. Thinakaran and E. H. Koo, "Amyloid precursor protein trafficking, processing, and function," *The Journal of Biological Chemistry*, vol. 283, no. 44, pp. 29615–29619, 2008.

[4] J. El Khoury, S. E. Hickman, C. A. Thomas, L. Cao, S. C. Silverstein, and J. D. Loike, "Scavenger receptor-mediated adhesion of microglia to β-amyloid fibrils," *Nature*, vol. 382, no. 6593, pp. 716–719, 1996.

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

[6] P. L. McGeer, S. Itagaki, H. Tago, and E. G. McGeer, "Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein hla-dr," *Neuroscience Letters*, vol. 79, no. 1-2, pp. 195–200, 1987.

[7] A. Matus, "Microtubule-associated proteins and the determination of neuronal form," *Journal De Physiologie*, vol. 84, no. 1, pp. 134–137, 1990.

[8] J. Götz and L. M. Ittner, "Animal models of Alzheimer's disease and frontotemporal dementia," *Nature Reviews Neuroscience*, vol. 9, no. 7, pp. 532–544, 2008.

[9] L. M. Ittner, Y. D. Ke, F. Delerue et al., "Dendritic function of tau mediates amyloid-β toxicity in Alzheimer's disease mouse models," *Cell*, vol. 142, no. 3, pp. 387–397, 2010.

[10] G. A. Jicha, R. Bowser, I. G. Kazam, and P. Davies, "Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau," *Journal of Neuroscience Research*, vol. 48, no. 2, pp. 128–132, 1997.

[11] J. D. Harper and P. T. Lansbury Jr., "Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins," *Annual Review of Biochemistry*, vol. 66, pp. 385–407, 1997.

[12] M. I. Fonseca, R. R. Ager, S. H. Chu et al., "Treatment with a c5ar antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease," *Journal of Immunology*, vol. 183, no. 2, pp. 1375–1383, 2009.

[13] M. Fiala, L. Zhang, X. Gan et al., "Amyloid-β induces chemokine secretion and monocyte migration across a human blood-brain barrier model," *Molecular Medicine*, vol. 4, no. 7, pp. 480–489, 1998.

[14] C. Hale, M. Véniant, Z. Wang et al., "Structural characterization and pharmacodynamic effects of an orally active 11β-hydroxysteroid dehydrogenase type 1 inhibitor," *Chemical Biology and Drug Design*, vol. 71, no. 1, pp. 36–44, 2008.

[15] T. Bolmont, F. Haiss, D. Eicke et al., "Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance," *Journal of Neuroscience*, vol. 28, no. 16, pp. 4283–4292, 2008.

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

[17] D. M. Paresce, R. N. Ghosh, and F. R. Maxfield, "Microglial cells internalize aggregates of the Alzheimer's disease amyloid β-protein via a scavenger receptor," *Neuron*, vol. 17, no. 3, pp. 553–565, 1996.

[18] R. H. Christie, M. Freeman, and B. T. Hyman, "Expression of the macrophage scavenger receptor, a multifunctional lipoprotein receptor, in microglia associated with senile plaques in Alzheimer's disease," *The American Journal of Pathology*, vol. 148, no. 2, pp. 399–403, 1996.

[19] J. L. Goldstein, Y. K. Ho, S. K. Basu, and M. S. Brown, "Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, no. 1, pp. 333–337, 1979.

[20] M. Z. Ashraf and N. Gupta, "Scavenger receptors: implications in atherothrombotic disorders," *International Journal of Biochemistry and Cell Biology*, vol. 43, no. 5, pp. 697–700, 2011.

[21] L. M. Stuart, J. Deng, J. M. Silver et al., "Response to staphylococcus aureus requires CD36-mediated phagocytosis triggered by the cooh-terminal cytoplasmic domain," *Journal of Cell Biology*, vol. 170, no. 3, pp. 477–485, 2005.

[22] L. M. Stuart and R. A. B. Ezekowitz, "Phagocytosis: elegant complexity," *Immunity*, vol. 22, no. 5, pp. 539–550, 2005.

[23] T. Kodama, M. Freeman, L. Rohrer, J. Zabrecky, P. Matsudaira, and M. Krieger, "Type I macrophage scavenger receptor contains α-helical and collagen-like coiled coils," *Nature*, vol. 343, no. 6258, pp. 531–535, 1990.

[24] L. Rohrer, M. Freeman, T. Kodama, M. Penman, and M. Krieger, "Coiled-coil fibrous domains mediate ligand binding by macrophage scavenger receptor type II," *Nature*, vol. 343, no. 6258, pp. 570–572, 1990.

[25] J. El Khoury, C. A. Thomas, J. D. Loike, S. E. Hickman, L. Cao, and S. C. Silverstein, "Macrophages adhere to glucose-modified basement membrane collagen iv via their scavenger receptors," *The Journal of Biological Chemistry*, vol. 269, no. 14, pp. 10197–10200, 1994.

[26] R. Y. Hampton, D. T. Golenbock, M. Penman, M. Krieger, and C. R. H. Raetz, "Recognition and plasma clearance of endotoxin by scavenger receptors," *Nature*, vol. 352, no. 6333, pp. 342–344, 1991.

[27] J. W. Greenberg, W. Fischer, and K. A. Joiner, "Influence of lipoteichoic acid structure on recognition by the macrophage scavenger receptor," *Infection and Immunity*, vol. 64, no. 8, pp. 3318–3325, 1996.

[28] C. A. Thomas, Y. Li, T. Kodama, H. Suzuki, S. C. Silverstein, and J. El Khoury, "Protection from lethal gram-positive infection by macrophage scavenger receptor-dependent phagocytosis," *Journal of Experimental Medicine*, vol. 191, no. 1, pp. 147–155, 2000.

[29] L. Peiser, M. P. J. De Winther, K. Makepeace et al., "The class A macrophage scavenger receptor is a major pattern recognition receptor for neisseria meningitidis which is independent of lipopolysaccharide and not required for secretory responses," *Infection and Immunity*, vol. 70, no. 10, pp. 5346–5354, 2002.

[30] N. Platt, H. Suzuki, Y. Kurihara, T. Kodama, and S. Gordon, "Role for the class A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes in vitro," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 22, pp. 12456–12460, 1996.

[31] H. Suzuki, Y. Kurihara, M. Takeya et al., "A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection," *Nature*, vol. 386, no. 6622, pp. 292–296, 1997.

[32] Y. Kobayashi, C. Miyaji, H. Watanabe et al., "Role of macrophage scavenger receptor in endotoxin shock," *Journal of Pathology*, vol. 192, no. 2, pp. 263–272, 2000.

[33] C. Sturchler-Pierrat, D. Abramowski, M. Duke et al., "Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 24, pp. 13287–13292, 1997.

[34] K. D. Bornemann, K. H. Wiederhold, C. Pauli et al., "Aβ-induced inflammatory processes in microglia cells of APP23 transgenic mice," *The American Journal of Pathology*, vol. 158, no. 1, pp. 63–73, 2001.

[35] J. Husemann and S. C. Silverstein, "Expression of scavenger receptor class B, type I, by astrocytes and vascular smooth muscle cells in normal adult mouse and human brain and in Alzheimer's disease brain," *The American Journal of Pathology*, vol. 158, no. 3, pp. 825–832, 2001.

[36] H. Chung, M. I. Brazil, M. C. Irizarry, B. T. Hyman, and F. R. Maxfield, "Uptake of fibrillar β-amyloid by microglia isolated from MSR-A (type I and type II) knockout mice," *Neuroreport*, vol. 12, no. 6, pp. 1151–1154, 2001.

[37] S. Mukhopadhyay, Y. Chen, M. Sankala et al., "Marco, an innate activation marker of macrophages, is a class A scavenger receptor for neisseria meningitidis," *European Journal of Immunology*, vol. 36, no. 4, pp. 940–949, 2006.

[38] R. F. Hamilton Jr., S. A. Thakur, J. K. Mayfair, and A. Holian, "Marco mediates silica uptake and toxicity in alveolar macrophages from c57bl/6 mice," *The Journal of Biological Chemistry*, vol. 281, no. 45, pp. 34218–34226, 2006.

[39] R. Alarcón, C. Fuenzalida, M. Santibáñez, and R. von Bernhardi, "Expression of scavenger receptors in glial cells: Comparing the adhesion of astrocytes and microglia from neonatal rats to surface-bound β-amyloid," *The Journal of Biological Chemistry*, vol. 280, no. 34, pp. 30406–30415, 2005.

[40] L. O. Brandenburg, M. Konrad, C. J. Wruck, T. Koch, R. Lucius, and T. Pufe, "Functional and physical interactions between formyl-peptide-receptors and scavenger receptor marco and their involvement in amyloid beta 1-42-induced signal transduction in glial cells," *Journal of Neurochemistry*, vol. 113, no. 3, pp. 749–760, 2010.

[41] P. Gruarin, R. F. Thorne, D. J. Dorahy, G. F. Burns, R. Sitia, and M. Alessio, "CD36 is a ditopic glycoprotein with the n-terminal domain implicated in intracellular transport," *Biochemical and Biophysical Research Communications*, vol. 275, no. 2, pp. 446–454, 2000.

[42] A. S. Asch, J. Barnwell, R. L. Silverstein, and R. L. Nachman, "Isolation of the thrombospondin membrane receptor," *Journal of Clinical Investigation*, vol. 79, no. 4, pp. 1054–1061, 1987.

[43] C. F. Ockenhouse and J. D. Chulay, "Plasmodium falciparum sequestration: okm5 antigen (CD36) mediates cytoadherence of parasitized erythrocytes to a myelomonocytic cell line," *Journal of Infectious Diseases*, vol. 157, no. 3, pp. 584–588, 1988.

[44] G. Endemann, L. W. Stanton, K. S. Madden, C. M. Bryant, R. T. White, and A. A. Protter, "CD36 is a receptor for oxidized low density lipoprotein," *The Journal of Biological Chemistry*, vol. 268, no. 16, pp. 11811–11816, 1993.

[45] S. Acton, A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Kriegert, "Identification of scavenger receptor SR-BI as a high density lipoprotein receptor," *Science*, vol. 271, no. 5248, pp. 518–520, 1996.

[46] N. A. Abumrad, M. R. El-Maghrabi, E. Z. Amri, E. Lopez, and P. A. Grimaldi, "Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. homology with human CD36," *The Journal of Biological Chemistry*, vol. 268, no. 24, pp. 17665–17668, 1993.

[47] C. M. Harmon and N. A. Abumrad, "Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins:

isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids," *Journal of Membrane Biology*, vol. 133, no. 1, pp. 43–49, 1993.

[48] T. K. Means, E. Mylonakis, E. Tampakakis et al., "Evolutionarily conserved recognition and innate immunity to fungal pathogens by the scavenger receptors scarf1 and CD36,' *Journal of Experimental Medicine*, vol. 206, no. 3, pp. 637–653, 2009.

[49] E. Scarselli, H. Ansuini, R. Cerino et al., "The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis c virus," *The EMBO Journal*, vol. 21, no. 19, pp. 5017–5025, 2002.

[50] M. B. Zeisel, G. Koutsoudakis, E. K. Schnober et al., "Scavenger receptor class B type I is a key host factor for hepatitis c virus infection required for an entry step closely linked to cd81," *Hepatology*, vol. 46, no. 6, pp. 1722–1731, 2007.

[51] N. Ohgami, R. Nagai, M. Ikemoto et al., "CD36, serves as a receptor for advanced glycation endproducts (AGE)," *Journal of Diabetes and its Complications*, vol. 16, no. 1, pp. 56–59, 2002.

[52] J. Husemann, J. D. Loike, T. Kodama, and S. C. Silverstein, "Scavenger receptor class B type I (SR-BI) mediates adhesion of neonatal murine microglia to fibrillar β-amyloid," *Journal of Neuroimmunology*, vol. 114, no. 1-2, pp. 142–150, 2001.

[53] K. Thanopoulou, A. Fragkouli, F. Stylianopoulou, and S. Georgopoulos, "Scavenger receptor class B type I (SR-BI) regulates perivascular macrophages and modifies amyloid pathology in an Alzheimer mouse model," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 48, pp. 20816–20821, 2010.

[54] J. B. El Khoury, K. J. Moore, T. K. Means et al., "CD36 mediates the innate host response to β-amyloid," *Journal of Experimental Medicine*, vol. 197, no. 12, pp. 1657–1666, 2003.

[55] I. S. Coraci, J. Husemann, J. W. Berman et al., "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," *The American Journal of Pathology*, vol. 160, no. 1, pp. 101–112, 2002.

[56] K. J. Moore, J. El Khoury, L. A. Medeiros et al., "A CD36-initiated signaling cascade mediates inflammatory effects of β-amyloid," *The Journal of Biological Chemistry*, vol. 277, no. 49, pp. 47373–47379, 2002.

[57] K. Wilkinson, J. D. Boyd, M. Glicksman, K. J. Moore, and J. El Khoury, "A high content drug screen identifies ursolic acid as an inhibitor of amyloid β protein interactions with its receptor CD36," *The Journal of Biological Chemistry*, vol. 286, no. 40, pp. 34914–34922, 2011.

[58] A. Pearson, A. Lux, and M. Krieger, "Expression cloning of dsR-CI, a class C macrophage-specific scavenger receptor from drosophila melanogaster," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 9, pp. 4056–4060, 1995.

[59] M. Rämet, A. Pearson, P. Manfruelli et al., "Drosophila scavenger receptor CI is a pattern recognition receptor for bacteria," *Immunity*, vol. 15, no. 6, pp. 1027–1038, 2001.

[60] L. Martinez-Pomares, N. Platt, A. J. Mcknight, R. P. Da Silva, and S. Gordon, "Macrophage membrane molecules: markers of tissue differentiation and heterogeneity," *Immunobiology*, vol. 195, no. 4-5, pp. 407–416, 1996.

[61] D. R. Greaves and S. Gordon, "Macrophage-specific gene expression: current paradigms and future challenges," *International Journal of Hematology*, vol. 76, no. 1, pp. 6–15, 2002.

[62] S. Gordon, "Macrophage-restricted molecules: role in differentiation and activation," *Immunology Letters*, vol. 65, no. 1-2, pp. 5–8, 1999.

[63] Y. Yamada, T. Doi, T. Hamakubo, and T. Kodama, "Scavenger receptor family proteins: roles for atherosclerosis, host defence and disorders of the central nervous system," *Cellular and Molecular Life Sciences*, vol. 54, no. 7, pp. 628–640, 1998.

[64] A. Argiles, G. Mourad, P. G. Kerr, M. Garcia, B. Collins, and J. G. Demaille, "Cells surrounding haemodialysis-associated amyloid deposits are mainly macrophages," *Nephrology Dialysis Transplantation*, vol. 9, no. 6, pp. 662–667, 1994.

[65] E. Zotova, C. Holmes, D. Johnston, J. W. Neal, J. A. R. Nicoll, and D. Boche, "Microglial alterations in human Alzheimer's disease following aβ42 immunization," *Neuropathology and Applied Neurobiology*, vol. 37, no. 5, pp. 513–524, 2011.

[66] T. Sawamura, N. Kume, T. Aoyama et al., "An endothelial receptor for oxidized low-density lipoprotein," *Nature*, vol. 386, no. 6620, pp. 73–77, 1997.

[67] H. Yoshida, N. Kondratenko, S. Green, D. Steinberg, and O. Quehenberger, "Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor," *Biochemical Journal*, vol. 334, no. 1, pp. 9–13, 1998.

[68] E. Luedecking-Zimmer, S. T. DeKosky, Q. Chen, M. M. Barmada, and M. I. Kamboh, "Investigation of oxidized IDI-receptor 1 (ORI1) as the candidate gene for Alzheimer's disease on chromosome 12," *Human Genetics*, vol. 111, no. 4-5, pp. 443–451, 2002.

[69] J. Shi, J. Tian, A. Pritchard et al., "A 3′-UTR polymorphism in the oxidized ldl receptor 1 gene increases Aβ40 load as cerebral amyloid angiopathy in Alzheimer's disease," *Acta Neuropathologica*, vol. 111, no. 1, pp. 15–20, 2006.

[70] T. Shimaoka, N. Kume, M. Minami et al., "LOX-1 supports adhesion of gram-positive and gram-negative bacteria," *Journal of Immunology*, vol. 166, no. 8, pp. 5108–5114, 2001.

[71] H. Adachi, M. Tsujimoto, H. Arai, and K. Inouel, "Expression cloning of a novel scavenger receptor from human endothelial cells," *The Journal of Biological Chemistry*, vol. 272, no. 50, pp. 31217–31220, 1997.

[72] J. G. Facciponte, X. Y. Wang, and J. R. Subjeck, "Hsp110 and Grp170, members of the Hsp70 superfamily, bind to scavenger receptor-A and scavenger receptor expressed by endothelial cell-I," *European Journal of Immunology*, vol. 37, no. 8, pp. 2268–2279, 2007.

[73] B. Berwin, Y. Delneste, R. V. Lovingood, S. R. Post, and S. V. Pizzo, "SREC-I, a type F scavenger receptor, is an endocytic receptor for calreticulin," *The Journal of Biological Chemistry*, vol. 279, no. 49, pp. 51250–51257, 2004.

[74] Z. Zhou, E. Hartwieg, and H. R. Horvitz, "CED-1 is a transmembrane receptor that mediates cell corpse engulfment in C. elegans," *Cell*, vol. 104, no. 1, pp. 43–56, 2001.

[75] C. Kocks, J. H. Cho, N. Nehme et al., "Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in drosophila," *Cell*, vol. 123, no. 2, pp. 335–346, 2005.

[76] T. D. Singh, S. Y. Park, J. S. Bae et al., "Megf10 functions as a receptor for the uptake of amyloid-β," *FEBS Letters*, vol. 584, no. 18, pp. 3936–3942, 2010.

[77] E. Suzuki and M. Nakayama, "The mammalian CED-1 ortholog MEGF10/KIAA1780 displays a novel adhesion pattern," *Experimental Cell Research*, vol. 313, no. 11, pp. 2451–2464, 2007.

[78] M. Neeper, A. M. Schmidt, J. Brett et al., "Cloning and expression of a cell surface receptor for advanced glycosylation

end products of proteins," *The Journal of Biological Chemistry*, vol. 267, no. 21, pp. 14998–15004, 1992.

[79] J. Brett, A. M. Schmidt, S. D. Yan et al., "Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues," *The American Journal of Pathology*, vol. 143, no. 6, pp. 1699–1712, 1993.

[80] S. D. Yan, X. Chen, J. Fu et al., "RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease," *Nature*, vol. 382, no. 6593, pp. 685–691, 1996.

[81] E. Leclerc, G. Fritz, S. W. Vetter, and C. W. Heizmann, "Binding of S100 proteins to RAGE: an update," *Biochimica Et Biophysica Acta*, vol. 1793, no. 6, pp. 993–1007, 2009.

[82] H. J. Huttunen, J. Kuja-Panula, and H. Rauvala, "Receptor for advanced glycation end products (RAGE) signaling induces creb-dependent chromogranin expression during neuronal differentiation," *The Journal of Biological Chemistry*, vol. 277, no. 41, pp. 38635–38646, 2002.

[83] H. J. Huttunen, C. Fages, and H. Rauvala, "Receptor for advanced glycation end products (RAGE)-mediated neurite outgrowth and activation of NF-κB require the cytoplasmic domain of the receptor but different downstream signaling pathways," *The Journal of Biological Chemistry*, vol. 274, no. 28, pp. 19919–19924, 1999.

[84] A. Taguchi, D. C. Blood, G. Del Toro et al., "Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases," *Nature*, vol. 405, no. 6784, pp. 354–360, 2000.

[85] Shi Du Yan, A. M. Schmidt, G. M. Anderson et al., "EnhanCED cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins," *The Journal of Biological Chemistry*, vol. 269, no. 13, pp. 9889–9897, 1994.

[86] H. Ariyoshi, N. Yoshikawa, Y. Aono et al., "Role of receptor for advanced glycation end-product (RAGE) and the JAK/STAT-signaling pathway in AGE-induced collagen production in NRK-49F cells," *Journal of Cellular Biochemistry*, vol. 81, no. 1, pp. 102–113, 2001.

[87] M. Andrassy, J. Igwe, F. Autschbach et al., "Posttranslationally modified proteins as mediators of sustained intestinal inflammation," *The American Journal of Pathology*, vol. 169, no. 4, pp. 1223–1237, 2006.

[88] J. B. Mackic, M. Stins, J. G. McComb et al., "Human blood-brain barrier receptors for Alzheimer's amyloid-β 1-40. asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer," *Journal of Clinical Investigation*, vol. 102, no. 4, pp. 734–743, 1998.

[89] R. Giri, Y. Shen, M. Stins et al., "β-amyloid-induced migration of monocytes across human brain endothelial cells involves RAGE and PECAM-1," *American Journal of Physiology*, vol. 279, no. 6, pp. C1772–C1781, 2000.

[90] O. Arancio, H. P. Zhang, X. Chen et al., "RAGE potentiates aβ-induced perturbation of neuronal function in transgenic mice," *The EMBO Journal*, vol. 23, no. 20, pp. 4096–4105, 2004.

[91] O. Arancio, H. P. Zhang, X. Chen et al., "RAGE potentiates aβ-induced perturbation of neuronal function in transgenic mice," *The EMBO Journal*, vol. 23, no. 20, pp. 4096–4105, 2004.

[92] N. Origlia, C. Bonadonna, A. Rosellini et al., "Microglial receptor for advanced glycation end product-dependent signal pathway drives β-amyloid-induced synaptic depression and long-term depression impairment in entorhinal cortex," *Journal of Neuroscience*, vol. 30, no. 34, pp. 11414–11425, 2010.

[93] E. Himeno, Y. Ohyagi, L. Ma et al., "Apomorphine treatment in Alzheimer mice promoting amyloid-β degradation," *Annals of Neurology*, vol. 69, no. 2, pp. 248–256, 2011.

[94] I. Vodopivec, A. Galichet, M. Knobloch, A. Bierhaus, C. W. Heizmann, and R. M. Nitsch, "RAGE does not affect amyloid pathology in transgenic ArcAβ mice," *Neurodegenerative Diseases*, vol. 6, no. 5-6, pp. 270–280, 2009.

[95] B. O. Fabriek, C. D. Dijkstra, and T. K. van den Berg, "The macrophage scavenger receptor CD163," *Immunobiology*, vol. 210, no. 2-4, pp. 153–160, 2005.

[96] M. M. J. Polfliet, B. O. Fabriek, W. P. Daniëls, C. D. Dijkstra, and T. K. van den Berg, "The rat macrophage scavenger receptor CD163: expression, regulation and role in inflammatory mediator production," *Immunobiology*, vol. 211, no. 6-8, pp. 419–425, 2006.

[97] B. O. Fabriek, R. V. Bruggen, D. M. Deng et al., "The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria," *Blood*, vol. 113, no. 4, pp. 887–892, 2009.

[98] E. S. Roberts, E. Masliah, and H. S. Fox, "CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE)," *Journal of Neuropathology and Experimental Neurology*, vol. 63, no. 12, pp. 1255–1264, 2004.

[99] B. O. Fabriek, E. S. Van Haastert, I. Galea et al., "CD163-positive perivascular macrophages in the human cns express molecules for antigen recognition and presentation," *Glia*, vol. 51, no. 4, pp. 297–305, 2005.

[100] M. Minami, N. Kume, T. Shimaoka et al., "Expression of SR-PSOX, a novel cell-surface scavenger receptor for phosphatidylserine and oxidized LDL in human atherosclerotic lesions," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 11, pp. 1796–1800, 2001.

[101] T. Shimaoka, N. Kume, M. Minami et al., "Molecular cloning of a novel scavenger receptor for oxidized low density lipoprotein, SR-PSOX, on macrophages," *The Journal of Biological Chemistry*, vol. 275, no. 52, pp. 40663–40666, 2000.

[102] D. M. Wuttge, X. Zhou, Y. Sheikine et al., "CXCL16/SR-PSOX Is an Interferon-γ-Regulated Chemokine and Scavenger Receptor Expressed in Atherosclerotic Lesions," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 4, pp. 750–755, 2004.

[103] M. Matloubian, A. David, S. Engel, J. E. Ryan, and J. G. Cyster, "A transmembrane CXC chemokine is a ligand for HIV-coreceptor bonzo," *Nature Immunology*, vol. 1, no. 4, pp. 298–304, 2000.

[104] S. Mukhopadhyay, J. Herre, G. D. Brown, and S. Gordon, "The potential for toll-like receptors to collaborate with other innate immune receptors," *Immunology*, vol. 112, no. 4, pp. 521–530, 2004.

[105] D. M. Underhill, "Toll-like receptors: networking for success," *European Journal of Immunology*, vol. 33, no. 7, pp. 1767–1775, 2003.

[106] B. N. Gantner, R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill, "Collaborative induction of inflammatory responses by dectin-1 and toll-like receptor 2," *Journal of Experimental Medicine*, vol. 197, no. 9, pp. 1107–1117, 2003.

[107] G. D. Brown, J. Herre, D. L. Williams, J. A. Willment, A. S. J. Marshall, and S. Gordon, "Dectin-1 mediates the biological effects of β-glucans," *Journal of Experimental Medicine*, vol. 197, no. 9, pp. 1119–1124, 2003.

[108] K. Hoebe, P. Georgel, S. Rutschmann et al., "CD36 is a sensor of diacylglycerides," *Nature*, vol. 433, no. 7025, pp. 523–527, 2005.

[109] H. Maxeiner, J. Husemann, C. A. Thomas, J. D. Loike, J. El Khoury, and S. C. Silverstein, "Complementary roles for scavenger receptor a and CD36 of human monocyte-derived macrophages in adhesion to surfaces coated with oxidized low-density lipoproteins and in secretion of H_2O_2," *Journal of Experimental Medicine*, vol. 188, no. 12, pp. 2257–2265, 1998.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 608732, 5 pages
doi:10.1155/2012/608732

Review Article

Traumatic Brain Injury, Microglia, and Beta Amyloid

Rebekah C. Mannix[1,2] and Michael J. Whalen[2,3]

[1] *Division of Emergency Medicine, Department of Medicine, Children's Hospital Boston, Harvard Medical School, Boston, MA 02124, USA*
[2] *Neuroscience Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA*
[3] *Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA*

Correspondence should be addressed to Rebekah C. Mannix, rebekah.mannix@childrens.harvard.edu

Received 30 November 2011; Accepted 2 March 2012

Academic Editor: Joseph El Khoury

Recently, there has been growing interest in the association between traumatic brain injury (TBI) and Alzheimer's Disease (AD). TBI and AD share many pathologic features including chronic inflammation and the accumulation of beta amyloid (Aβ). Data from both AD and TBI studies suggest that microglia play a central role in Aβ accumulation after TBI. This paper focuses on the current research on the role of microglia response to Aβ after TBI.

1. Introduction

Recently, there has been growing interest in the association between traumatic brain injury (TBI) and Alzheimer's Disease (AD). The interest grew from several lines of evidence, including epidemiological studies that demonstrated an association of TBI and the development of AD later in life [1–7] and autopsy studies that showed acute and chronic AD-like pathology in TBI victims [8, 9]. While most of the studies investigating the association of AD and TBI have focused on the accumulation and clearance amyloid-β (Aβ) [2, 8, 9], chronic neuroinflammation is also a common feature of AD and TBI, and microglia likely play a central role [10, 11]. In AD, microglia are recruited to newly formed Aβ plaques, where microglial activation functions as a double-edged sword, promoting beneficial responses such as Aβ clearance [12–14] while also eliciting a proinflammatory response [12]. Similar patterns of microglia activation have been demonstrated both acutely and chronically after TBI [15, 16].

This paper will explore the current research on the role of microglia response to Aβ after TBI. Although there are few studies that directly examine microglial reaction to trauma-induced Aβ, data from TBI and AD experimental and human studies will be used to make an argument for a central role of microglia in acute and chronic responses to Aβ-mediated secondary injury after TBI.

2. General Microglial Response after TBI

TBI is a disease process in which mechanical injury initiates cellular and biochemical changes that perpetuate neuronal injury and death over time, a process known as secondary injury. Secondary injury begins minutes after injury and can continue years after the initial insult. Mechanisms implicated in secondary injury after TBI include glutamate excitotoxicity, blood-brain barrier disruption, secondary hemorrhage, ischemia, mitochondrial dysfunction, apoptotic and necrotic cell death, and inflammation [17].

As the primary mediators of the brain's innate immune response to infection, injury, and disease, microglia react to injury within minutes. In fact, microglia may represent the first line of defense following injury [18]. Microglial activation has been demonstrated as early as 72 hours after injury in human TBI victims and can persist for years after injury [15, 16, 19, 20]. Experimental models have recapitulated these findings, with chronic microglial activation being demonstrated weeks to months after injury [21–24].

Gene-profiling studies also strongly implicate early microglial activation after TBI.

Markers of microglial activation (CD68, MHC-II), stress responses (p22phox, heme oxygenase 1), and chemokine expression (CXCL10, CXCL6) have been shown to markedly increase after experimental models of TBI [25]. Consistent

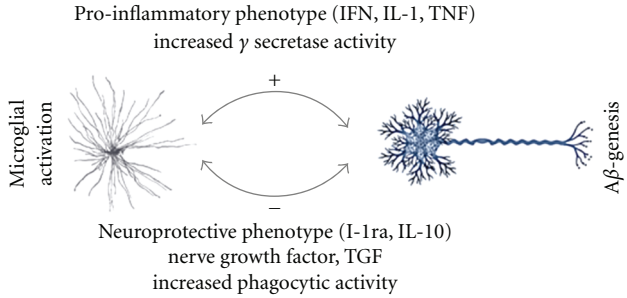


Figure 1: Schematic depiction of the beneficial and detrimental effects of the interaction Aβ -genesis and microglia after traumatic brain injury.

with early microglial activation after injury, experimental models have demonstrated rapid increases in expression of both IL-1β and TNF-α after injury [26, 27]. Much of this proinflammatory cascade may be mediated by Il-1IR, which is strongly expressed on microglia [27–29]. In addition to proinflammatory cytokines, activated microglia also produce other neurotoxic products after injury such as nitric oxide (NO) and superoxide free radicals that generate reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Microglia also produce a number of neuroprotective substances after injury, including anti-inflammatory cytokines (IL-10, IL-1 receptor antagonist (Il-1ra)) and neurotrophic factors (nerve growth factor, transforming growth factor β (TGF-β). IL-10, which is elevated acutely after injury in humans [30], has been shown to have beneficial effects in experimental models of injury [31]).

These neuroprotective effects may be a result of suppressed microglial production of proinflammatory cytokines [31, 32]. TGF-β also has also been shown to have neuroprotective effects after injury, including improved function, decreased lesion size, and decreased iNOS production [33, 34].

3. Microglial Response to Aβ after TBI

Aβ, which is elevated acutely after TBI, may be a key mediator of microglial activation in this setting [35]. In autopsy studies, Aβ plaques, a hallmark of AD, were present in as many as 30% of TBI victims (including children) [8, 9]. The plaques found in TBI patients, which are strikingly similar to those observed in the early stages of AD, develop rapidly and can appear within a few hours after injury [9, 36]. TBI-induced increases in Aβ have been successfully replicated in animal models of brain trauma [37–40]. Moreover, Aβ accumulation after TBI has also been shown to be associated with increases in the enzymes necessary for Aβ-genesis, including BACE1 protein (β-secretase) and the gamma secretase complex proteins [41–43].

Microglia may have a dual role in Aβ accumulation and clearance (Figure 1). Following closed head injury, microglia have been shown to have increased expression of the gamma secretase complex proteins, suggesting a role for microglia in posttraumatic Aβ-genesis [43]. Polymorphisms in the Aβ-degrading enzyme neprilysin have been shown to affect rates of Aβ accumulation after TBI [2], suggesting the possibility that changes in microglial neprilysin expression may be a pathologic mechanism in post-TBI Aβ accumulation in addition to the known relevance to AD [12]. Moreover, proinflammatory cytokines expressed by microglia, including interferon-γ, interleukin-1β, and tumor necrosis factor-α, can specifically stimulate gamma-secretase activity, concomitant with increased production of Aβ and the intracellular domain of APP (AICD) [44]. Further evidence suggests that increases in the numbers of neurons with elevated ß-APP concentrations after TBI correlate with increases in the number of activated microglia expressing IL-1α and that clusters of dystrophic neurites containing ß-APP are nearly universally associated with activated microglia expressing IL-1α [45]. Microglia containing Aβ have also been described in association with TBI-induced Aβ plaques, suggesting that phagocytic clearance of plaques may occur [46].

In addition to the temporospatial relationship of microglial and Aβ after TBI, much of the evidence regarding the important role of microglia in the modulation of TBI-induced Aβ is indirect. Most of this indirect evidence is derived from drug studies, which, while not designed to demonstrate the important interaction of microglial with TBI-induced Aβ, have served to demonstrate this vital interaction. Many of the studies that have indirectly elucidated the interaction of microglia and Aβ after TBI have focused on therapeutics targeting postinjury inflammation. Minocycline, a compound whose anti-inflammatory properties (including attenuating microglial activation) have been widely demonstrated in different models of TBI [47, 48], has also been shown to preclude formation of Aβ through restoration of the nonamyloidogenic α-secretase pathway of APP processing [49]. Interestingly, it has been shown that some other anti-inflammatory compounds that exert neuroprotective effects in TBI, such as nonsteroidal anti-inflammatory drugs, cholesterol-lowering drugs, and steroid hormones, also enhance the α-secretase pathway [50–53]. It is unclear from these studies whether the anti-inflammatory and proα-secretase effects are merely parallel processes, although certainly diversion of APP from the production of Aβ, which has known proinflammatory properties including stimulation of microglial activation, may itself adequately explain both the anti inflammatory and anti amyloidogentic effects.

Additional studies support the observation that suppression of microglial activation after TBI is also associated with decreases in injury-induced Aβ. Apoe mimetic peptides, liver X-receptor (LXR) agonists, and 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have all been shown to attenuate microglial activation and mitigate increases in Aβ after TBI [54–56]. It should be noted, however that none of these earlier studies can definitively lead one to conclude whether Aβ is a cause, product, or mere marker of microglial activation and secondary injury after TBI. However, what can be concluded is that Aβ is an excellent indicator of microglial activation following TBI.

4. Conclusions and Future Directions

The link between trauma, microglial activation, and Aβ is likely to be extremely complex, and work in this field remains

in its infancy. Much of the work is derived from studies in AD, though the time course and long-term sequelae of TBI and AD may require separate lines of investigation. One recurring issue is the role of Aβ in the pathogenesis of microglial activation after brain injury which parallels the increasing emphasis on microglial function in the pathogenesis of AD.

Prior studies in AD suggest that microglial clearance of Aβ declines with aging [12]. It is therefore important to understand how TBI-induced Aβ alters long-term microglial function and Aβ-clearance. Future work should focus on whether blocking Aβ-genesis after TBI alters short- and long-term microglial activation. In addition, therapeutics targeting microglial-mediated Aβ clearance, such as the new AD therapeutic bexarotene [57], may hold promise as new modalities to treat TBI patients.

In this paper, we have attempted to show how a mechanistic understanding of the interaction of Aβ and microglia after TBI could have significant implications for therapeutics, especially for those at the highest risk for TBI, including those in the military and those who engage in contact sports. Furthermore, the advancement of drug discoveries in the field of TBI, such as sex steroids or Apoe mimetics which alter both microglial function and Aβ metabolism, may have potentially important roles in TBI as well as other neurodegenerative diseases. Finally, as we advance our mechanistic understanding of the interaction between microglia and Aβ after TBI, it is important to understand whether therapeutic interventions targeting microglia and Aβ will have any effect on long-term cognitive sequelae in TBI victims.

References

[1] V. E. Johnson, W. Stewart, and D. H. Smith, "Traumatic brain injury and amyloid-β pathology: a link to Alzheimer's disease?" *Nature Reviews Neuroscience*, vol. 11, no. 5, pp. 361–370, 2010.

[2] V. E. Johnson, W. Stewart, D. I. Graham, J. E. Stewart, A. H. Praestgaard, and D. H. Smith, "A neprilysin polymorphism and amyloid-β plaques after traumatic brain injury," *Journal of Neurotrauma*, vol. 26, no. 8, pp. 1197–1202, 2009.

[3] J. A. Mortimer, L. R. French, J. T. Hutton, and L. M. Schuman, "Head injury as a risk factor for Alzheimer's disease," *Neurology*, vol. 35, no. 2, pp. 264–267, 1985.

[4] A. B. Graves, E. White, T. D. Koepsell et al., "The association between head trauma and Alzheimer's disease," *American Journal of Epidemiology*, vol. 131, no. 3, pp. 491–501, 1990.

[5] J. A. Mortimer, C. M. van Duijn, V. Chandra et al., "Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group," *International Journal of Epidemiology*, vol. 20, supplement 2, pp. S28–S35, 1991.

[6] E. Salib and V. Hillier, "Head injury and the risk of Alzheimer's disease: a case control study," *International Journal of Geriatric Psychiatry*, vol. 12, pp. 363–368, 1997.

[7] Z. Guo, L. A. Cupples, A. Kurz et al., "Head injury and the risk of AD in the MIRAGE study," *Neurology*, vol. 54, no. 6, pp. 1316–1323, 2000.

[8] G. W. Roberts, S. M. Gentleman, A. Lynch, and D. I. Graham, "βA4 amyloid protein deposition in brain after head trauma," *The Lancet*, vol. 338, no. 8780, pp. 1422–1423, 1991.

[9] G. W. Roberts, S. M. Gentleman, A. Lynch, L. Murray, M. Landon, and D. I. Graham, "β amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 57, no. 4, pp. 419–425, 1994.

[10] M. T. Giordana, A. Attanasio, P. Cavalla, A. Migheli, M. C. Vigliani, and D. Schiffer, "Reactive cell proliferation and microglia following injury to the rat brain," *Neuropathology and Applied Neurobiology*, vol. 20, no. 2, pp. 163–174, 1994.

[11] W. J. Streit, "The role of microglia in brain injury," *NeuroToxicology*, vol. 17, no. 3-4, pp. 671–678, 1996.

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

[13] S. Mandrekar, Q. Jiang, C. Y. D. Lee, J. Koenigsknecht-Talboo, D. M. Holtzman, and G. E. Landreth, "Microglia mediate the clearance of soluble aβ through fluid phase macropinocytosis," *The Journal of Neuroscience*, vol. 29, no. 13, pp. 4252–4262, 2009.

[14] J. C. M. Schlachetzki and M. Hüll, "Microglial activation in Alzheimer's disease," *Current Alzheimer Research*, vol. 6, no. 6, pp. 554–563, 2009.

[15] S. Engel, H. Schluesener, M. Mittelbronn et al., "Dynamics of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage-related proteins MRP8 and MRP14," *Acta Neuropathologica*, vol. 100, no. 3, pp. 313–322, 2000.

[16] S. M. Gentleman, P. D. Leclercq, L. Moyes et al., "Long-term intracerebral inflammatory response after traumatic brain injury," *Forensic Science International*, vol. 146, no. 2-3, pp. 97–104, 2004.

[17] T. K. McIntosh, K. E. Saatmann, R. Raghupathi et al., "The Dorothy Russell memorial lecture. The molecular and cellular sequelae of experimental traumatic brain injury: pathogenetic mechanisms," *Neuropathology and Applied Neurobiology*, vol. 24, no. 4, pp. 251–267, 1998.

[18] D. Davalos, J. Grutzendler, G. Yang et al., "ATP mediates rapid microglial response to local brain injury in vivo," *Nature Neuroscience*, vol. 8, no. 6, pp. 752–758, 2005.

[19] R. Beschorner, T. D. Nguyen, F. Gözalan et al., "CD14 expression by activated parenchymal microglia/macrophages and infiltrating monocytes following human traumatic brain injury," *Acta Neuropathologica*, vol. 103, no. 6, pp. 541–549, 2002.

[20] A. F. Ramlackhansingh, D. J. Brooks, R. J. Greenwood et al., "Inflammation after trauma: microglial activation and traumatic brain injury," *Annals of Neurology*, 2011.

[21] E. Csuka, V. H. J. Hans, E. Ammann, O. Trentz, T. Kossmann, and M. C. Morganti-Kossmann, "Cell activation and inflammatory response following traumatic axonal injury in the rat," *NeuroReport*, vol. 11, no. 11, pp. 2587–2590, 2000.

[22] J. Maeda, M. Higuchi, M. Inaji et al., "Phase-dependent roles of reactive microglia and astrocytes in nervous system injury as delineated by imaging of peripheral benzodiazepine receptor," *Brain Research*, vol. 1157, no. 1, pp. 100–111, 2007.

[23] J. Gehrmann, "Microglia: a sensor to threats in the nervous system?" *Research in Virology*, vol. 147, no. 2-3, pp. 79–88, 1996.

[24] M. Koshinaga, Y. Katayama, M. Fukushima, H. Oshima, T. Suma, and T. Takahata, "Rapid and widespread microglial activation induced by traumatic brain injury in rat brain slices," *Journal of Neurotrauma*, vol. 17, no. 3, pp. 183–192, 2000.

[25] C. Israelsson, H. Bengtsson, A. Kylberg et al., "Distinct cellular patterns of upregulated chemokine expression supporting a prominent inflammatory role in traumatic brain injury," *Journal of Neurotrauma*, vol. 25, no. 8, pp. 959–974, 2008.

[26] J. F. Stover, B. Schöning, T. F. Beyer, C. Woiciechowsky, and A. W. Unterberg, "Temporal profile of cerebrospinal fluid glutamate, interleukin-6, and tumor necrosis factor-α in relation to brain edema and contusion following controlled cortical impact injury in rats," *Neuroscience Letters*, vol. 288, no. 1, pp. 25–28, 2000.

[27] K. T. Lu, Y. W. Wang, J. T. Yang, Y. L. Yang, and H. I. Chen, "Effect of interleukin-1 on traumatic brain injury-induced damage to hippocampal neurons," *Journal of Neurotrauma*, vol. 22, no. 8, pp. 885–895, 2005.

[28] E. Pinteaux, L. C. Parker, N. J. Rothwell, and G. N. Luheshi, "Expression of interleukin-1 receptors and their role in interleukin-1 actions in murine microglial cells," *Journal of Neurochemistry*, vol. 83, no. 4, pp. 754–763, 2002.

[29] N. Rothwell, "Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential," *Brain, Behavior, and Immunity*, vol. 17, no. 3, pp. 152–157, 2003.

[30] E. Csuka, M. C. Morganti-Kossmann, P. M. Lenzlinger, H. Joller, O. Trentz, and T. Kossmann, "IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-α, TGF-β1 and blood-brain barrier function," *Journal of Neuroimmunology*, vol. 101, no. 2, pp. 211–221, 1999.

[31] S. M. Knoblach and A. I. Faden, "Interleukin-10 improves outcome and alters proinflammatory cytokine expression after experimental traumatic brain injury," *Experimental Neurology*, vol. 153, no. 1, pp. 143–151, 1998.

[32] S. G. Kremlev and C. Palmer, "Interleukin-10 inhibits endotoxin-induced pro-inflammatory cytokines in microglial cell cultures," *Journal of Neuroimmunology*, vol. 162, no. 1-2, pp. 71–80, 2005.

[33] Y. Hamada, T. Ikata, S. Katoh et al., "Effects of exogenous transforming growth factor-β1 on spinal cord injury in rats," *Neuroscience Letters*, vol. 203, no. 2, pp. 97–100, 1996.

[34] W. R. Tyor, N. Avgeropoulos, G. Ohlandt, and E. L. Hogan, "Treatment of spinal cord impact injury in the rat with transforming growth factor-β," *Journal of the Neurological Sciences*, vol. 200, no. 1-2, pp. 33–41, 2002.

[35] J. Rogers and L. F. Lue, "Microglial chemotaxis, activation, and phagocytosis of amyloid β-peptide as linked phenomena in Alzheimer's disease," *Neurochemistry International*, vol. 39, no. 5-6, pp. 333–340, 2001.

[36] M. D. Ikonomovic, K. Uryu, E. E. Abrahamson et al., "Alzheimer's pathology in human temporal cortex surgically excised after severe brain injury," *Experimental Neurology*, vol. 190, no. 1, pp. 192–203, 2004.

[37] A. Iwata, X. H. Chen, T. K. McIntosh, K. D. Browne, and D. H. Smith, "Long-term accumulation of amyloid-β in axons following brain trauma without persistent upregulation of amyloid precursor protein genes," *Journal of Neuropathology and Experimental Neurology*, vol. 61, no. 12, pp. 1056–1068, 2002.

[38] K. Uryu, H. Laurer, T. McIntosh et al., "Repetitive mild brain trauma accelerates Abeta deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis," *The Journal of Neuroscience*, vol. 22, pp. 446–454, 2002.

[39] D. J. Loane, A. Pocivavsek, C. E. H. Moussa et al., "Amyloid precursor protein secretases as therapeutic targets for traumatic brain injury," *Nature Medicine*, vol. 15, no. 4, pp. 377–379, 2009.

[40] R. C. Mannix, J. Zhang, J. Park et al., "Age-dependent effect of apolipoprotein E4 on functional outcome after controlled cortical impact in mice," *Journal of Cerebral Blood Flow and Metabolism*, vol. 31, no. 1, pp. 351–361, 2011.

[41] I. Blasko, R. Beer, M. Bigl et al., "Experimental traumatic brain injury in rats stimulates the expression, production and activity of Alzheimer's disease β-secretase (BACE-1)," *Journal of Neural Transmission*, vol. 111, no. 4, pp. 523–536, 2004.

[42] X. H. Chen, R. Siman, A. Iwata, D. F. Meaney, J. Q. Trojanowski, and D. H. Smith, "Long-term accumulation of amyloid-β, β-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma," *American Journal of Pathology*, vol. 165, no. 2, pp. 357–371, 2004.

[43] Y. Nadler, A. Alexandrovich, N. Grigoriadis et al., "Increased expression of the γ-secretase components presenilin-1 and nicastrin in activated astrocytes and microglia following traumatic brain injury," *GLIA*, vol. 56, no. 5, pp. 552–567, 2008.

[44] Y. F. Liaoi, B. J. Wang, H. T. Cheng, L. H. Kuo, and M. S. Wolfe, "Tumor necrosis factor-α, interleukin-1β, and interferon-γ stimulate γ-secretase-mediated cleavage of amyloid precursor protein through a JNK-dependent MAPK pathway," *Journal of Biological Chemistry*, vol. 279, no. 47, pp. 49523–49532, 2004.

[45] W. S. T. Griffin, J. G. Sheng, S. M. Gentleman, D. I. Graham, R. E. Mrak, and G. W. Roberts, "Microglial interleukin-1α expression in human head injury: correlations with neuronal and neuritic P-amyloid precursor protein expression," *Neuroscience Letters*, vol. 176, no. 2, pp. 133–136, 1994.

[46] X. H. Chen, V. E. Johnson, K. Uryu, J. Q. Trojanowski, and D. H. Smith, "A lack of amyloid β plaques despite persistent accumulation of amyloid β in axons of long-term survivors of traumatic brain injury," *Brain Pathology*, vol. 19, no. 2, pp. 214–223, 2009.

[47] R. O. Sanchez Mejia, V. O. Ona, M. Li, and R. M. Friedlander, "Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction," *Neurosurgery*, vol. 48, no. 6, pp. 1393–1401, 2001.

[48] N. Bye, M. D. Habgood, J. K. Callaway et al., "Transient neuroprotection by minocycline following traumatic brain injury is associated with attenuated microglial activation but no changes in cell apoptosis or neutrophil infiltration," *Experimental Neurology*, vol. 204, no. 1, pp. 220–233, 2007.

[49] E. Siopi, A. H. Cho, S. Homsi et al., "Minocycline restores sAPPalpha levels and reduces the late histopathological consequences of traumatic brain injury in mice," *Journal of Neurotrauma*, vol. 28, pp. 2135–2143, 2011.

[50] A. B. Jaffe, C. D. Toran-Allerand, P. Greengard, and S. E. Gandy, "Estrogen regulates metabolism of Alzheimer amyloid β precursor protein," *The Journal of Biological Chemistry*, vol. 269, no. 18, pp. 13065–13068, 1994.

[51] E. Kojro, G. Gimpl, S. Lammich, W. März, and F. Fahrenholz, "Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the α-secretase ADAM 10," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 10, pp. 5815–5820, 2001.

[52] B. S. Kasturi and D. G. Stein, "Progesterone decreases cortical and sub-cortical edema in young and aged ovariectomized rats with brain injury," *Restorative Neurology and Neuroscience*, vol. 27, no. 4, pp. 265–275, 2009.

[53] B. Li, A. Mahmood, D. Lu et al., "Simvastatin attenuates microglial cells and astrocyte activation and decreases interleukin-1B level after traumatic brain injury," *Neurosurgery*, vol. 65, no. 1, pp. 179–185, 2009.

[54] H. Wang, L. Durham, H. Dawson et al., "An apolipoprotein E-based therapeutic improves outcome and reduces Alzheimer's

disease pathology following closed head injury: evidence of pharmacogenomic interaction," *Neuroscience*, vol. 144, no. 4, pp. 1324–1333, 2007.

[55] E. E. Abrahamson, M. D. Ikonomovic, C. Edward Dixon, and S. T. DeKosky, "Simvastatin therapy prevents brain trauma-induced increases in β-amyloid peptide levels," *Annals of Neurology*, vol. 66, no. 3, pp. 407–414, 2009.

[56] D. J. Loane, P. M. Washington, L. Vardanian et al., "Modulation of ABCA1 by an LXR agonist reduces beta-amyloid levels and improves outcome after traumatic brain injury," *Journal of Neurotrauma*, vol. 28, no. 2, pp. 225–236, 2011.

[57] P. E. Cramer, J. R. Cirrito, D. W. Wesson et al., "ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models," *Science*, vol. 335, no. 6075, pp. 1503–1506, 2012.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 891087, 11 pages
doi:10.1155/2012/891087

Review Article

Sweepers in the CNS: Microglial Migration and Phagocytosis in the Alzheimer Disease Pathogenesis

Mariko Noda and Akio Suzumura

Department of Neuroimmunology, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

Correspondence should be addressed to Mariko Noda, noda@riem.nagoya-u.ac.jp

Received 11 January 2012; Accepted 2 March 2012

Academic Editor: Lee-Way Jin

Microglia are multifunctional immune cells in the central nervous system (CNS). In the neurodegenerative diseases such as Alzheimer's disease (AD), accumulation of glial cells, gliosis, occurs in the lesions. The role of accumulated microglia in the pathophysiology of AD is still controversial. When neuronal damage occurs, microglia exert diversified functions, including migration, phagocytosis, and production of various cytokines and chemokines. Among these, microglial phagocytosis of unwanted neuronal debris is critical to maintain the healthy neuronal networks. Microglia express many surface receptors implicated in phagocytosis. It has been suggested that the lack of microglial phagocytosis worsens pathology of AD and induces memory impairment. The present paper summarizes recent evidences on implication of microglial chemotaxis and phagocytosis in AD pathology and discusses the mechanisms related to chemotaxis toward injured neurons and phagocytosis of unnecessary debris.

1. Introduction

Microglia are macrophage-like resident immune cells in the central nervous system (CNS) and possess both neurotoxic and neuroprotective function. Microglia accumulate in the lesions of a variety of neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease, and multiple sclerosis, and are thought to play both toxic and protective functions for neuronal survival [1]. Microglia are considered to be a first line defense and respond quickly to various stimuli. When activated, microglia undergo morphological changes to ameboid, proliferate, migrate toward injured areas, and release many soluble factors and phagocytosis of foreign substances or unwanted self-debris. Appropriate migration of microglia to damaged area is controlled by chemokines and nucleotide ATP [2, 3]. Phagocytosis seems to be important to prevent the senile plaque expansion in AD by removing amyloid β (Aβ) deposit [4]. Microglia not only engulf the Aβ protein but also phagocytose apoptotic cells and degenerated neuronal debris. Phagocytosis of apoptotic or degenerated neuronal debris is crucial to reduce inflammation and maintain healthy neuronal networks. Another type of phagocytosis, phagocytosis with inflammation, occurs in chronic inflammatory-related neurodegenerative disorders including Alzheimer disease [5–7].

Degenerated neurons releases several signaling molecules, including nucleotides, cytokines, and chemokines, to recruit microglia and enhance their activities [8, 9]. The phenomenon are now termed as find-me, eat-me, and help-me signals. In this paper, we focused on find-me, and eat-me signals from degenerated neurons to microglia. Most distinguished and examined eat-me signal is phosphatidylserine (PS), which is a component of cellular membrane and is everted on apoptotic cell membrane [10, 11]. Nucleotides are also considered as the eat-me signal lately; microglia expresse various P2X and P2Y receptors, nucleotide receptors, which regulate not only chemotaxis but also phagocytosis [8, 12].

Microglia express many other surface receptors, which have direct interaction with the target to initiate phagocytosis, including PS receptor [6], lipopolysaccharide (LPS) receptor CD14 [13], the scavenger receptor CD36 [14], the purine receptor P2Y6 [8], and the toll-like receptors (TLRs) [15] (Table 1, Figure 1). Another surface receptor, the CX3C

Table 1: Various chemotaxis or phagocytosis-related receptors in microglia and its ligand(s) or interacted factors. Microglia are activated with various stimuli through the specific receptor of each stimuli. For detailed review of chemokines, pathogens, and factors associated with tissue damage recognized by microglia, refer to [19–21].

Receptor type	Subtypes	Ligand(s)/interacted factors
Chemokine receptor	CCR1	CCL3 (MIP-1α), CCL5 (RANTES), CCL7 (MCP-3), CCL9 (MIP-1γ), CCL14 (HCC-1), CCL15 (HCC-2/leukotactin-1), CCL16 (HCC-4/LEC), CCL23 (MPIF-1)
	CCR2	CCL2 (MCP-1), CCL7 (MCP-3), CCL8 (MCP-2), CCL13 (MCP-4), CCL16 (HCC-4/LEC)
	CCR5	CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES)
	CXCR3	CXCL9 (Mig), CXCL10 (IP-10), CXCL11 (I-TAC)
	CX3CR1	CX3CL1 (Fractalkine)
Purinergic receptor	P2X4, P2Y7, P2Y12	ATP, ADP
	P2Y6	UDP
TLR	TLR1	Triacyl lipopeptides
	TLR2	Glycolipids, Hsp70, HMGB1, Aβ
	TLR4	LPS, Hsp
	TLR6	Diacyl lipopeptides
	TLR9	CpG-DNA
Phosphatidylserine (PS) receptor	MFG-E8, Tim1, Tim4	PS of apoptotic cells
	RAGE	Aβ, AGE, HMGB1 PS of apoptotic cells
Scavenger receptor (SR)	SR-AI/II (CD204), SR-BI, CD36	Cellular debris, apoptotic cells, Aβ (CD36)
Immunoglobulin (Ig) receptor	FcγRI, FcγRIII	Ig-opsonized particles
Complement receptor (CR)	CR3 (MAC-1; CD11b/CD18)	Complement components, opsonized particles
Other phagocytosis-related receptor	CD14	LPS, Aβ
	CD47	SIRPα (CD172a)
	CD200R	CD200
	TREM2	Hsp60, DAP12

chemokine fractalkine receptor CX3CR1, is almost exclusively expressed in microglia throughout the CNS, which is involved in progression of neurodegenerative disease by altering microglial activities [16, 17] (Figure 2). Deletion of CX3CR1 expression in microglia results in progressive neuronal cell death in an animal model of neurodegenerative disease, by inducing microglial dysfunction. It has been identified recently that neurons themselves produce cytokine and chemokine, such as fractalkine. As shown in Figure 2, we previously reported that interleukin-34 (IL-34), a newly discovered cytokine, is produced by neurons, and that its receptor, colony-stimulating factor 1 receptor, is primarily expressed on microglia [18]. Fractalkine and IL-34 might be important mediator between neurons and microglia, and it is important to clarify this cellular crosstalk signaling pathways for seeking future therapeutic target of neurodegenerative diseases including AD. In the following sections, we will discuss about recent advances of microglial chemotaxis and phagocytosis and their implications for AD therapy.

2. Microglial Chemotaxis in CNS Injury

Chemokine and its receptors are expressed in broad area of the CNS. The expression levels were increased under pathological conditions, which seem to facilitate the recruitment and trafficking of glial cells to the damaged area [22]. Microglia constitutively express several chemokine receptors (Table 1), which are implicated in the recruitment and accumulation microglia in AD lesions. When exposed to Aβ, microglia are induced to produce several chemokines, such as CCL2, CCL4, and CXCL12 [23]. CCR2, the receptor of CCL2, deficiency resulted in reduction of microglial accumulation and higher brain Aβ levels in mouse model of AD, which might be mediated via suppression of anti-inflammatory molecule, transforming growth factor β (TGF-β) [3]. However, there is a conflicting report showing increased TGF-β signaling in microglia surrounding Aβ plaques in CCR2 knockout in AD model mouse (APP_{Swe}/PS1/CCR2$^{-/-}$) [24]. The other chemokine receptor CX3CR1 expression in microglia was also increased in the mice. This AD model has been shown to have accumulation of oligomeric Aβ and memory impairment [24]. CX3CR1 is the sole receptor of CX3C chemokine CX3CL1 (fractalkine). The roles of CX3CL1-CX3CR1 signaling on AD pathology are discussed in next section. CCL2 expression level is also related to another neurodegenerative disorder, multiple sclerosis (MS); CCL2 level is downregulated in cerebrospinal fluid from MS patients [25].

CCL21 is a neuronal chemokine, expressed in neurons. Expression of CCL21 is upregulated in neurons undergoing degeneration [26, 27]. CCL21 triggers chemotaxis of microglia through CXCR3, but not CCR7 which implicated in peripheral lymphoid organs [28].

CXCR3-CXCL10 interaction is also implicated in microglial migration [29]. CXCR3 knockout mice reveal impairment of the microglial migration but no change in proliferation. CXCL10 is also expressed in neurons. CXCL10 and CCL21 synergistically induce microglial homing through

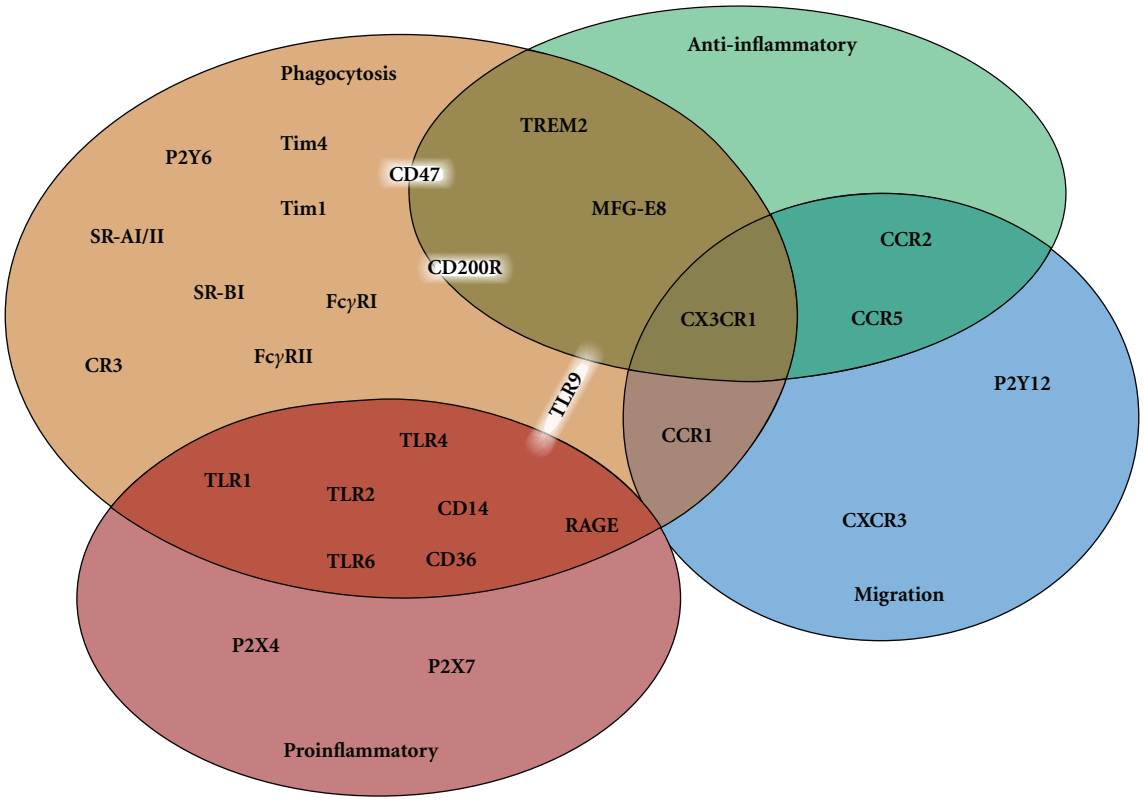


Figure 1: Chemotaxis or phagocytosis-involved receptors in microglia and correlation of the inflammatory or anti-inflammatory response. Many of the receptors correlated with microglial activities of chemotaxis (migration) or phagocytosis, respectively. Among these, some of the receptors possess not merely single function; CCR1 is the migration-inducing receptor that also possesses phagocytotic activity. CCR2 and CCR5 are also the migration-inducting receptors that lead to anti-inflammatory response. CX3CR1 contributes to migration, phagocytosis, and anti-inflammatory response. TREM2 and MFG-E8 induce phagocytosis and anti-inflammatory response. CD47 and CD200R usually induce phagocytosis under pathological condition, so that they indirectly contribute to anti-inflammatory status. TLR9 activates microglia to induce phagocytosis with producing proinflammatory and anti-inflammatory molecules. There are receptors inducing not only phagocytosis but also inflammatory response (CD14, CD36, RAGE, TLR1, TLR2, TLR4, and TLR6). Within these receptors that the synergistic signaling involved Aβ-triggering inflammatory response are CD14-TLR2-TLR4 and CD36-TLR2-TLR6.

the receptor CXCR3 [30]. CXCL10 inhibits CCL21-induced migration in microglia through CXCR3 [31]. Neuronal CCL21 upregulates P2X4 receptor, the nucleotide receptor, expressed in microglia [32]. This cascade is implicated in pathophysiology of tactile allodynia to cause chronic neuropathic pain.

CCR5 also plays a role in neuronal survival [33]. In ischemic stroke models, brain damage is severer by CCR5 deficiency [34].

Inter- and intracellular transmitter nucleotides can influence microglial migration and phagocytosis. Microglia express various nucleotide receptors, P2X and P2Y receptors (Table 1) [35]. Aβ-induced microglial activation is mediated through P2X7 receptor that is reported as proinflammatory response conductive receptor [36]. ATP predominantly induced microglial migration among nucleotides through P2Y receptors, especially P2Y12 [2, 37]. Following CNS injury, expression of P2Y12 in microglia drastically reduced after microglial activation, suggesting that P2Y12 is a primary and temporary receptor to induce microglial chemotaxis at early stages of the local CNS injury [37]. The other nucleotide UDP increases mainly microglial phagocytosis (uptake of microspheres) via P2Y6 receptor [8]. In the condition brain damage by kainic acid administration, the P2Y6 receptor is upregulated and can act as a sensor for phagocytosis [38].

3. CX3CL1-CX3CR1

The CX3C chemokine CX3CL1 (fractalkine, also called as neurotactin), which has been identified as two forms, soluble or membrane-anchored forms, plays a pivotal role in signaling between degenerating neurons and microglia [39]. CX3CL1 and its receptor CX3CR1 are highly expressed in brain tissue, particularly in neurons and microglia [40–42]. CX3CL1 directly induces various microglial functions including migration [41], proliferation [43], inhibition of Fas-ligand-induced cell death [44] and glutamate-induced neurotoxicity [45, 46], and inhibition of proinflammatory cytokine production [42, 47]. Recently, we have shown that soluble form of CX3CL1 also directly enhances microglial clearance of degenerated neuronal debris, which is mediated

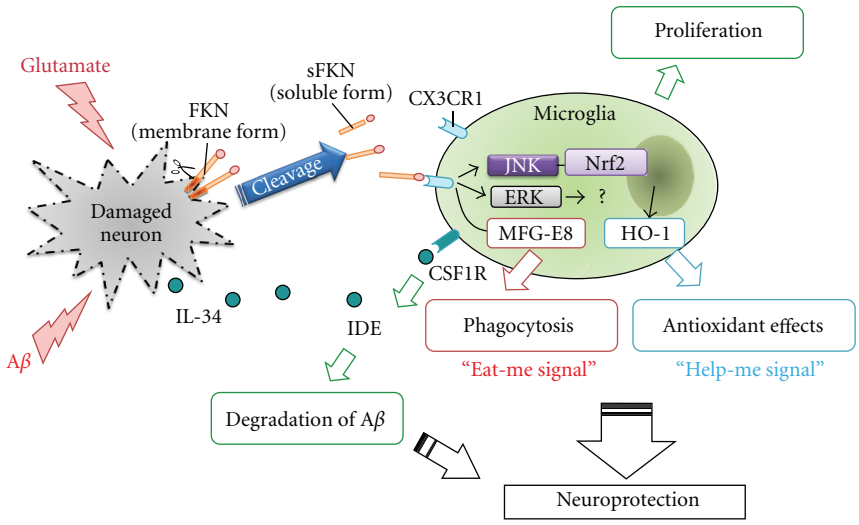


Figure 2: Model of the role of neuronal chemokine (FKN) and neuronal cytokine (IL-34) in microglial phagocytosis and neuroprotection. Neuronal cells primary produce chemokine fractalkine (CX3CL1; FKN) and cytokine IL-34. Microglia predominantly express its receptor, CX3CR1, and colony-stimulating factor 1 receptor (CSF1R). Soluble form of FKN (sFKN) is secreted from damaged neurons and promotes microglial phagocytosis of neuronal debris through the release of MFG-E8. sFKN also induces the expression of the antioxidant enzyme HO-1 in microglia via Nrf2 recruitment and activation of the JNK MAPK signaling pathway. The neuroprotective effects of sFKN are also mediated in part by activation of ERK MAPK, although the downstream signaling pathway has not yet been elucidated. IL-34 promoted microglial proliferation and clearance of Aβ which mediates insulin-degrading enzyme (IDE) expression. Therefore, sFKN and IL-34 may be an intrinsic neuroprotectant for damaged yet surviving neurons.

through phosphatidylserine (PS) receptor and production of Milk fat globule-EGF factor 8 protein (MFG-E8) [46] (Figure 2). The source of soluble form of CX3CL1 is neuron. The membrane-anchored CX3CL1 is cleaved by several proteases including a disintegrin and metalloprotease (ADAM) family, ADAM-10 and ADAM-17 [48–50], and cathepsin S [51]. When neurons are injured or exposed to glutamate, shedding of CX3CL1 occurs immediately [41, 52]. However, little is known about direct connection with Aβ-induced neuronal toxicity and the CX3CL1-shedding.

Another important function of ADAM family enzyme, except for CX3CL1 shedding, is an α-secretase. It cleaves APP in the centre of the Aβ domain, and generated α-APP is considered to have neurotrophic function [53–56]. The ADAMs include ADAM-9, ADAM-10, and ADAM-17 [57, 58]. Cathepsin S is expressed predominantly in microglia and implicated in microglial activation of neuropathic pain [51]. Therefore, these CX3CL1-shedding proteases may regulate the microglial phagocytosis directly or indirectly through CX3CL1 expression, and may also play a role on pathogenesis of AD.

Microglia respond to CX3CL1 through CX3CR1. In a previous study, we have shown that CX3CL1 functions neuroprotective against activated microglia-induced neurotoxicity [46]. There are some reports showing that CCL2 activates CX3CR1 expression, and the induction of CX3CR1 expression by CCL2-CCR2 axis is mediated through p38 MAPK activation [59]. CX3CR1 deficiency increases susceptibility to neurotoxicity in mouse models of Parkinson's disease, amyotrophic lateral sclerosis, and systemic LPS administration [16]. CX3CL1-induced neuroprotection in a rat model of Parkinson's disease has also been reported recently [60]. In addition, there are some reports showing that pathologic features of AD mouse model are worsened by knockout of CX3CR1 [17, 61].

4. Microglial Receptors Involving Phagocytosis with or without Inflammation: Possible Implication to AD Pathology

Microglial phagocytosis is classified into two categories, with or without inflammation [62]. Some receptors are involved in both types of phagocytosis (Table 1). The receptor leading to inflammatory status includes CD14, CD36, the receptor for advanced glycation end products (RAGEs), and toll-like receptor (TLR) 1, TLR2, TLR4, and TLR6. The receptor leading to anti-inflammatory status includes triggering receptor expressed on myeloid cells 2 (TREM2) and PS receptor (PSR), MFG-E8. Microglia also express many other phagocytosis-related receptors which are not yet unclear to the correlation of inflammatory status.

CR3. Microglia express classical phagocytosis-related receptor, the $\alpha_M\beta_2$ integrin complement-receptor-3 (CR3; MAC-1; CD11b/CD18), and scavenger-receptor (SR)-AI/II (SR-AI/II; CD204). CR3 synergistically activated SR-AI/II-mediated myelin phagocytosis by microglia [63, 64]. CR3 is implicated in clearance of bacteria through induction of major histocompatibility complex class II (MHC II) antigens [65]. CR3 is involved in endocytosis in normal conditions and MHC II antigens in an inflammatory state. Microglia express SR-AI/II and SR-BI during neonatal period, while in adult they lack the expression. However SR-AI/II expression in microglia is upregulated in AD [66].

CD14. CD14 is the LPS receptor which is also considered as classical phagocytosis-related receptor in macrophage and microglia [67]. CD14-mediated phagocytosis of apoptotic cells occurred in both normal and inflammatory conditions [67]. CD14-mediated phagocytosis does not require expression of PS receptor and possibly induces inflammatory conditions through activation of CD14 signaling [68]. CD14 also contributes phagocytosis of Aβ by microglia [13]. However, deletion of CD14 reportedly attenuates pathological features of AD mouse model. The authors suggested that it might be due to reduction of insoluble, but not soluble, Aβ [69].

FcγReceptor. Exposure of fibrillar Aβ to microglia induces phagocytosis through the receptors distinct from those used by the classical phagocytosis: immunoglobulin receptors (FcγRI and FcγRIII) or complement receptors [70].

CD36 and CD47. Aβ directly interacted with microglial cell surface receptor complex comprising the class B scavenger receptor CD36, $\alpha_6\beta_1$ integrin, and integrin-associated protein CD47, all of them involved in migration and phagocytosis of microglia [70–72]. CD36 is required for fibrillar Aβ-induced chemotaxis and proinflammatory molecules including reactive oxygen species (ROS), TNFα, IL-1β, and several chemokines in microglia [23]. Aβ activates microglial recruitment to amyloid deposition site through CD36-dependent signaling cascade involving the Src kinase family members, Lyn and Fyn, and the ERK1/2 [73]. CD47 is a membrane glycoprotein, broadly expressed in the various cell types in the CNS, including neurons and microglia. Signal regulatory protein-α (SIRPα; CD172a) is a receptor binding to CD47, which is also expressed on neurons and myeloid cells. SIRPα is an inhibitory molecule of CD47 that downregulates MAPK phosphorylation which is a downstream pathway of CD47 [74]. SIRPα interacts with the protein tyrosine phosphatases SHP-1 and SHP-2, which are predominantly expressed in neurons, dendritic cells, and macrophages [75]. Intact myelin expresses CD47 to suppress myelin phagocytosis by microglia via SIRPα-CD47 interaction [76]. Therefore, CD47 functions normally as a marker of "self" to protect intact body component [76, 77].

RAGE. In has been reported that the direct interaction of Aβ peptide with the receptor for RAGE is important in AD pathophysiology. In AD brains, RAGE expression is increased, and RAGE directly induces neurotoxicity by production of oxidative stressors and indirectly by activating microglia [78]. RAGE increases macrophage-colony stimulating factor (M-CSF) from neurons via nuclear-factor-κB- (NF-κB-) dependent pathway and released M-CSF induced interaction of cognate receptor c-fms on microglia which prolongs survival of microglia [79]. RAGE recognizes multiple ligands other than Aβ peptide, such as advanced glycation end products (AGEs), PS, and high-mobility group box 1 protein (HMGB1) [80, 81]. These molecules act as an agonist of RAGE on microglia, by inducing proinflammatory molecules, such as NO, TNF-α, IL-1β, and IL-6, via MAP-kinase-kinase (MEK) and phosphatidylinositol 3-kinase (PI3K) pathways [81]. Activation of RAGE leaded to NF-κB and MAPK-mediated signaling to propagate and perpetuate inflammation status [82]. RAGE also mediates the transport of peripheral Aβ into the brain across the blood-brain barrier (BBB) [83].

CD200R. CD200 is a transmembrane glycoprotein and is expressed on many different cell types including neurons, endothelial cells, lymphocytes, and dendritic cells [84]. The receptor of CD200, CD200R expression, is predominant in myeloid cells, macrophages, and microglia [84, 85]. As in the case of SIRPα-CD47, CD200 exerts inhibitory effect on CD200R, so that CD200-CD200R interaction can downregulate activity of microglia. In retina, it has been shown that activation of CD200R in microglia does not show direct effect on migration, but CD200-CD200R signaling restores LPS/IFNγ-induced loss of migration [86]. CD200 knockout leads to an expansion of the myeloid population in several tissues and increased expression of the activation markers in microglia, including the signaling adaptor protein DNAX-activating protein of 12 kDa (DAP12), CD11b, CD45, CD68, and inducible NO synthase (iNOS) [87]. Blocking of CD200R increases neurodegeneration in mouse model of Parkinson's disease [88]. These observations suggest that CD200-CD200R signaling leads to anti-inflammatory state and protection against neurotoxic stimuli. CD200 and CD200R expression levels (neurons and microglia, resp.) are decreased in AD hippocampus and inferior temporal gyrus, indicating that inhibition of CD200-CD200R axis contributes to AD pathology [89].

TREM2. TREM2, a recently identified innate immune receptor, and its adaptor protein DAP12 are expressed on microglia and cortical neurons, but not on hippocampal neurons, astrocytes, and oligodendrocytes. Their expression correlates with clearance of apoptotic neurons by microglia without inflammation [90–92]. However, endogenous ligand or specific agonist of TREM2 had not been identified until recently. Heat shock protein 60 (Hsp60) is a mitochondrial chaperone, which interacts with TREM2 directly. Hsp60-induced phagocytosis is only found in microglia which have robust expression of TREM2 [93]. In AD model mouse, TREM2 expression was highest in the outer zone in Aβ plaques, and the expression level correlated with the size of Aβ plaque [94]. Forced expression of TREM2 positively

regulated microglial phagocytosis, the ability of microglia to stimulate $CD4^+$ T-cell proliferation, TNF-α and CCL2 production, but not IFNγ production [94].

5. TLRs

TLRs are class of pattern-recognition receptors in the innate immune system to induce inflammatory responses. 13 TLRs have been identified in human and mouse to date, except for TLR10 which is expressed only in human [95, 96]. TLRs may also contribute to the microglial inflammatory response to promote AD pathogenesis [15].

CD11b, a marker of macrophages and microglia, has been shown to interact with TLR signaling. CD11b knockout mouse exhibited enhanced TLR-mediated responses and subsequent more susceptibility to endotoxin shock [97].

Among the TLRs, LPS receptor TLR4 potently activates microglia in various aspects, such as Aβ phagocytosis and proinflammatory molecules production [98, 99]. Activation of TLR1, TLR2, TLR3, and TLR9 by each selective agonist also increased phagocytosis and several cytokines and chemokine production [98, 100]. CD14 is a coreceptor of TLR4. The response of microglia to fibrillar Aβ is mediated via CD14, which act together with TLR4 and TLR2 to bind fibrillar Aβ and induced microglial activation through p38 MAPK [101]. Deficiency of CD200 induces the expression of TLR2 and TLR4 in glial cells and proliferation of $CD11b^+/MHCII^+/CD40^+$ activated microglia [102]. These mice show memory impairment, suggesting that dual activation of TLR2 and TLR4 may induce an inflammatory phenotype of microglia which negatively regulate synaptic plasticity in the AD model. Aβ triggers the inflammatory status in microglia via heterodimer of TLR4 and TLR6, which is regulated by CD36 [103]. Therefore, CD14-TLR2-TLR4 and CD36-TLR4-TLR6 signaling are crucial to Aβ-induced inflammatory response, and also in microglial phagocytosis.

Bacterial DNA containing motifs of unmethylated CpG dinucleotides (CpG-DNA) is a ligand of TLR9, which is initially identified to activate microglia and strongly induces TNF-α and IL-12 production [104]. However, we have shown that CpG activated microglia to produce neuroprotective molecule, such as hemeoxygenase-1 (HO-1) and matrix metalloproteinase 9 (MMP-9) without producing neurotoxic molecules, such as TNF-α, glutamate and nitric oxide (NO), and enhanced Aβ clearance to protect memory disturbance *in vivo* [105]. There are the discrepancies about CpG effect between aforementioned two reports despite using the same origin cells (mouse primary microglia). It may be due to concentrations of this TLR9 ligand used: higher concentration (10 μM) in former report [104], whereas lower concentrations (1 to 100 nM) in latter report [105]. Moreover, the latter report revealed the difference on neuroprotective effect of CpG among synthetic oligodeoxynucleotides (ODNs) classes (A to C). Class A CpG did not activate microglia, but classes B and C CpGs increased microglial neuroprotective effect through induction of clearance of Aβ and production of neuroprotectant. These suggested that the CpG sequence-dependent microglial activation and responses are present.

CpG increased chemokine CCL9 and its receptor CCR1 expression in macrophages and microglia via TLR9/MyD88 signaling involving ERK, p38 MAPK, and PI3K pathways. Thus it can enhance microglial migration as well as phagocytosis [106].

6. PSR

Phagocytotic cells recognize apoptotic cells by several mechanisms, including recognition of PS expressed on the cells. PS is receiving much attention because it is responsible for phagocytosis without inducing inflammation [10]. The receptors of PS (PSRs) had not been clarified for a long time but have uncovered in recent years. These include MFG-E8 (the lactadherin homolog in humans) and T-cell immunoglobulin mucin domain 4 (Tim4) [107, 108]. These act as a bridge between PS-expressing apoptotic cells and PSR expressing phagocytes and trigger engulfment of cellular debris.

MFG-E8 is expressed on various macrophage subsets in the periphery and on microglia in the CNS. Recently, we have shown that CX3CL1 induces MFG-E8 expression in primary mouse microglia to lead to the microglial clearance of degenerated neuronal debris [46]. Others also reported the induction of MFG-E8 by CX3CL1 in macrophages and rat microglia [109, 110]. MFG-E8 bridges PS and integrins $\alpha_v\beta_3$ or $\alpha_v\beta_5$ on the surface of phagocytes [107, 111]. High-mobility group box 1 protein (HMGB1) is an intracellular protein that activates transcriptional factors, including p53 and NF-κB. HMGB1 reportedly suppresses the interaction between MFG-E8 and $\alpha_v\beta_3$ integrin in macrophage and inhibits the phagocytosis of apoptotic cells through ERK-mediated signaling pathway [112]. MFG-E8 may also be involved in Aβ phagocytosis, since its expression is reduced in AD [113]. We previously showed the neutralization of MFG-E8 progressed neuronal degeneration [46]. MFG-E8 reportedly induces anti-inflammation status in the periphery. Therefore, MFG-E8 may possibly lead to targeted clearance of unwanted molecules, such as Aβ, without inflammation.

The other well-studied PSR, Tim4, is expressed in $MAC\text{-}1^+$ cells in various mouse tissues, including spleen, lymph nodes, and fetal liver [108]. Among the other Tim family members, Tim1, but neither Tim2 nor Tim3, also specifically binds to PS. Tim1-PS subsequently connects with exosomes to recognize and engulf apoptotic cells [114–116].

It has been shown recently that RAGE also recognizes PS and induces apoptotic cell clearance [80]. However as mentioned previously, RAGE-guided intracellular signaling pathway induces prolonged inflammatory status.

7. γ-Secretase and Phagocytosis in AD Pathology

γ-secretase is a protein complex of four essential membrane proteins: aph-1, pen-2, nicastrin, and presenilin. A recent study suggests that presenilin increases microglial phagocytosis of Aβ, and this γ-secretase has dual role for AD pathogenesis: one is cleavage of amyloid precursor protein

(APP) to produce pathologic Aβ, and the other is reduction of microglial phagocytosis of Aβ by γ-secretase inhibitor [117]. Vertebrates have two presenilin genes, PSEN1 (located on chromosome 14 in humans; encoded presenilin 1) and PSEN2 (located on chromosome 1; encoded presenilin 2). There is a report that presenilin 2 is identified as the predominant γ-secretase in mouse microglia (but not presenilin 1), which repressed microglial activation via its function as a γ-secretase, and its expression is increased by inflammatory stimuli (IFN-γ) [118].

In order to explore the detailed mechanism how γ-secretase regulates microglial activity, further studies are needed since γ-secretase is a therapeutic target for AD. In traumatic injury of the brain, presenilin and nicastrin expressions are elevated in activated microglia and astrocytes [119]. γ-secretase mainly cleaves APP to lead to accumulation of A$\beta_{1\text{-}42}$, which then results in aggregation of Aβ protein to worsen AD pathology. However A$\beta_{1\text{-}42}$ is also a target of microglial phagocytosis.

8. Concluding Remarks

Microglia express a wide array of receptors characteristic to immune cell, such as CD molecules, integrins, chemokine receptors, and PSR (Figure 1). These receptors are involved in multiple functions of microglia. Chemokine receptors not only induce migration of microglia but also contribute directly to AD pathogenesis through regulation of phagocytosis and neuroprotective activity. PS acts as eat-me signal, and PSR-mediated phagocytosis so far is regarded as inducing anti-inflammatory responses. However, according to some recent reports, RAGE interacts with PSR and facilitates phagocytosis with robust inflammation status [80, 120]. Therefore, if the other phagocytosis-related receptors including TLRs interact with PSR, microglia would be activated to outbreak excessive phagocytosis with robust inflammation.

Microglia from old APP/PS1 mouse, but not from younger ones, show the reduction of SRA, CD36, RAGE, and the Aβ-degrading enzymes including insulysin, neprilysin, and MMP9 [121]. Dysfunction of microglia after progression of disease development may lead to neurodegeneration. Thus, it is important to consider the microglial status depending on the disease stage, to treat AD effectively. As shown in Figure 2, FKN and IL-34 may be an intrinsic neuroprotectant for damaged but still surviving neurons through activation of microglia. Therefore, it is important to elucidate neurons-microglia crosstalk in neurodegenerative condition.

References

[1] D. Farfara, V. Lifshitz, and D. Frenkel, "Neuroprotective and neurotoxic properties of glial cells in the pathogenesis of Alzheimer's disease," *Journal of Cellular and Molecular Medicine*, vol. 12, no. 3, pp. 762–780, 2008.

[2] S. Honda, Y. Sasaki, K. Ohsawa et al., "Extracellular ATP or ADP induce chemotaxis of cultured microglia through $G_{i/o}$-coupled P2Y receptors," *Journal of Neuroscience*, vol. 21, no. 6, pp. 1975–1982, 2001.

[3] J. El Khoury and A. D. Luster, "Mechanisms of microglia accumulation in Alzheimer's disease: therapeutic implications," *Trends in Pharmacological Sciences*, vol. 29, no. 12, pp. 626–632, 2008.

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

[5] J. J. Hoarau, P. Krejbich-Trotot, M. C. Jaffar-Bandjee et al., "Activation and control of CNS innate immune responses in health and diseases: a balancing act finely tuned by neuroimmune regulators (NIReg)," *CNS & neurological disorders drug targets*, vol. 10, no. 1, pp. 25–43, 2011.

[6] A. D. Fuller and L. J. van Eldik, "MFG-E8 regulates microglial phagocytosis of apoptotic neurons," *Journal of Neuroimmune Pharmacology*, vol. 3, no. 4, pp. 246–256, 2008.

[7] S. McArthur, E. Cristante, M. Paterno et al., "Annexin A1: a central player in the anti-inflammatory and neuroprotective role of microglia," *Journal of Immunology*, vol. 185, no. 10, pp. 6317–6328, 2010.

[8] S. Koizumi, Y. Shigemoto-Mogami, K. Nasu-Tada et al., "UDP acting at $P2Y_6$ receptors is a mediator of microglial phagocytosis," *Nature*, vol. 446, no. 7139, pp. 1091–1095, 2007.

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

[10] D. R. Green and H. M. Beere, "Apoptosis: gone but not forgotten," *Nature*, vol. 405, no. 6782, pp. 28–29, 2000.

[11] G. R. Sambrano and D. Steinberg, "Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 5, pp. 1396–1400, 1995.

[12] R. J. Horvath and J. A. DeLeo, "Morphine enhances microglial migration through modulation of $P2X_4$ receptor signaling," *Journal of Neuroscience*, vol. 29, no. 4, pp. 998–1005, 2009.

[13] Y. Liu, S. Walter, M. Stagi et al., "LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide," *Brain*, vol. 128, pp. 1778–1789, 2005.

[14] A. Stolzing and T. Grune, "Neuronal apoptotic bodies: phagocytosis and degradation by primary microglial cells," *The FASEB journal*, vol. 18, no. 6, pp. 743–745, 2004.

[15] G. E. Landreth and E. G. Reed-Geaghan, "Toll-like receptors in Alzheimer's disease," *Current Topics in Microbiology and Immunology*, vol. 336, no. 1, pp. 137–153, 2009.

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

[17] S. Lee, N. H. Varvel, M. E. Konerth et al., "CX_3CR1 deficiency alters microglial activation and reduces β-amyloid deposition in two Alzheimer's disease mouse models," *American Journal of Pathology*, vol. 177, no. 5, pp. 2549–2562, 2010.

[18] T. Mizuno, Y. Doi, H. Mizoguchi et al., "Interleukin-34 selectively enhances the neuroprotective effects of microglia to attenuate oligomeric amyloid-β neurotoxicity," *The American Journal of Pathology*, vol. 179, no. 4, pp. 2016–2027, 2011.

[19] U. K. Hanisch, "Microglia as a source and target of cytokines," *Glia*, vol. 40, no. 2, pp. 140–155, 2002.

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

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

[22] L. Cartier, O. Hartley, M. Dubois-Dauphin, and K. H. Krause, "Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases," *Brain Research Reviews*, vol. 48, no. 1, pp. 16–42, 2005.

[23] J. B. El Khoury, K. J. Moore, T. K. Means et al., "CD36 mediates the innate host response to β-amyloid," *Journal of Experimental Medicine*, vol. 197, no. 12, pp. 1657–1666, 2003.

[24] G. Naert and S. Rivest, "CC chemokine receptor 2 deficiency aggravates cognitive impairments and amyloid pathology in a transgenic mouse model of Alzheimer's disease," *Journal of Neuroscience*, vol. 31, no. 16, pp. 6208–6220, 2011.

[25] C. Savarin-Vuaillat and R. M. Ransohoff, "Chemokines and chemokine receptors in neurological disease: raise, retain, or reduce?" *Neurotherapeutics*, vol. 4, no. 4, pp. 590–601, 2007.

[26] E. K. de Jong, J. Vinet, V. S. Stanulovic et al., "Expression, transport, and axonal sorting of neuronal CCL21 in large dense-core vesicles," *The FASEB Journal*, vol. 22, no. 12, pp. 4136–4145, 2008.

[27] E. K. de Jong, I. M. Dijkstra, M. Hensens et al., "Vesicle-mediated transport and release of CCL21 in endangered neurons: a possible explanation for microglia activation remote from a primary lesion," *Journal of Neuroscience*, vol. 25, no. 33, pp. 7548–7557, 2005.

[28] A. Rappert, K. Biber, C. Nolte et al., "Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a Cl-current and chemotaxis in murine microglia," *Journal of Immunology*, vol. 168, no. 7, pp. 3221–3226, 2002.

[29] A. Rappert, I. Bechmann, T. Pivneva et al., "CXCR3-dependent microglial recruitment is essential for dendrite loss after brain lesion," *Journal of Neuroscience*, vol. 24, no. 39, pp. 8500–8509, 2004.

[30] J. Vinet, E. K. de Jong, H. W. G. M. Boddeke et al., "Expression of CXCL10 in cultured cortical neurons," *Journal of Neurochemistry*, vol. 112, no. 3, pp. 703–714, 2010.

[31] I. M. Dijkstra, S. Hulshof, P. van der Valk, H. W. G. M. Boddeke, and K. Biber, "Cutting edge: activity of human adult microglia in response to CC chemokine ligand 21," *Journal of Immunology*, vol. 172, no. 5, pp. 2744–2747, 2004.

[32] K. Biber, M. Tsuda, H. Tozaki-Saitoh et al., "Neuronal CCL21 up-regulates microglia $P2X_4$ expression and initiates neuropathic pain development," *The EMBO Journal*, vol. 30, no. 9, pp. 1864–1873, 2011.

[33] S. Sorce, R. Myburgh, and K. H. Krause, "The chemokine receptor CCR5 in the central nervous system," *Progress in Neurobiology*, vol. 93, no. 2, pp. 297–311, 2011.

[34] S. Sorce, J. Bonnefont, S. Julien et al., "Increased brain damage after ischaemic stroke in mice lacking the chemokine receptor CCR5," *British Journal of Pharmacology*, vol. 160, no. 2, pp. 311–321, 2010.

[35] J. M. Crain, M. Nikodemova, and J. J. Watters, "Expression of P2 nucleotide receptors varies with age and sex in murine brain microglia," *Journal of Neuroinflammation*, vol. 6, article no. 1742, p. 24, 2009.

[36] J. M. Sanz, P. Chiozzi, D. Ferrari et al., "Activation of microglia by amyloid β requires $P2X_7$ receptor expression," *Journal of Immunology*, vol. 182, no. 7, pp. 4378–4385, 2009.

[37] S. E. Haynes, G. Hollopeter, G. Yang et al., "The P2Y12 receptor regulates microglial activation by extracellular nucleotides," *Nature Neuroscience*, vol. 9, no. 12, pp. 1512–1519, 2006.

[38] K. Inoue, S. Koizumi, A. Kataoka, H. Tozaki-Saitoh, and M. Tsuda, "Chapter 12 $P2Y_6$-evoked microglial phagocytosis," *International Review of Neurobiology*, vol. 85, pp. 159–163, 2009.

[39] Y. Pan, C. Lloyd, H. Zhou et al., "Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation," *Nature*, vol. 387, no. 6633, pp. 611–617, 1997.

[40] A. Nishiyori, M. Minami, Y. Ohtani et al., "Localization of fractalkine and CX_3CR1 mRNAs in rat brain: does fractalkine play a role in signaling from neuron to microglia?" *The FEBS Letters*, vol. 429, no. 2, pp. 167–172, 1998.

[41] J. K. Harrison, Y. Jiang, S. Chen et al., "Role for neuronally derived fractalkine in mediating interactions between neurons and CX_3CR1-expressing microglia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 18, pp. 10896–10901, 1998.

[42] T. Mizuno, J. Kawanokuchi, K. Numata, and A. Suzumura, "Production and neuroprotective functions of fractalkine in the central nervous system," *Brain Research*, vol. 979, no. 1-2, pp. 65–70, 2003.

[43] K. Hatori, A. Nagai, R. Heisel, J. K. Ryu, and S. U. Kim, "Fractalkine and fractalkine receptors in human neurons and glial cells," *Journal of Neuroscience Research*, vol. 69, no. 3, pp. 418–426, 2002.

[44] S. A. Boehme, F. M. Lio, D. Maciejewski-Lenoir, K. B. Bacon, and P. J. Conlon, "The chemokine fractalkine inhibits Fas-mediated cell death of brain microglia," *Journal of Immunology*, vol. 165, no. 1, pp. 397–403, 2000.

[45] C. Lauro, S. D. Angelantonio, R. Cipriani et al., "Activity of adenosine receptors type 1 is required for CX_3CL1-mediated neuroprotection and neuromodulation in hippocampal neurons," *Journal of Immunology*, vol. 180, no. 11, pp. 7590–7596, 2008.

[46] M. Noda, Y. Doi, J. Liang et al., "Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression," *Journal of Biological Chemistry*, vol. 286, no. 3, pp. 2308–2319, 2011.

[47] V. Zujovic, J. Benavides, X. Vige et al., "Fractalkine modulates TNF-α secretion and neurotoxicity induced by microglial activation," *Glia*, vol. 29, no. 4, pp. 305–315, 2000.

[48] K. J. Garton, P. J. Gough, C. P. Blobel et al., "Tumor necrosis factor-α-converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX_3CL1)," *Journal of Biological Chemistry*, vol. 276, no. 41, pp. 37993–38001, 2001.

[49] C. Hundhausen, D. Misztela, T. A. Berkhout et al., "The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX_3CL1 (fractalkine) and regulates CX_3CL1-mediated cell-cell adhesion," *Blood*, vol. 102, no. 4, pp. 1186–1195, 2003.

[50] C. L. Tsou, C. A. Haskell, and I. F. Charo, "Tumor necrosis factor-α-converting enzyme mediates the inducible cleavage of fractalkine," *Journal of Biological Chemistry*, vol. 276, no. 48, pp. 44622–44626, 2001.

[51] A. K. Clark, P. K. Yip, J. Grist et al., "Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 25, pp. 10655–10660, 2007.

[52] G. A. Chapman, K. Moores, D. Harrison, C. A. Campbell, B. R. Stewart, and P. J. Strijbos, "Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage," *Journal of Neuroscience*, vol. 20, no. 15, p. RC87, 2000.

[53] F. Fahrenholz, "α-secretase as a therapeutic target," *Current Alzheimer Research*, vol. 4, no. 4, pp. 412–417, 2007.

[54] R. Postina, A. Schroeder, I. Dewachter et al., "A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model," *Journal of Clinical Investigation*, vol. 113, no. 10, pp. 1456–1464, 2004.

[55] E. A. Milward, R. Papadopoulos, S. J. Fuller et al., "The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth," *Neuron*, vol. 9, no. 1, pp. 129–137, 1992.

[56] L. W. Jin, H. Ninomiya, J. M. Roch et al., "Peptides containing the RERMS sequence of amyloid β/A4 protein precursor bind cell surface and promote neurite extension," *Journal of Neuroscience*, vol. 14, no. 9, pp. 5461–5470, 1994.

[57] J. Walter, C. Kaether, H. Steiner, and C. Haass, "The cell biology of Alzheimer's disease: uncovering the secrets of secretases," *Current Opinion in Neurobiology*, vol. 11, no. 5, pp. 585–590, 2001.

[58] R. E. Tanzi and L. Bertram, "Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective," *Cell*, vol. 120, no. 4, pp. 545–555, 2005.

[59] S. R. Green, K. H. Han, Y. Chen et al., "The CC chemokine MCP-1 stimulates surface expression of CX_3CR1 and enhances the adhesion of monocytes to fractalkine/CX_3CL1 via p38 MAPK," *Journal of Immunology*, vol. 176, no. 12, pp. 7412–7420, 2006.

[60] M. M. Pabon, A. D. Bachstetter, C. E. Hudson, C. Gemma, and P. C. Bickford, "CX_3CL1 reduces neurotoxicity and microglial activation in a rat model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 8, article 9, 2011.

[61] M. Fuhrmann, T. Bittner, C. K. E. Jung et al., "Microglial CX_3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease," *Nature Neuroscience*, vol. 13, no. 4, pp. 411–413, 2010.

[62] H. Neumann, M. R. Kotter, and R. J. M. Franklin, "Debris clearance by microglia: an essential link between degeneration and regeneration," *Brain*, vol. 132, pp. 288–295, 2009.

[63] F. Reichert and S. Rotshenker, "Complement-receptor-3 and scavenger-receptor-AI/II mediated myelin phagocytosis in microglia and macrophages," *Neurobiology of Disease*, vol. 12, no. 1, pp. 65–72, 2003.

[64] S. Rotshenker, "Microglia and macrophage activation and the regulation of complement-receptor-3 (CR3/MAC-1)-mediated myelin phagocytosis in injury and disease," *Journal of Molecular Neuroscience*, vol. 21, no. 1, pp. 65–72, 2003.

[65] C. Kaur, H. F. Too, and E. A. Ling, "Phagocytosis of *Escherichia coli* by amoeboid microglial cells in the developing brain," *Acta Neuropathologica*, vol. 107, no. 3, pp. 204–208, 2004.

[66] J. Husemann, J. D. Loike, R. Anankov, M. Febbraio, and S. C. Silverstein, "Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system," *Glia*, vol. 40, no. 2, pp. 195–205, 2002.

[67] R. A. Schlegel, S. Krahling, M. K. Callahan, and P. Williamson, "CD14 is a component of multiple recognition systems used by macrophages to phagocytose apoptotic lymphocytes," *Cell Death and Differentiation*, vol. 6, no. 6, pp. 583–592, 1999.

[68] A. Devitt, S. Pierce, C. Oldreive, W. H. Shingler, and C. D. Gregory, "CD14-dependent clearance of apoptotic cells by human macrophages: the role of phosphatidylserine," *Cell Death and Differentiation*, vol. 10, no. 3, pp. 371–382, 2003.

[69] E. G. Reed-Geaghan, Q. W. Reed, P. E. Cramer, and G. E. Landreth, "Deletion of CD14 attenuates Alzheimer's disease pathology by influencing the brain's inflammatory milieu," *Journal of Neuroscience*, vol. 30, no. 46, pp. 15369–15373, 2010.

[70] J. Koenigsknecht and G. Landreth, "Microglial phagocytosis of fibrillar β-amyloid through a β1 integrin-dependent mechanism," *Journal of Neuroscience*, vol. 24, no. 44, pp. 9838–9846, 2004.

[71] M. E. Bamberger, M. E. Harris, D. R. McDonald, J. Husemann, and G. E. Landreth, "A cell surface receptor complex for fibrillar β-amyloid mediates microglial activation," *Journal of Neuroscience*, vol. 23, no. 7, pp. 2665–2674, 2003.

[72] D. A. Persaud-Sawin, L. Banach, and G. J. Harry, "Raft aggregation with specific receptor recruitment is required for microglial phagocytosis of Aβ42," *Glia*, vol. 57, no. 3, pp. 320–335, 2009.

[73] K. J. Moore, J. El Khoury, L. A. Medeiros et al., "A CD36-initiated signaling cascade mediates inflammatory effects of β-amyloid," *Journal of Biological Chemistry*, vol. 277, no. 49, pp. 47373–47379, 2002.

[74] A. Kharitonenkov, Z. Chen, I. Sures, H. Wang, J. Schilling, and A. Ullrich, "A family of proteins that inhibit signalling through tyrosine kinase receptors," *Nature*, vol. 386, no. 6621, pp. 181–186, 1997.

[75] T. Matozaki, Y. Murata, H. Okazawa, and H. Ohnishi, "Functions and molecular mechanisms of the CD47-SIRPα signalling pathway," *Trends in Cell Biology*, vol. 19, no. 2, pp. 72–80, 2009.

[76] M. Gitik, S. Liraz-Zaltsman, P. A. Oldenborg, F. Reichert, and S. Rotshenker, "Myelin down-regulates myelin phagocytosis by microglia and macrophages through interactions between CD47 on myelin and SIRPα (signal regulatory protein-α) on phagocytes," *Journal of Neuroinflammation*, vol. 8, article 24, 2011.

[77] P. A. Oldenborg, A. Zheleznyak, Y. F. Fang, C. F. Lagenaur, H. D. Gresham, and F. P. Lindberg, "Role of CD47 as a marker of self on red blood cells," *Science*, vol. 288, no. 5473, pp. 2051–2054, 2000.

[78] S. D. Yan, X. Chen, J. Fu et al., "RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease," *Nature*, vol. 382, no. 6593, pp. 685–691, 1996.

[79] S. D. Yan, H. Zhu, J. Fu et al., "Amyloid-β peptide-receptor for advanced glycation endproduct interaction elicits neuronal expression of macrophage-colony stimulating factor: a proinflammatory pathway in Alzheimer disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 10, pp. 5296–5301, 1997.

[80] A. Friggeri, S. Banerjee, S. Biswas et al., "Participation of the receptor for advanced glycation end products in efferocytosis," *Journal of Immunology*, vol. 186, no. 11, pp. 6191–6198, 2011.

[81] S. Dukic-Stefanovic, J. Gasic-Milenkovic, W. Deuther-Conrad, and G. Münch, "Signal transduction pathways in mouse microglia N-11 cells activated by advanced glycation endproducts (AGEs)," *Journal of Neurochemistry*, vol. 87, no. 1, pp. 44–55, 2003.

[82] R. Kang, D. Tang, N. E. Schapiro et al., "The receptor for advanced glycation end products (RAGE) sustains autophagy

and limits apoptosis, promoting pancreatic tumor cell survival," *Cell Death and Differentiation*, vol. 17, no. 4, pp. 666–676, 2010.

[83] R. Dearie, A. Sagare, and B. V. Zlokovic, "The role of the cell surface LRP and soluble LRP in blood-brain barrier Aβ clearance in Alzheimer's disease," *Current Pharmaceutical Design*, vol. 14, no. 16, pp. 1601–1605, 2008.

[84] N. Koning, D. F. Swaab, R. M. Hoek, and I. Huitinga, "Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron-glia and glia-glia interactions," *Journal of Neuropathology and Experimental Neurology*, vol. 68, no. 2, pp. 159–167, 2009.

[85] H. Matsumoto, Y. Kumon, H. Watanabe et al., "Expression of CD200 by macrophage-like cells in ischemic core of rat brain after transient middle cerebral artery occlusion," *Neuroscience Letters*, vol. 418, no. 1, pp. 44–48, 2007.

[86] D. A. Carter and A. D. Dick, "CD200 maintains microglial potential to migrate in adult human retinal explant model," *Current Eye Research*, vol. 28, no. 6, pp. 427–436, 2004.

[87] T. Langmann, "Microglia activation in retinal degeneration," *Journal of Leukocyte Biology*, vol. 81, no. 6, pp. 1345–1351, 2007.

[88] S. Zhang, X. J. Wang, L. P. Tian et al., "CD200-CD200R dysfunction exacerbates microglial activation and dopaminergic neurodegeneration in a rat model of Parkinson's disease," *Journal of Neuroinflammation*, vol. 8, no. 1, p. 154, 2011.

[89] D. G. Walker, J. E. Dalsing-Hernandez, N. A. Campbell, and L. F. Lue, "Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation," *Experimental Neurology*, vol. 215, no. 1, pp. 5–19, 2009.

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

[91] G. Sessa, P. Podini, M. Mariani et al., "Distribution and signaling of TREM2/DAP12, the receptor system mutated in human polycystic lipomembraneous osteodysplasia with sclerosing leukoencephalopathy dementia," *European Journal of Neuroscience*, vol. 20, no. 10, pp. 2617–2628, 2004.

[92] K. Takahashi, M. Prinz, M. Stagi, O. Chechneva, and H. Neumann, "TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis," *PLoS Medicine*, vol. 4, no. 4, p. e124, 2007.

[93] L. Stefano, G. Racchetti, F. Bianco et al., "The surface-exposed chaperone, Hsp60, is an agonist of the microglial TREM2 receptor," *Journal of Neurochemistry*, vol. 110, no. 1, pp. 284–294, 2009.

[94] B. Melchior, A. E. Garcia, B. K. Hsiung et al., "Dual induction of TREM2 and tolerance-related transcript, Tmem176b, in amyloid transgenic mice: Implications for vaccine-based therapies for Alzheimer's disease," *ASN Neuro*, vol. 2, no. 3, Article ID e00037, 2010.

[95] B. B. Mishra, U. M. Gundra, and J. M. Teale, "Expression and distribution of Toll-like receptors 11–13 in the brain during murine neurocysticercosis," *Journal of Neuroinflammation*, vol. 5, article 53, 2008.

[96] Z. Shi, Z. Cai, A. Sanchez et al., "A novel toll-like receptor that recognizes vesicular stomatitis virus," *Journal of Biological Chemistry*, vol. 286, no. 6, pp. 4517–4524, 2011.

[97] C. Han, J. Jin, S. Xu, H. Liu, N. Li, and X. Cao, "Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b," *Nature Immunology*, vol. 11, no. 8, pp. 734–742, 2010.

[98] S. Ribes, S. Ebert, T. Regen et al., "Toll-like receptor stimulation enhances phagocytosis and intracellular killing of nonencapsulated and encapsulated *Streptococcus pneumoniae* by murine microglia," *Infection and Immunity*, vol. 78, no. 2, pp. 865–871, 2010.

[99] M. Song, J. Jin, J. E. Lim et al., "TLR4 mutation reduces microglial activation, increases Aβ deposits and exacerbates cognitive deficits in a mouse model of Alzheimer's disease," *Journal of Neuroinflammation*, vol. 8, p. 92, 2011.

[100] S. Ribes, N. Adam, S. Ebert et al., "The viral TLR3 agonist poly(I:C) stimulates phagocytosis and intracellular killing of *Escherichia coli* by microglial cells," *Neuroscience Letters*, vol. 482, no. 1, pp. 17–20, 2010.

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

[102] D. A. Costello, A. Lyons, S. Denieffe et al., "Long term potentiation is impaired in membrane glycoprotein CD200-deficient mice: a role for Toll-like receptor activation," *The Journal of Biological Chemistry*, vol. 286, no. 40, pp. 34722–34732, 2011.

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

[104] A. H. Dalpke, M. K. Schafer, M. Frey et al., "Immunostimulatory CpG-DNA activates murine microglia," *The Journal of Immunology*, vol. 168, no. 10, pp. 4854–4863, 2002.

[105] Y. Doi, T. Mizuno, Y. Maki et al., "Microglia activated with the toll-like receptor 9 ligand CpG attenuate oligomeric amyloid β neurotoxicity in *in vitro* and *in vivo* models of Alzheimer's disease," *American Journal of Pathology*, vol. 175, no. 5, pp. 2121–2132, 2009.

[106] C. Ravindran, Y. C. Cheng, and S. M. Liang, "CpG-ODNs induces up-regulated expression of chemokine CCL9 in mouse macrophages and microglia," *Cellular Immunology*, vol. 260, no. 2, pp. 113–118, 2010.

[107] R. Hanayama, M. Tanaka, K. Miwa, A. Shinohara, A. Iwamatsu, and S. Nagata, "Identification of a factor that links apoptotic cells to phagocytes," *Nature*, vol. 417, no. 6885, pp. 182–187, 2002.

[108] M. Miyanishi, K. Tada, M. Koike, Y. Uchiyama, T. Kitamura, and S. Nagata, "Identification of Tim4 as a phosphatidylserine receptor," *Nature*, vol. 450, no. 7168, pp. 435–439, 2007.

[109] F. Leonardi-Essmann, M. Emig, Y. Kitamura, R. Spanagel, and P. J. Gebicke-Haerter, "Fractalkine-upregulated milk-fat globule EGF factor-8 protein in cultured rat microglia," *Journal of Neuroimmunology*, vol. 160, no. 1-2, pp. 92–101, 2005.

[110] M. Miksa, D. Amin, R. Wu, W. Dong, T. S. Ravikumar, and P. Wang, "Fractalkine-induced MFG-E8 leads to enhanced apoptotic cell clearance by macrophages," *Molecular Medicine*, vol. 13, no. 11-12, pp. 553–560, 2007.

[111] S. Akakura, S. Singh, M. Spataro et al., "The opsonin MFG-E8 is a ligand for the $\alpha v\beta 5$ integrin and triggers DOCK180-dependent Rac1 activation for the phagocytosis of apoptotic cells," *Experimental Cell Research*, vol. 292, no. 2, pp. 403–416, 2004.

[112] A. Friggeri, Y. Yang, S. Banerjee, Y. J. Park, G. Liu, and E. Abraham, "HMGB1 inhibits macrophage activity in efferocytosis through binding to the $\alpha v\beta 3$-integrin," *American Journal of Physiology*, vol. 299, no. 6, pp. C1267–C1276, 2010.

[113] J. Boddaert, K. Kinugawa, J. C. Lambert et al., "Evidence of a role for lactadherin in Alzheimer's disease," *American Journal of Pathology*, vol. 170, no. 3, pp. 921–929, 2007.

[114] D. Feng, W. L. Zhao, Y. Y. Ye et al., "Cellular internalization of exosomes occurs through phagocytosis," *Traffic*, vol. 11, no. 5, pp. 675–687, 2010.

[115] J. Martinez, J. Almendinger, A. Oberst et al., "Microtubule-associated protein 1 light chain 3 α (LC3)-associated phagocytosis is required for the efficient clearance of dead cells," *Proceedings of the National Academy of Sciences of the United States*, vol. 108, no. 42, pp. 17396–17401, 2011.

[116] Y. Yamanishi, J. Kitaura, K. Izawa et al., "TIM1 is an endogenous ligand for LMIR5/CD300b: LMIR5 deficiency ameliorates mouse kidney ischemia/reperfusion injury," *Journal of Experimental Medicine*, vol. 207, no. 7, pp. 1501–1511, 2010.

[117] D. Farfara, D. Trudler, N. Segev-Amzaleg, R. Galron, R. Stein, and D. Frenkel, "γ-secretase component presenilin is important for microglia β-amyloid clearance," *Annals of Neurology*, vol. 69, no. 1, pp. 170–180, 2011.

[118] S. Jayadev, A. Case, A. J. Eastman et al., "Presenilin 2 is the predominant γ-secretase in microglia and modulates cytokine release," *PLoS One*, vol. 5, no. 12, Article ID e15743, 2010.

[119] Y. Nadler, A. Alexandrovich, N. Grigoriadis et al., "Increased expression of the γ-secretase components presenilin-1 and nicastrin in activated astrocytes and microglia following traumatic brain injury," *Glia*, vol. 56, no. 5, pp. 552–567, 2008.

[120] M. He, H. Kubo, K. Morimoto et al., "Receptor for advanced glycation end products binds to phosphatidylserine and assists in the clearance of apoptotic cells," *The EMBO Reports*, vol. 12, no. 4, pp. 358–364, 2011.

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

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 165021, 6 pages
doi:10.1155/2012/165021

Review Article

Microglia in Alzheimer Brain: A Neuropathological Perspective

Robert E. Mrak

Department of Pathology, University of Toledo College of Medicine, 3000 Arlington Avenue, Toledo, OH 43614, USA

Correspondence should be addressed to Robert E. Mrak, robert.mrak@utoledo.edu

Received 31 January 2012; Accepted 19 February 2012

Academic Editor: Lee-Way Jin

Microglia have long been noted to be present and activated in Alzheimer brain. Demonstrations that these microglia are associated with the specific lesions of Alzheimer disease—Aβ plaques and neurofibrillary tangles—and that these microglia overexpress the potent proinflammatory cytokine interleukin-1 led to the recognition of a potential pathogenic role for these cells in initiation and progression of disease. Activated, cytokine-overexpressing microglia are near-universal components of Aβ plaques at early (diffuse) and mid (neuritic) stages of progression in Alzheimer brain, and only decline in end-stage, dense core plaques. They correlate with plaque distribution across cerebral cortical cytoarchitectonic layers and across brain regions. They also show close associations with tangle-bearing neurons in Alzheimer brain. Microglial activation is a consistent feature in conditions that confer increased risk for Alzheimer disease or that are associated with accelerated appearance of Alzheimer-type neuropathological changes. These include normal ageing, head injury, diabetes, heart disease, and chronic intractable epilepsy. The neuropathological demonstration of microglial activation in Alzheimer brain and in Alzheimer-related conditions opened the field of basic and applied investigations centered on the idea of a pathogenically important neuroinflammatory process in Alzheimer disease.

1. Introduction

Microglia have been known to be present in the characteristic plaques of Alzheimer disease since the first descriptions of these cells by del Rio Hortega and Penfield in the 1920s [1], but half a century would pass before attention returned to these cells. The first suggestion of a causative role for microglia in Alzheimer disease came from Glenner, who hypothesized in 1979 that the amyloid found in Alzheimer brain was produced by these cells [2]. This idea dominated several subsequent studies that identified microglia associated with amyloid plaques in the brains of Alzheimer patients [3–5]. The idea was largely abandoned when the neuronal origin of Aβ was elucidated [6], although occasional studies have returned to this idea [7].

The first evidence that microglia may have an immunological—rather than a phagocytic or Aβ-processing—function in Alzheimer brain was the demonstration in 1989 by Griffin and colleagues that these microglia express the potent immunomodulatory cytokine interleukin-1 [8] (Figure 1). This report, together with the finding that interleukin-1 regulates the synthesis of the Aβ precursor protein [9], immediately suggested that microglia and their cytokines might play a role in driving plaque development, a concept very different from ideas about amyloid production or phagocytosis and protein degradation that had been previously attributed to microglia. Over the next several years, additional cytokines were added to the listing of proteins that are elevated in Alzheimer brain. These include interleukin-6 [10], transforming growth factor β_1 [11], interferon α [12, 13], and interleukins-2 and -3 [14].

Ideas regarding the role of microglia in Alzheimer disease have continued to evolve over the 20 years since these seminal studies. Neuropathological investigations, in particular, have both suggested and supported ideas about the potential roles of inflammatory mechanisms in Aβ plaque formation and progression in Alzheimer disease, and the potential roles of microglial activation in progressive plaque-associated neuritic damage, neuronal damage, and neuronal death. This review will highlight these neuropathological studies.

2. Microglial Identification in Human Brain

Microglia were first described in 1899 by Nissl, who distinguished these cells from other neural components based on

the shape of their nuclei [15]. The definitive identification and characterization of these cells were done in the 1920s by del Rio Hortega and Penfield, using a silver carbonate staining technique [1]. Microglia are now known to express a wide variety of immune-related molecules and antigens [16], many of which can be used to immunolabel microglia in histological tissue sections. "Resting" microglia, found throughout normal brain parenchyma, express many of these molecules either at very low levels or not at all.

In contrast to the low levels of expression of immune-related molecules by resting microglia, immunological challenge or tissue injury leads to upregulation of many of these factors, a process known as microglial activation. With further activation, microglia undergo morphological changes that include enlargement and withdrawal of their ramified processes. Activated microglia can be identified through their expression of such factors. In general, however, antibodies against secreted products such as interleukin-1β (IL-1β) or tumor necrosis factor-α generally yield poor results in paraffin sections as these soluble peptides are lost during tissue processing. In contrast, the cytokine IL-1α is expressed by microglia as a membrane-bound peptide, and immunohistochemistry using antibodies against IL-1α is very effective at labeling activated microglia while producing little or no labeling of resting microglia (Figure 1) [17]. Other techniques that have been used to identify microglia include MHC class II cell surface receptors [18], Fc receptors [19], various lectins [20–22], and other monocyte markers [23, 24]. More recently, immunohistochemistry for ionized calcium binding adapter molecule 1 (Iba1) has been identified as a reliable marker for microglia, although this technique labels resting as well as activated microglia and is thus not specific for activated forms [25, 26]. A subset of microglia express ferritin and can be immunolabelled with anti-ferritin antibodies. Such expression, however, appears to represent a degenerative, or "dystrophic" change in microglia rather than an activated state [27].

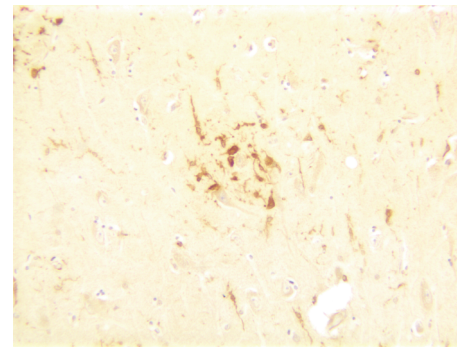


Figure 1: Activated microglia, overexpressing interleukin-1, within an Aβ plaque in Alzheimer brain. Immunohistochemistry using an antibody specific for IL-1α.

3. Microglial Associations with Aβ Plaques

Activated microglia are near-universal components of Aβ plaques in Alzheimer brain. In Alzheimer brain, microglia accumulate fragmented DNA, presumably originating from neuronal injury and death [28]. Such accumulation, together with cytokine stimulation, is a potent microglial activating stimulus [28]. Microglial activation has been shown to progress with clinical (CDR) stage of dementia [29, 30], with neuropathological (Braak and Braak [31]) stage of disease severity [29], and with stage of progression of individual Aβ plaques [32]. The distribution of activated microglia across different brain regions closely parallels that of neuritic plaques in Alzheimer brain, with involvement of hippocampus > temporal lobe > frontal and occipital lobes [33]. Moreover, there is variation in the distribution of microglia in different cortical cytoarchitectonic layers [34]. In Alzheimer brain, this distribution correlates closely with the distribution of neuritic plaques across these same layers, with both showing greater involvement of layers III–VI than of layers I-II. Of even greater interest, normal individuals—without Alzheimer disease and without neuritic plaques—show a similar (but much less pronounced) variation in content of (resting) microglia across these cortical layers. This latter finding suggests that the normal distribution of microglia across cerebral cortical layers may influence the pattern of development of neuritic plaques in Alzheimer brain [34].

The association of activated microglia with Aβ plaques is also a function of plaque type. The Aβ plaques present in Alzheimer brain show different morphological appearances, which are thought to represent different stages of plaque progression, commencing with diffuse deposits of Aβ peptide, progressing to complex plaques with congophilic amyloid and with evidence of damage to neurons and their processes ("dystrophic neurites"), and terminating in a dense core of condensed amyloid without diffuse Aβ peptide and without adjacent dystrophic neurites [32, 35]. This schemata is widely (but not universally [7]) accepted.

Both the numbers of microglia and the degree of activation of microglia vary with plaque type, or stage [32]. Early plaques in Alzheimer brain—those which contain diffuse deposits of Aβ peptide without formation of true (fibrillar) amyloid and without evidence of neuritic injury—already contain small numbers of microglia that overexpress interleukin-1 (IL-1) [32]. These microglia are not enlarged or phagocytic [36] and thus are easily mistaken for normal "resting" microglia in the absence of specific immunohistochemical demonstration of their cytokine upregulation [26]. Of interest is the fact that similar-appearing diffuse deposits of Aβ peptide can sometimes be found, in the absence of the later plaque forms and in the absence of clinical dementia. This includes occasional otherwise normal, cognitively intact elderly individuals, and includes individuals with the disease hereditary cerebral hemorrhage with amyloidosis (Dutch) (HCHA-D), a disease that is characterized by extensive amyloid angiopathy and consequent cerebral hemorrhage. In contrast to the ubiquitous presence of activated microglia in diffuse plaques present in Alzheimer brain [32], activated

microglia have not been identified in either the similar-appearing deposits in the brains of neurologically normal elderly individuals [37] or in the similar-appearing deposits in the brains of patients with HCHA-D [23]. This observation suggests that Aβ deposition alone is not sufficient for the development of Alzheimer disease. Further, this observation suggests that activated microglia, overexpressing IL-1, may be a key element in Aβ plaque progression, and hence a key element in the clinical conversion to, and progression of, clinical Alzheimer disease.

It remains unclear why the diffuse Aβ deposits in normal elderly or in HCHA-D differ in their ability to attract and activate microglia. One possibility is the lack of some codeposited, immunogenic protein, possibly originating outside the brain. In support of this idea is the observation that Alzheimer brain shows diffuse immunoreactivity for the serum-derived proteins fibrinogen and IgG, suggesting breakdown of blood-brain barrier function in Alzheimer disease [38]. This fibrinogen immunoreactivity is enhanced in Aβ deposits and is associated with microglial activation [38]. A second possibility is that there is a difference in some physical characteristic of the Aβ peptide (e.g., oligomer or fibrillar state), as may be the case for patients with HCHA-D. Finally, for those unusual elderly individuals with only diffuse Aβ deposits, there is the third possibility that there is an inherent difference in the responsiveness of microglia in these individuals.

Early, diffuse plaques progress to neuritic forms, characterized by condensation of Aβ peptide into true, fibrillar amyloid, and by the appearance of injured (dystrophic) neural processes (neurites). At this stage, there are increases both in the number of microglia associated with individual plaques, and in the degree of activation of these associated microglia [32, 36, 39]. In addition to overexpression of IL-1, these microglia become enlarged with fewer ramified processes, thus meeting classic morphological criteria (in addition to immunohistochemical criteria) for activation. With continued condensation of Aβ peptide, a "dense core" of amyloid is formed. This process appears to mask or reverse the immunogenic properties of the plaque, as the numbers of microglia associated with such plaques are less than that seen prior to the formation of the dense core [32]. Further, the microglia found in these cored plaques are phagocytic in appearance [36], although the appearance of microglia in these later plaques has also been interpreted as a degenerative or senescent change [26]. Microglia are clearly capable of phagocytosing and even removing Aβ, as trials of Aβ vaccines have suggested in both animals and humans [40].

The final, end-stage of plaque progression is a solitary dense core of amyloid, without surrounding diffuse Aβ peptide and without associated dystrophic neurites. These end-stage plaques are also devoid of microglia, suggesting that the immunogenic stimulus that attracts and activates microglia has abated [32]. This waxing and waning of numbers of activated microglia with plaque progression has its parallel in the finding that microglia in Alzheimer brain appear early in the disease course (as measured by premortem assessments using the Blessed test score) and decline late in the disease [41], at which point there is severe dementia from the accumulated neuronal loss. A similar observation is that there is a slight decrease in microglial activation in end-stage (Braak and Braak stage VI) Alzheimer brain, after showing increases over the first five Braak and Braak stages [42].

The concurrent absence of microglia and dystrophic neurites in end-stage, dense-core plaques also suggests that plaque-associated microglia may play a role in damaging surrounding neural elements and generating the dystrophic neurites of Alzheimer amyloid plaques. Interleukin-1 is known to be neurotoxic at high concentrations, as coculture of lipopolysaccharide-activated microglia with primary rat cortical neurons results in neuronal death, and this effect is attenuated in the presence of the naturally occurring IL-1 receptor antagonist IL-1ra [43]. In human brain, the association of activated, IL-1-expressing microglia with Aβ plaques correlates with histochemical evidence of progressive neuronal damage, as assessed using the TUNEL technique [44]. Cortical neurons contained within or adjacent to Aβ plaques frequently show TUNEL positivity, and the percentage of such neurons that are positive by this technique increases with plaque progression, reaching nearly 100% in end-stage dense core plaques. Further, the total number of plaque-associated neurons declines significantly in such end-stage plaques, suggesting that there is neuronal death and loss associated with plaque progression [44]. Plaques are generally much more numerous than tangles in Alzheimer brain, and neuronal loss in Alzheimer brain has long been known to exceed the incidence of neurofibrillary tangles [45]. Thus, such plaque-associated neuronal injury and loss, mediated at least in part by microglia-derived cytokine overexpression, may be a significant—and perhaps even major—source of neuronal loss in Alzheimer brain.

4. Microglial Associations with Neurofibrillary Tangles

Neuropathological investigations have also offered evidence that microglia may be involved in the pathogenesis and progression of neurofibrillary tangle formation. Just as there are correlations between the distribution of activated microglia and distribution of Aβ plaques in Alzheimer brain, so, too are there correlations between the distribution of microglia and the distribution of neurofibrillary tangles [46]. Further, and again as for Aβ plaques, a progressive association has been shown between activated microglia and neurofibrillary tangle stage [47]. Neurofibrillary tangles can be classified into different stages, representing a pathological progression [47, 48]. In Alzheimer brain, there is a progressive association of activated, IL-1-overexpressing microglia with neurofibrillary tangles across this spectrum of tangle progression [47]. *In vitro* work has shown that IL-1 increases neuronal expression of the tau-phosphorylating enzyme MAPK-p38 and also increases the phosphorylation (activation) of this enzyme [43]. This, together with the neuropathological observations, suggests that neuron-associated microglia may be important in driving or sustaining neuronal tangle formation in Alzheimer disease.

5. Microglia in Other Neurodegenerative Diseases

Chronic microglial activation as a potentially neurotoxic driving force is not likely to be restricted to Alzheimer disease. Following the seminal observations of microglial activation in Alzheimer brain, similar microglial activation has been found in other chronic neurological diseases, including amyotrophic lateral sclerosis, spinocerebellar ataxia, and Huntington's disease [49–52], and especially in diseases characterized by α-synuclein pathology: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Activated, cytokine-overexpressing microglia are found in the substantia nigra of Parkinson patients (for reviews, see references [53–55]). Activated microglia also show close associations with inclusion-bearing neurons in dementia with Lewy bodies [56] and with inclusion-containing oligodendrocytes in multiple system atrophy [57]. These findings suggest a common final pathway of neuronal injury and loss in a number of chronic neurodegenerative diseases [58].

6. Origins of Neuroinflammatory Processes

Alzheimer disease, and Alzheimer-type neuropathological changes, show important associations with a number of other conditions. These include ageing, head injury, diabetes, heart disease, and epilepsy. All of these conditions have been shown to enhance or accelerate microglial activation in the brain. In normal human ageing, for instance, there is a progressive increase in the numbers of activated microglia overexpressing IL-1 [59]. This effect may serve to lower the threshold required to subsequently initiate disease [60] and may thus explain in part the strong age association of Alzheimer disease. Similarly, head injury results in activation of microglia overexpressing IL-1, together with expression of the Aβ precursor protein by neurons and neuronal processes [61]. For patients with established Alzheimer disease, and microglial activation, there is further enhancement of this microglial activation in the presence of concurrent diabetes [62] or heart disease [63]. Patients with chronic intractable temporal lobe epilepsy or with HIV infection show increased proinflammatory activation of microglia [64, 65], and both of these conditions have been associated with accelerated appearance of age-associated, Alzheimer-type "senile" changes [66, 67]. Collectively, these findings suggest that proinflammatory processes of various etiologies can contribute to a summative inflammatory state—characterized by microglial activation—that lowers the threshold and increases the risk for the subsequent development of neurodegenerative disease.

7. Conclusion

Microglial activation is a universal feature of Alzheimer disease, as well as a number of other chronic neurodegenerative diseases. These microglia show specific patterns of association with the neuropathological features of Alzheimer disease, which collectively suggest a pathogenic role in driving the progression of such pathology. Further, microglial activation is a prominent feature of several other conditions that have been associated with increased risk for Alzheimer disease or with accelerated appearance of Alzheimer-type neuropathological changes.

Acknowledgment

This work was supported in part by Grants AG12411 and AG19606 from the National Institutes of Health.

References

[1] P. del Rio Hortega and W. Penfield, "Cerebral cicatrix: the reaction of neuroglia and microglia to brain wounds," *Bulletin of the Johns Hopkins Hospital*, vol. 41, pp. 278–282, 1997.

[2] G. G. Glenner, "Congophilic microangiopathy in the pathogenesis of alzheimer's syndrome (presenile dementia)," *Medical Hypotheses*, vol. 5, no. 11, pp. 1231–1236, 1979.

[3] V. M. Vostrikov, "lektronno-tsitokhimicheskoe issledovanie mikroglii pri bolezni Al'tsgeimera i senil'noi dementsii," *Zhurnal Nevropatologii i Psikhiatrii Imeni S-S-Korsakova*, vol. 85, pp. 974–976, 1985.

[4] J. M. Rozemuller, P. Eikelenboom, and F. C. Stam, "Role of microglia in plaque formation in senile dementia of the alzheimer type. an immunohistochemical study," *Virchows Archiv Abteilung B Cell Pathology*, vol. 51, no. 3, pp. 247–254, 1986.

[5] D. W. Dickson, J. Farlo, P. Davies, H. Crystal, P. Fuld, and S. H. C. Yen, "Alzheimer's disease. a double-labeling immunohistochemical study of senile plaques," *American Journal of Pathology*, vol. 132, no. 1, pp. 86–101, 1988.

[6] J. Wegiel, K. C. Wang, M. Tarnawski, and B. Lach, "Microglial cells are the driving force in fibrillar plaque formation, whereas astrocytes are a leading factor in plaque degradation," *Acta Neuropathologica*, vol. 100, no. 4, pp. 356–364, 2000.

[7] S. Bahmanyar, G. A. Higgins, and D. Goldgaber, "Localization of amyloid β protein messenger RNA in brains from patients with Alzheimer's disease," *Science*, vol. 237, no. 4810, pp. 77–80, 1987.

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

[9] D. Goldgaber, H. W. Harris, T. Hla et al., "Interleukin 1 regulates synthesis of amyloid β-protein precursor mrna in human endothelial cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 19, pp. 7606–7610, 1989.

[10] J. Bauer, S. Strauss, U. Schreiter-Gasser et al., "Interleukin-6 and α-2-macroglobulin indicate an acute-phase state in alzheimer's disease cortices," *Febs Letters*, vol. 285, no. 1, pp. 111–114, 1991.

[11] E. A. Van Der Wal, F. Gomez-Pinilla, and C. W. Cotman, "Transforming growth factor-β1 is in plaques in alzheimer and down pathologies," *Neuroreport*, vol. 4, no. 1, pp. 69–72, 1993.

[12] H. Akiyama, K. Ikeda, M. Katoh, E. G. McGeer, and P. L. McGeer, "Expression of mrp14, 27e10, interferon-α and leukocyte common antigen by reactive microglia in postmortem human brain tissue," *Journal of Neuroimmunology*, vol. 50, no. 2, pp. 195–201, 1994.

[13] T. Yamada, M. A. Horisberger, N. Kawaguchi, I. Moroo, and T. Toyoda, "Immunohistochemistry using antibodies to α-interferon and its induced protein, mxa, in alzheimer's and parkinson's disease brain tissues," *Neuroscience Letters*, vol. 181, no. 1-2, pp. 61–64, 1994.

[14] D. M. Araujo, "Induction of immune system mediators in the hippocampal formation in alzheimer's and parkinson's diseases: selective effects on specific interleukins and interleukin receptors," *Neuroscience*, vol. 61, no. 4, pp. 745–754, 1994.

[15] F. Nissl, "Ueber einige Beziehungen zwischen Nervenzellerkrankungen und glioesen Erscheinungen bei verschiedenen Psychosen," *Archiv fur Psychiatrie*, vol. 32, no. 2, pp. 1–21, 1899.

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

[17] L. C. Stanley, R. E. Mrak, R. C. Woody et al., "Glial cytokines as neuropathogenic factors in hiv infection: pathogenic similarities to alzheimer's disease," *Journal of Neuropathology and Experimental Neurology*, vol. 53, no. 3, pp. 231–238, 1994.

[18] A. F. Carpenter, P. W. Carpenter, and W. R. Markesbery, "Morphometric analysis of microglia in alzheimer's disease," *Journal of Neuropathology and Experimental Neurology*, vol. 52, no. 6, pp. 601–608, 1993.

[19] E. Ulvestad, K. Williams, R. Matre, H. Nyland, A. Olivier, and J. Antel, "Fc receptors for igg on cultured human microglia mediate cytotoxicity and phagocytosis of antibody-coated targets," *Journal of Neuropathology and Experimental Neurology*, vol. 53, no. 1, pp. 27–36, 1994.

[20] C. A. Colton, C. Abel, J. Patchett, J. Keri, and J. Yao, "Lectin staining of cultured cns microglia," *Journal of Histochemistry and Cytochemistry*, vol. 40, no. 4, pp. 505–512, 1992.

[21] H. Mannoji, H. Yeger, and L. E. Becker, "A specific histochemical marker (lectin ricinus communis agglutinin-1) for normal human microglia, and application to routine histopathology," *Acta Neuropathologica*, vol. 71, no. 3-4, pp. 341–343, 1986.

[22] W. J. Streit and G. W. Kreutzberg, "Lectin binding by resting and reactive microglia," *Journal of Neurocytology*, vol. 16, no. 2, pp. 249–260, 1987.

[23] M. L. C. Maat-Schieman, A. J. Rozemuller, S. G. Van Duinen, J. Haan, P. Eikelenboom, and R. A. C. Roos, "Microglia in diffuse plaques in hereditary cerebral hemorrhage with amyloidosis (dutch). an immunohistochemical study," *Journal of Neuropathology and Experimental Neurology*, vol. 53, no. 5, pp. 483–491, 1994.

[24] H. Muhleisen, J. Gehrmann, and R. Meyermann, "Reactive microglia in creutzfeldt-jakob disease," *Neuropathology and Applied Neurobiology*, vol. 21, no. 6, pp. 505–517, 1995.

[25] J. Scholz, A. Abele, C. Marian et al., "Low-dose methotrexate reduces peripheral nerve injury-evoked spinal microglial activation and neuropathic pain behavior in rats," *Pain*, vol. 138, no. 1, pp. 130–142, 2008.

[26] W. J. Streit, H. Braak, Q. S. Xue, and I. Bechmann, "Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in alzheimer's disease," *Acta Neuropathologica*, vol. 118, no. 4, pp. 475–485, 2009.

[27] K. O. Lopes, D. L. Sparks, and W. J. Streit, "Microglial dystrophy in the aged and alzheimer's disease brain is associated with ferritin immunoreactivity," *Glia*, vol. 56, no. 10, pp. 1048–1060, 2008.

[28] Y. Li, L. Liu, D. Liu et al., "Microglial activation by uptake of fdna via a scavenger receptor," *Journal of Neuroimmunology*, vol. 147, no. 1-2, pp. 50–55, 2004.

[29] Z. Xiang, V. Haroutunian, L. Ho, D. Purohit, and G. M. Pasinetti, "Microglia activation in the brain as inflammatory biomarker of alzheimer's disease neuropathology and clinical dementia," *Disease Markers*, vol. 22, no. 1-2, pp. 95–102, 2006.

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

[31] H. Braak and E. Braak, "Neuropathological stageing of alzheimer-related changes," *Acta Neuropathologica*, vol. 82, no. 4, pp. 239–259, 1991.

[32] W. S. T. Griffin, Jin Gen Sheng, G. W. Roberts, and R. E. Mrak, "Interleukin-1 expression in different plaque types in alzheimer's disease: significance in plaque evolution," *Journal of Neuropathology and Experimental Neurology*, vol. 54, no. 2, pp. 276–281, 1995.

[33] J. G. Sheng, R. E. Mrak, and W. S. T. Griffin, "Microglial interleukin-1α expression in brain regions in alzheimer's disease: correlation with neuritic plaque distribution," *Neuropathology and Applied Neurobiology*, vol. 21, no. 4, pp. 290–301, 1995.

[34] J. G. Sheng, W. S. T. Griffin, M. C. Royston, and R. E. Mrak, "Distribution of interleukin-1-immunoreactive microglia in cerebral cortical layers: implications for neuritic plaque formation in alzheimer's disease," *Neuropathology and Applied Neurobiology*, vol. 24, no. 4, pp. 278–283, 1998.

[35] J. M. Rozemuller, P. Eikelenboom, F. C. Stam, K. Beyreuther, and C. L. Masters, "A4 protein in alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition," *Journal of Neuropathology and Experimental Neurology*, vol. 48, no. 6, pp. 674–691, 1989.

[36] J. G. Sheng, R. E. Mrak, and W. S. T. Griffin, "Neuritic plaque evolution in alzheimer's disease is accompanied by transition of activated microglia from primed to enlarged to phagocytic forms," *Acta Neuropathologica*, vol. 94, no. 1, pp. 1–5, 1997.

[37] I. R. A. Mackenzie, C. Hao, and D. G. Munoz, "Role of microglia in senile plaque formation," *Neurobiology of Aging*, vol. 16, no. 5, pp. 797–804, 1995.

[38] J. K. Ryu and J. G. McLarnon, "A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed alzheimer's disease brain," *Journal of Cellular and Molecular Medicine*, vol. 13, no. 9 A, pp. 2911–2925, 2009.

[39] H. Fukumoto, A. Asami-Odaka, N. Suzuki, and T. Iwatsubo, "Association of aβ40-positive senile plaques with microglial cells in the brains of patients with alzheimer's disease and in non-demented aged individuals," *Neurodegeneration*, vol. 5, no. 1, pp. 13–17, 1996.

[40] D. M. Wilcock and C. A. Colton, "Anti-amyloid-β immunotherapy in alzheimer's disease: relevance of transgenic mouse studies to clinical trials," *Journal of Alzheimer's Disease*, vol. 15, no. 4, pp. 555–569, 2008.

[41] Y. M. Arends, C. Duyckaerts, J. M. Rozemuller, P. Eikelenboom, and J. J. Hauw, "Microglia, amyloid and dementia in alzheimer disease: a correlative study," *Neurobiology of Aging*, vol. 21, no. 1, pp. 39–47, 2000.

[42] D. R. Thal, T. Arendt, G. Waldmann et al., "Progression of neurofibrillary changes and phf-τ in end-stage alzheimer's disease is different from plaque and cortical microglial pathology," *Neurobiology of Aging*, vol. 19, no. 6, pp. 517–525, 1998.

[43] Y. Li, L. Liu, S. W. Barger, and W. S. T. Griffin, "Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-mapk pathway," *Journal of Neuroscience*, vol. 23, no. 5, pp. 1605–1611, 2003.

[44] J. G. Sheng, X. Q. Zhou, R. E. Mrak, and W. S. T. Griffin, "Progressive neuronal injury associated with amyloid plaque formation in alzheimer disease," *Journal of Neuropathology and Experimental Neurology*, vol. 57, no. 7, pp. 714–717, 1998.

[45] J. C. Troncoso, R. R. Sukhov, C. H. Kawas, and V. E. Koliatsos, "In situ labeling of dying cortical neurons in normal aging and in alzheimer's disease: correlations with senile plaques and disease progression," *Journal of Neuropathology and Experimental Neurology*, vol. 55, no. 11, pp. 1134–1142, 1996.

[46] L. G. Sheffield, J. G. Marquis, and N. E. J. Berman, "Regional distribution of cortical microglia parallels that of neurofibrillary tangles in alzheimer's disease," *Neuroscience Letters*, vol. 285, no. 3, pp. 165–168, 2000.

[47] J. G. Sheng, R. E. Mrak, and W. S. T. Griffin, "Glial-neuronal interactions in alzheimer disease: progressive association of il-1α+ microglia and s100β+ astrocytes with neurofibrillary tangle stages," *Journal of Neuropathology and Experimental Neurology*, vol. 56, no. 3, pp. 285–290, 1997.

[48] A. Alzheimer, "Ueber eigenartige Krankheitsfaelle des spaeteren Alters," *Zeitschrift fur Die Gesamte Neurologie und Psychiatrie*, vol. 4, pp. 356–385, 1911.

[49] C. S. Lobsiger and D. W. Cleveland, "Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease," *Nature Neuroscience*, vol. 10, no. 11, pp. 1355–1360, 2007.

[50] K. Moisse and M. J. Strong, "Innate immunity in amyotrophic lateral sclerosis," *Biochimica Et Biophysica Acta*, vol. 1762, no. 11-12, pp. 1083–1093, 2006.

[51] S. A. Sargsyan, P. N. Monk, and P. J. Shaw, "Microglia as potential contributors to motor neuron injury in amyotrophic lateral sclerosis," *Glia*, vol. 51, no. 4, pp. 241–253, 2005.

[52] Y. F. Tai, N. Pavese, A. Gerhard et al., "Microglial activation in presymptomatic Huntington's disease gene carriers," *Brain*, vol. 130, no. 7, pp. 1759–1766, 2007.

[53] M. Sawada, K. Imamura, and T. Nagatsu, "Role of cytokines in inflammatory process in parkinson's disease," *Journal of Neural Transmission, Supplement*, no. 70, pp. 373–381, 2006.

[54] P. L. McGeer and E. G. McGeer, "Inflammation and neurodegeneration in parkinson's disease," *Parkinsonism and Related Disorders*, vol. 10, no. 1, pp. S3–S7, 2004.

[55] M. Vila, V. Jackson-Lewis, C. Guégan et al., "The role of glial cells in parkinson's disease," *Current Opinion in Neurology*, vol. 14, no. 4, pp. 483–489, 2001.

[56] E. Iseki, W. Marui, H. Akiyama, K. Uéda, and K. Kosaka, "Degeneration process of lewy bodies in the brains of patients with dementia with lewy bodies using α-synuclein-immunohistochemistry," *Neuroscience Letters*, vol. 286, no. 1, pp. 69–73, 2000.

[57] K. Ishizawa, T. Komori, S. Sasaki, N. Arai, T. Mizutani, and T. Hirose, "Microglial activation parallels system degeneration in multiple system atrophy," *Journal of Neuropathology and Experimental Neurology*, vol. 63, no. 1, pp. 43–52, 2004.

[58] R. E. Mrak and W. S. T. Griffin, "Common inflammatory mechanisms in lewy body disease and alzheimer disease," *Journal of Neuropathology and Experimental Neurology*, vol. 66, no. 8, pp. 683–686, 2007.

[59] J. G. Sheng, R. E. Mrak, and W. S. T. Griffin, "Enlarged and phagocytic, but not primed, interleukin-1α-immunoreactive microglia increase with age in normal human brain," *Acta Neuropathologica*, vol. 95, no. 3, pp. 229–234, 1998.

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

[61] W. S. T. Griffin, J. G. Sheng, S. M. Gentleman, D. I. Graham, R. E. Mrak, and G. W. Roberts, "Microglial interleukin-1α expression in human head injury: correlations with neuronal and neuritic p-amyloid precursor protein expression," *Neuroscience Letters*, vol. 176, no. 2, pp. 133–136, 1994.

[62] T. Valente, A. Gella, X. Fernàndez-Busquets, M. Unzeta, and N. Durany, "Immunohistochemical analysis of human brain suggests pathological synergism of alzheimer's disease and diabetes mellitus," *Neurobiology of Disease*, vol. 37, no. 1, pp. 67–76, 2010.

[63] W. J. Streit and D. L. Sparks, "Activation of microglia in the brains of humans with heart disease and hypercholesterolemic rabbits," *Journal of Molecular Medicine*, vol. 75, no. 2, pp. 130–138, 1997.

[64] J. G. Sheng, F. A. Boop, R. E. Mrak, and W. S. T. Griffin, "Increased neuronal β-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1α immunoreactivity," *Journal of Neurochemistry*, vol. 63, no. 5, pp. 1872–1879, 1994.

[65] L. C. Stanley, R. E. Mrak, R. C. Woody et al., "Glial cytokines as neuropathogenic factors in hiv infection: pathogenic similarities to alzheimer's disease," *Journal of Neuropathology and Experimental Neurology*, vol. 53, no. 3, pp. 231–238, 1994.

[66] I. R. A. Mackenzie and L. A. Miller, "Senile plaques in temporal lobe epilepsy," *Acta Neuropathologica*, vol. 87, no. 5, pp. 504–510, 1994.

[67] M. M. Esiri, S. C. Biddolph, and C. S. Morris, "Prevalence of Alzheimer plaques in AIDS," *Journal of Neurology, Neurosurgery & Psychiatyry*, vol. 65, no. 1, pp. 29–33, 1998.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 737846, 9 pages
doi:10.1155/2012/737846

Review Article

The Biphasic Role of Microglia in Alzheimer's Disease

Tetsuya Mizuno

Department of Neuroimmunology, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

Correspondence should be addressed to Tetsuya Mizuno, tmizuno@riem.nagoya-u.ac.jp

Received 28 December 2011; Accepted 29 February 2012

Academic Editor: Akio Suzumura

Neuroinflammation is involved in the pathogenesis of Alzheimer's disease (AD). Microglia, macrophage-like resident immune cells in the brain, play critical roles in the inflammatory aspects of AD. Microglia may be activated by oligomeric and fibrillar species of amyloid β (Aβ) that are constituents of senile plaques and by molecules derived from degenerated neurons, such as purines and chemokines, which enhance their migration and phagocytosis. The main neurotoxic molecules produced by activated microglia may be reactive oxygen species, glutamate, and inflammatory cytokines such as tumor-necrosis-factor-α and interleukin- (IL-) 1β These molecules differentially induce neurotoxicity. Aβ itself directly damages neurons. In terms of neuroprotective properties, microglia treated with fractalkine or IL-34 attenuate Aβ neurotoxicity by Aβ clearance and the production of antioxidants. Therefore, regulation of the microglial role in neuroprotection may be a useful therapeutic strategy for AD.

1. Introduction

Microglia, macrophage-like immune cells in the central nervous system (CNS), cluster around the senile plaques that along with polymorphous amyloid β (Aβ) deposits are the pathological hallmarks of Alzheimer's disease (AD). Microglia have a biphasic neurotoxic-neuroprotective role in the pathogenesis of AD. In regard to their neurotoxic properties, microglia may be involved in the inflammatory component of AD [1, 2]. In AD, the trigger molecule for microglial activation may be Aβ and molecules derived from degenerated neurons may enhance microglial neurotoxicity [3]. Aβ exists in different assembly forms including monomers, oligomers, and fibrils. Both oligomeric Aβ (oAβ) and fibrillar Aβ (fAβ) have been shown to stimulate microglial secretion of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor-necrosis-factor-α (TNF-α); chemokines including monocyte chemotactic-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1); complement components; free radicals such as reactive oxygen species (ROS), including superoxide anions and hydroxy radicals [4, 5]. Glutamate also plays an important role in microglial neurotoxicity in AD. Activated microglia produce large amounts of glutamate, which induces excitotoxicity via N-methyl-D-aspartate (NMDA) receptor signaling [6–9]. Chronic activation of extrasynaptic NMDA receptors leads to increased Aβ production [10].

Aβ itself is toxic to neurons in AD, with oAβ being more toxic than fAβ (Figure 1). The toxicity of oAβ manifests itself in terms of synaptic dysfunction, including inhibition of hippocampal long-term potentiation, facilitation of long-term depression, and disruption of synaptic plasticity [11, 12]. It is therefore necessary to evaluate microglial neurotoxicity apart from Aβ neurotoxicity.

In regard to its neuroprotective properties, microglia attenuate Aβ neurotoxicity by Aβ clearance, including phagocytosis and degradation of Aβ and the production of antioxidants and neurotrophic factors [13–15]. In the present paper, we focus on the trigger molecules that mediate microglial activation and the mechanisms of microglial neurotoxicity mediated by ROS glutamate, and inflammatory cytokines. We also discuss the neuroprotective role of microglia in AD.

2. The Trigger Molecules That Mediate Microglial Activation

2.1. Fibrillar Aβ. Aβ, the key mediator of AD, is processed from the amyloid precursor protein (APP). The most common isoforms are Aβ1-40 and Aβ1-42, which are the

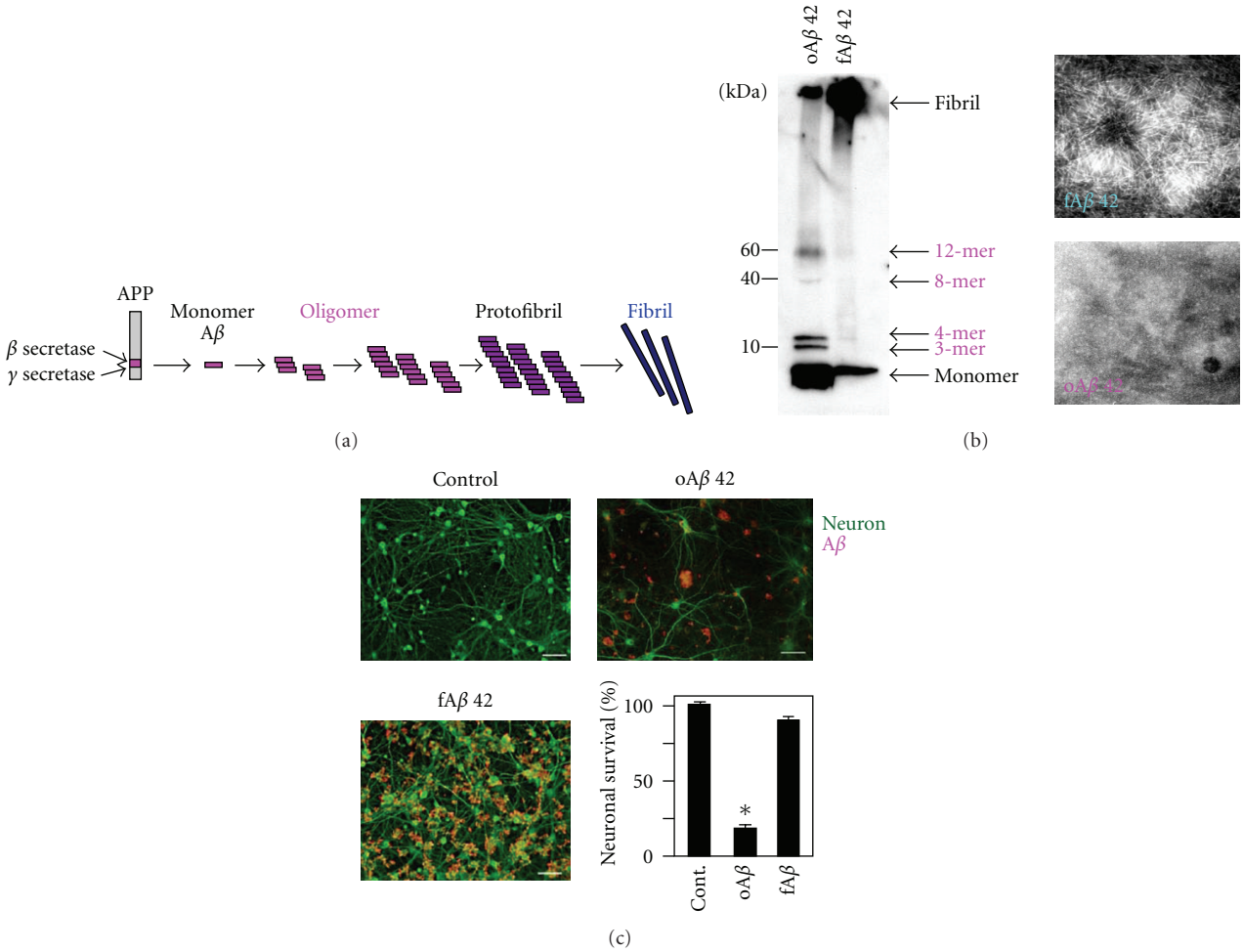


Figure 1: Aβ neurotoxicity. Aβ is derived from APP by enzymatic cleavage. Aβ consists of 40 or 42 amino acids. Aβ40 is soluble and of lower toxicity, but Aβ42 is prone to aggregate and form fibrillar structures via oligomers (a). Oligomeric Aβ (oAβ42) and fibrillar Aβ (fAβ42) are detected by western blotting and an electron microscope (b). Administration of 5 μM oAβ42 to cortical cultures induces significant neuronal death. In contrast, administration of fAβ42 does not induce neuronal cell death, although Aβ deposition is observed on dendrites (c).

major constituents of senile plaques. Aβ1-42 is more prone to aggregate than Aβ1-40. Initial Aβ deposition begins with Aβ1-42, but not with Aβ1-40. In the process of Aβ1-42 aggregation, many types of soluble oAβ are formed: dimers, trimers, tetramers, dodecamers, Aβ-derived diffusible ligands, and annular protofibrils [16–18]. Stimulation of microglia with fAβ reportedly results in the Syk kinase- and NFκB-dependent production of TNF-α, iNOS, and peroxynitrite [19]. However, the ability of fAβ to activate microglia is generally low or absent when fAβ is used as the sole stimulant. Recent reports have clarified that fAβ can activate microglia via Toll-like receptor 2 (TLR2) [20] or interaction with cell surface receptor complexes. TLR2, TLR4, and TLR6 have been shown to be essential components of the receptor complexes for microglial activation. The coreceptor CD14 and TLR2 and 4 complex is required for fAβ-stimulated microglial activation [21]. The class B scavenger receptor CD36 and TLR4 and 6 complex is also involved in the activation of microglia by fAβ. The signals mediated by this receptor complex induce IL-1β production in microglia [22].

2.2. Oligomeric Aβ. The patterns of microglial activation caused by fAβ and oAβ are different. In addition, studies of microglial activation with oAβ have yielded controversial results. oAβ activates microglia by increasing levels of phosphorylated Lyn, Syk kinase, and p38 MAP kinase, which results in the production of IL-6 and a decrease in MCP-1 [23]. On the other hand, oAβ does not produce several proinflammatory mediators commonly induced by lipopolysaccharides (LPS), such as prostaglandin E2, glutamate, TNF-α, IL-1β, and IL-6. There is a report that oAβ at low nanomolar concentrations induces neurotoxicity by increasing the production of nitric oxide (NO) as well as the activity of scavenger receptor A and the Ca^{2+}-activated potassium channel KCa3.1 [24]. oAβ induces neuronal ROS through a mechanism requiring NMDA receptor activation [25]. ROS is also induced by fAβ and oAβ in microglia [26]. In contrast to the above reports, our data showed that both fAβ and oAβ failed to induce toxic molecules such as TNF-α, NO, and glutamate in microglia and to enhance these molecules in LPS-activated microglia (Figure 2) [15]. The synthetic oAβ that is used

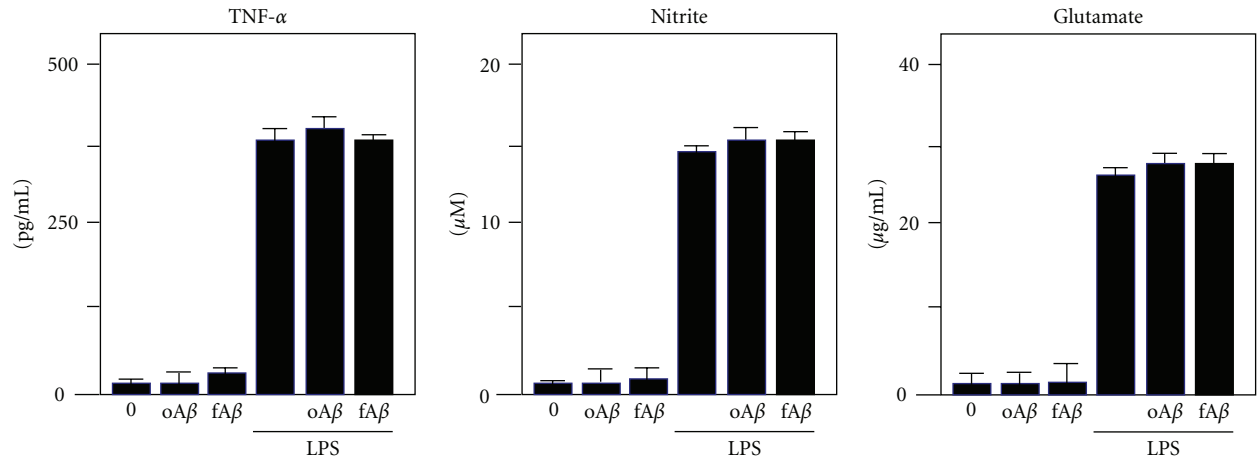


Figure 2: Inflammatory molecules produced by Aβ- or LPS-activated microglia. In primary microglial culture, administration of 5 μM oAβ42 or 5 μM fAβ42 for 24 h does not induce the production of neurotoxic mediators such as TNF-α, glutamate, or nitrite, a stable breakdown product of NO. While microglia activated with 1 μg/mL LPS produce these molecules, both oAβ42 and fAβ42 do not enhance the production.

for in vitro studies contains oligomers of different sizes and shapes, and microglia may respond to this heterogeneous oAβ mixture in various ways. Moreover, synthetic oAβ preparations are less potent than oAβ isolated from the supernatant of transfected cell cultures.

2.3. The Molecules Derived from Degenerated Neurons. Recent findings have indicated that damaged neurons are not merely passive targets of microglia but rather regulate microglial activity through nucleotides and chemokines [27]. Damaged neurons release several substances that activate microglia, for instance purines, including ATP [28] and UDP [29]; chemokines, such as CCL-21 [30]; glutamate [31]. However, these molecules do not always induce microglial neurotoxicity. ATP regulates microglial branch dynamics and mediates a rapid microglial response toward injured neurons. UDP triggers microglial phagocytosis via P2Y6 receptors. The chemokine CCL-21, released by damaged neurons, activates microglia via the CXCR3 receptor. Excessive neuronal glutamate is released as a result of neurodegenerative processes. Microglial cells express the AMPA and kainate subtypes of ionotropic glutamate receptors [32]. Metabotropic glutamate (mGlu) receptor 2, mGlu3, and mGlu5 are expressed in microglia [33, 34]. Glutamate serves as an activation signal for microglia, and activation of mGluR2 on microglia promotes neurotoxicity. However, microglial mGluR5 provides neuronal protection by suppressing the NO and TNF-α production induced by blood protein fibrinogen [34].

3. The Neurotoxic Molecules Produced by Microglia

3.1. ROS. Oxidative damage to protein, lipids, polysaccharides, and DNA is involved in the pathogenesis of AD [35]. The expression of NADPH oxidase, a multisubunit enzyme complex responsible for the production of ROS, is upregulated in AD [36]. Microglial NADPH oxidase is activated by fAβ [37, 38], and NADPH oxidase activation subsequently causes neurotoxicity through two mechanisms: (1) extracellular ROS produced by microglia are directly toxic to neurons, and (2) intracellular ROS function as a signaling mechanism in microglia to amplify the production of several proinflammatory and neurotoxic cytokines [39]. ROS are induced in the mitochondrial membranes of both neurons and microglia, causing subsequent oxidative damage in the early stages of disease progression. Loss of mitochondrial membrane potential and increase of ROS production have been demonstrated in studies of AD patients as well as in transgenic mice models of AD [40, 41]. An increase in hydrogen peroxide and a decrease in cytochrome oxidase activity were found in young Tg2576 mice prior to the appearance of Aβ plaques [40]. Oxidative stress has been shown to contribute to the onset of cognitive dysfunction caused by Aβ [42].

A recent report showed that fAβ and oAβ induced ROS in microglia through the TRPV1 cation channel, also known as the capsaisin receptor, and that pretreatment with fAβ or oAβ induced microglial priming through Kv1.3 K(+) channels, that is, increased ROS production upon secondary stimulation with the phorbol ester PMA [26]. The translocation of chloride intracellular channel 1 from the cytosol to the plasma membrane is also involved in microglial ROS generation [43].

Neuronal ROS induced by Aβ can be selectively dysfunctional as well as degenerative. Overstimulation of excitatory NMDA receptors can lead to excessive ROS. Antioxidative enzyme heme oxygenase-1 (HO-1) is a microsomal enzyme that oxidatively cleaves heme to produce biliverdin, carbon monoxide, and iron [44]. Aβ binds to heme to promote a functional heme deficiency and induces mitochondrial dysfunction and neurotoxicity [45]. APP also binds to HO, and oxidative neurotoxicity is markedly enhanced in cerebral

cortical cultures from APP Swedish mutant transgenic mice [46].

3.2. Glutamate. Activated microglia release large amounts of glutamate through upregulation of glutaminase expression and induce excitoneurotoxicity through NMDA receptor signaling [7, 47, 48]. Microglial glutamate production is reported to be induced by APP, but not Aβ [48]. Excessive glutamate increases Ca^{2+} influx via the NMDA receptor, resulting in Ca^{2+}/calmodulin-dependent protein kinase (CaMK) activation. NO induced by CaMK inhibits mitochondrial function. Stimulation of synaptic NMDA receptors enhances prosurvival signals through the activation of cAMP response element-binding protein (CREB) and the extracellular signal-regulated kinase (ERK) cascade [49, 50], whereas calcium flux through extrasynaptic NMDA receptors overrides these functions, causing mitochondrial dysfunction and neuronal cell death [51, 52]. A recent report suggested that chronic activation of extrasynaptic NMDA receptors leads to sustained neuronal Aβ release via amyloidogenic APP expression [53].

Focal bead-like swelling in dendrites and axons, known as neuritic beading, is a neuropathological sign that is a feature of neuronal cell dysfunction preceding neuronal death in various diseases such as ischemia, epilepsy, brain tumor, and AD [54–57]. We found that glutamate from activated microglia induces neuritic beading by impairing dendritic and axonal transport through NMDA receptor signaling [7]. Moreover, we demonstrated that gap junction hemichannels are the main avenue of excessive glutamate release from neurotoxic activated microglia [6]. The blockade of gap junction hemichannels by glycyrrhetinic acid derivatives significantly prevents activated microglia-mediated neuronal death in vitro [7, 58, 59] and in vivo in rodent models of transient ischemic brain injury, multiple sclerosis, amyotrophic lateral sclerosis, and AD [60–62]. In the APP/PS1 transgenic mouse model of AD, glycyrrhetinic acid derivatives improved memory impairments without altering Aβ deposition [62].

oAβ is directly neurotoxic as a result of inducing glutamate release from hippocampal neurons and may contribute to dysregulation of excitatory signaling in neurons [63].

3.3. Inflammatory Cytokines. oAβ, but not fAβ, has been shown to increase levels of TNF-α and IL-1β in rat microglial cultures [64]. However, gene expression analysis of microglia using cDNA arrays has confirmed that the upregulation of TNF-α and IL-1β is caused by both oAβ and fAβ [65]. TNF-α is a well-characterized proinflammatory cytokine involved in many neuroinflammatory cascades, including autocrine activation of microglia [66] and direct apoptosis via activation of extrinsic pathway-associated TNF receptors [67, 68]. TNF-α enhances microglial glutaminase expression, glutamate production, and cell-surface expression of gap junction hemichannels [6]. Synergistic and autocrine activities of TNF-α may cause the release of large amounts of glutamate, resulting in excitotoxic neuronal death [69]. TNF-α has been shown to directly upregulate the expression of the AMPA receptor GluR1 subunit in mouse hippocampus and cerebral cortex neurons [70] and to exacerbate AMPA-induced neuronal death at high doses [71]. TNF-α has also been reported to enhance excitotoxicity through synergistic stimulation of the TNF and NMDA receptors [72].

IL-1β is known to be a driving force in the inflammatory process in AD, and it promotes the synthesis and processing of APP [73]. IL-1β affects ion currents, intracellular Ca^{2+} homeostasis, and membrane potentials and suppresses long-term potentiation, thus contributing to dysfunction and inflammation [74]. A recent report indicated that the cytoplasmic receptor NALP3 inflammasome is involved in the innate immune response in AD [75]. Activation of the microglial NALP3 inflammasome is initiated by phagocytosis of fAβ and induces lysosomal damage and cathepsin B release. Moreover, it leads to the cleavage of pro-IL-1β/pro-IL-18 into IL-1β/IL-18 by caspase-1. Subsequently, IL-1β activates the secretion of several proinflammatory and chemotactic mediators [75].

Wnt signaling plays an important role in neural development, including synaptic differentiation. Wnt 5a and its receptor Frizzled-5 have been shown to be upregulated in the AD mouse brain [76], and activation of Wnt5a signaling enhances Aβ-evoked neurotoxicity by induced TNF-α and IL-1β [76]. In contrast, postsynaptic damage induced by oAβ in hippocampal neurons is reported to be prevented by Wnt5a [77]. This discrepancy can be explained by the fact that basal Wnt5a has synaptoprotective activity, but excessive Wnt5a may induce proinflammatory factors.

4. Phagocytosis of Microglia

Microglial phagocytosis of neuronal debris and Aβ plays a pivotal role in AD. Phagocytosis is associated with inflammation during uptake of microbes via TLRs and Fc receptors, while phagocytosis of apoptotic cells is executed without inflammation via phosphatidylserine receptors such as T-cell-immunoglobulin-mucin-4 (TIM-4) [78, 79]. Milk-fat-globule-EGF-factor-8 (MFG-E8), secreted by activated microglia or macrophages, also binds to phosphatidylserine exposed on plasma membranes of apoptotic cells [80, 81]. Phagocytosis with inflammation may be toxic to neurons because of the production of inflammatory molecules such as proinflammatory cytokines, NO, and ROS. However, phagocytosis of Aβ contributes to microglial neuroprotection in AD. Peptidoglycan, the TLR2 ligand, and unmethylated DNA CpG motifs, the TLR9 ligand, increase Aβ phagocytosis through protein-coupled formyl peptide receptor-like 2 [82, 83]. Similarly, LPS, the TLR4 ligand, increases phagocytosis through the CD14 receptor [84]. TLR4 mutation exacerbates the Aβ burden in mouse models of AD [85].

5. Molecules Able to Induce Microglial Neuroprotective Properties

5.1. Fractalkine. Degenerating neurons produce signaling molecules that regulate microglial phagocytosis and neuroprotection. Some of this signaling may be controlled by chemokines and chemokine receptors, which are widely

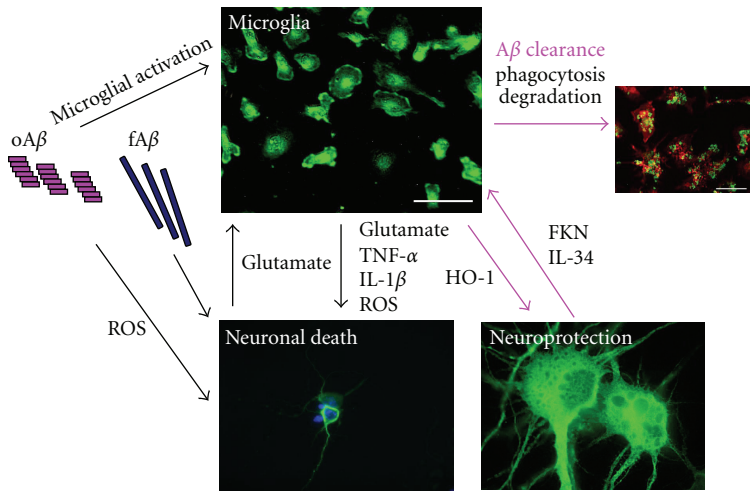


Figure 3: Aβ toxicity in neurons and microglia. Aβ is directly toxic to neurons by ROS, and enhances the production of microglia-derived neurotoxic molecules such as glutamate, TNF-α, and IL-1β. Glutamate from degenerated neurons also induces microglial neurotoxicity. IL-34 and FKN induce microglial neuroprotection via Aβ clearance and antioxidant activity.

expressed throughout the central nervous system [86]. We have shown that the soluble CX3C chemokine fractalkine (FKN), secreted from damaged neurons, promotes microglial phagocytosis of neuronal debris through the release of MFG-E8 and induces the expression of the antioxidant enzyme HO-1 in microglia, resulting in neuroprotection against glutamate toxicity [87]. The end-products of HO-1 including biliverdin, carbon monoxide, and iron provide cellular and tissue protection through antiinflammatory, antiapoptotic, or antioxidative effects [88]. Numerous studies have demonstrated that upregulation of HO-1 expression in the CNS may be beneficial to counteract neuroinflammation and neurodegenerative diseases [89]. The neuroprotective effect of FKN is abolished by treatment with the HO-1 inhibitor tin-mesoporphyrin IX (SnMP). Moreover, FKN suppresses microglial NO, IL-6, and TNF-α production [90]. FKN signaling is deficient in AD brains and is downregulated by Aβ. CX3CR1, the fractalkine receptor, is a key member of the microglial pathway that protects against AD-related cognitive deficits that are associated with aberrant microglial activation and elevated inflammatory cytokines [91]. Mice lacking the CX3CR1 receptor show cognitive dysfunction as demonstrated by contextual fear conditioning and Morris water maze tests, deficits in motor learning, and a significant impairment in long-term potentiation via increase in IL-1β [92]. CX3CR1 deficiency worsens the AD-related neuronal and behavioral deficits [91]. In contrast, CX3CR1 deficiency is reported to reduce Aβ deposition in AD mouse models [93]. Thus, FKN-CX3CR1 signaling in AD is still controversial.

5.2. IL-34. The dimeric glycoprotein IL-34, which is mainly expressed in neurons, may also be a neuronal cytokine that regulates microglial function. The major function of IL-34 is to stimulate the differentiation and proliferation of monocytes and macrophages via the colony-stimulating-factor-(CSF-) 1 receptor [94]. We have shown that IL-34 induces microglial proliferation and antioxidant HO-1 production and enhances the degradation of Aβ via insulin degrading enzyme (IDE) (also known as Aβ degrading enzyme) and that IL-34 reduces the amount of oAβ and ROS present in the supernatant of neuron-microglia cocultures, resulting in microglial neuroprotection against oAβ toxicity [95]. IDE activity is critical in determining the level of Aβ. The levels of hippocampal IDE protein and activity have been shown to be reduced in AD [96]. Enhanced IDE activity in IDE/APP double-transgenic mice decreased Aβ levels and prevented the development of AD pathology [97]. Moreover, single intracerebroventricular injection of IL-34 effectively suppressed the impairment of associative learning in an APP/PS1 transgenic mouse model of AD [95]. The injection of IL-34 may act directly on microglia, which can rapidly eliminate oAβ via upregulation of IDE and exert antioxidant effect via HO-1.

5.3. M-CSF. CSF-1, also known as macrophage colony-stimulating factor (M-CSF), is another ligand of the CSF-1 receptor. M-CSF enables the acidification of macrophage lysosomes and subsequently the degradation of internalized Aβ [98]. Intraperitoneal injection of M-CSF prevents memory disturbance in APP/PS1 mice by inducing microglial phagocytosis of Aβ [99]. A recent report showed that IL-34 and M-CSF differ in their structure and the CSF-1 receptor domains that they bind, causing different bioactivities and signal activation kinetics [100]. Both IL-34 and M-CSF are useful molecules in terms of inducing microglial neuroprotective properties.

5.4. CpG. TLR9, which is located in the intracellular endosomal-lysosomal compartment in innate immune cells, detects single-stranded DNA containing unmethylated CpG. Microglia express TLR9 at higher levels than do astrocytes and neuronal cells. Thus, CpG mainly acts on microglia in the CNS. We have also shown that microglia activated by

CpG attenuate oAβ neurotoxicity [15]. While high concentrations of CpG (10 μM) induce TNF-α, IL-12, and NO in microglia and enhance neuronal damage [101, 102], lower concentrations of CpG (1 nM–100 nM) enhance microglial phagocytosis of Aβ without inflammation. Intracerebroventricular administration of CpG ameliorates both the cognitive impairments induced by oAβ and the impairment of associative learning in a Tg2576 mouse model of AD [15].

Taken together, these molecules induce neuroprotective properties in microglia through the antioxidant effect of HO-1 and Aβ clearance. HO-1 is induced by nuclear translocation of Nrf2, which is a transcription factor and reportedly plays a pivotal role in cellular survival [103, 104]. Nrf2 gene therapy also has been shown to improve memory in the mouse model of AD [105]. Moreover, Aβ clearance including phagocytosis and degradation of Aβ by microglia can decrease Aβ plaque formation and Aβ toxicity.

6. Conclusion

While Aβ, especially oAβ, is directly toxic to neurons, it may also enhance microglial neurotoxic effects by inducing inflammatory mediators. Degenerated neurons produce molecules other than Aβ that enhance microglial neurotoxicity. However, the microglial neuroprotective effect resulting from Aβ clearance and antioxidant activity is obvious in AD (Figure 3). The conditions that determine microglial toxic or protective effects remain to be elucidated. Clarifying these issues may contribute to the understanding of AD pathophysiology. A useful therapeutic strategy for AD may be to regulate microglia toward neuroprotection, specifically, Aβ clearance without inflammation.

Acknowledgments

This work was supported in part by the Global COE program Integrated Functional Molecular Medicine for Neuronal and Neoplastic Disorders, funded by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

References

[1] L. Meda, M. A. Cassatella, G. I. Szendrei et al., "Activation of microglial cells by β-amyloid protein and interferon-γ," *Nature*, vol. 374, no. 6523, pp. 647–650, 1995.

[2] H. Akiyama, S. Barger, S. Barnum et al., "Key issues in Alzheimer's disease inflammation," *Neurobiology of Aging*, vol. 21, no. 3, pp. 383–421, 2000.

[3] T. F. Pais, C. Figueiredo, R. Peixoto, M. H. Braz, and S. Chatterjee, "Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway," *Journal of Neuroinflammation*, vol. 5, p. 43, 2008.

[4] P. L. McGeer and E. G. McGeer, "Inflammation, autotoxicity and Alzheimer disease," *Neurobiology of Aging*, vol. 22, no. 6, pp. 799–809, 2001.

[5] V. Della Bianca, S. Dusi, E. Bianchini, I. Dal Prà, and F. Rossi, "β-amyloid activates the O_2forming NADPH oxidase in microglia, monocytes, and neutrophils. A possible inflammatory mechanism of neuronal damage in Alzheimer's disease," *Journal of Biological Chemistry*, vol. 274, no. 22, pp. 15493–15499, 1999.

[6] H. Takeuchi, S. Jin, J. Wang et al., "Tumor necrosis factor-α induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner," *Journal of Biological Chemistry*, vol. 281, no. 30, pp. 21362–21368, 2006.

[7] H. Takeuchi, T. Mizuno, G. Zhang et al., "Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport," *Journal of Biological Chemistry*, vol. 280, no. 11, pp. 10444–10454, 2005.

[8] S. W. Barger and A. S. Basile, "Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function," *Journal of Neurochemistry*, vol. 76, no. 3, pp. 846–854, 2001.

[9] D. Piani, M. Spranger, K. Frei, A. Schaffner, and A. Fontana, "Macrophage-induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines," *European Journal of Immunology*, vol. 22, no. 9, pp. 2429–2436, 1992.

[10] K. Bordji, J. Becerril-Ortega, O. Nicole, and A. Buisson, "Activation of extrasynaptic, but not synaptic, NMDA receptors modifies amyloid precursor protein expression pattern and increases amyloid-β production," *Journal of Neuroscience*, vol. 30, no. 47, pp. 15927–15942, 2010.

[11] D. M. Walsh, I. Klyubin, J. V. Fadeeva et al., "Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation *in vivo*," *Nature*, vol. 416, no. 6880, pp. 535–539, 2002.

[12] S. Li, S. Hong, N. E. Shepardson, D. M. Walsh, G. M. Shankar, and D. Selkoe, "Soluble oligomers of amyloid β protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake," *Neuron*, vol. 62, no. 6, pp. 788–801, 2009.

[13] P. Yan, X. Hu, H. Song et al., "Matrix metalloproteinase-9 degrades amyloid-β fibrils *in vitro* and compact plaques *in situ*," *Journal of Biological Chemistry*, vol. 281, no. 134, pp. 24566–24574, 2006.

[14] K. L. Richard, M. Filali, P. Préfontaine, and S. Rivest, "Toll-like receptor 2 acts as a natural innate immune receptor to clear amyloid β1-42 and delay the cognitive decline in a mouse model of Alzheimer's disease," *Journal of Neuroscience*, vol. 28, no. 22, pp. 5784–5793, 2008.

[15] Y. Doi, T. Mizuno, Y. Maki et al., "Microglia activated with the toll-like receptor 9 ligand CpG attenuate oligomeric amyloid β neurotoxicity in *in vitro* and *in vivo* models of Alzheimer's disease," *American Journal of Pathology*, vol. 175, no. 5, pp. 2121–2132, 2009.

[16] G. M. Shankar, S. Li, T. H. Mehta et al., "Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory," *Nature Medicine*, vol. 14, no. 8, pp. 837–842, 2008.

[17] S. Lesné, T. K. Ming, L. Kotilinek et al., "A specific amyloid-β protein assembly in the brain impairs memory," *Nature*, vol. 440, no. 7082, pp. 352–357, 2006.

[18] R. Kayed, A. Pensalfini, L. Margol et al., "Annular protofibrils area structurally and functionally distinct type of amyloid oligomer," *Journal of Biological Chemistry*, vol. 284, no. 7, pp. 4230–4237, 2009.

[19] C. K. Combs, J. Colleen Karlo, S. C. Kao, and G. E. Landreth, "β-amyloid stimulation of microglia anti monocytes results in TNF α-dependent expression of inducible nitric oxide synthase and neuronal apoptosis," *Journal of Neuroscience*, vol. 21, no. 4, pp. 1179–1188, 2001.

[20] M. Jana, C. A. Palencia, and K. Pahan, "Fibrillar amyloid-β peptides activate microglia via TLR2: Implications for Alzheimer's disease," *Journal of Immunology*, vol. 181, no. 10, pp. 7254–7262, 2008.

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

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

[23] C. M. Sondag, G. Dhawan, and C. K. Combs, "β amyloid oligomers and fibrils stimulate differential activation of primary microglia," *Journal of Neuroinflammation*, vol. 6, no. 1, pp. 3693–3706, 2009.

[24] I. Maezawa, P. I. Zimin, H. Wulff, and L. W. Jin, "Amyloid-β protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity," *Journal of Biological Chemistry*, vol. 286, no. 5, pp. 3693–3706, 2011.

[25] F. G. de Felice, P. T. Velasco, M. P. Lambert et al., "Aβ oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine," *Journal of Biological Chemistry*, vol. 282, no. 15, pp. 11590–11601, 2007.

[26] T. Schilling and C. Eder, "Amyloid-β-induced reactive oxygen species production and priming are differentially regulated by ion channels in microglia," *Journal of Cellular Physiology*, vol. 226, no. 12, pp. 3295–3302, 2011.

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

[28] D. Davalos, J. Grutzendler, G. Yang et al., "ATP mediates rapid microglial response to local brain injury *in vivo*," *Nature Neuroscience*, vol. 8, no. 6, pp. 752–758, 2005.

[29] S. Koizumi, Y. Shigemoto-Mogami, K. Nasu-Tada et al., "UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis," *Nature*, vol. 446, no. 7139, pp. 1091–1095, 2007.

[30] E. K. de Jong, I. M. Dijkstra, M. Hensens et al., "Vesicle-mediated transport and release of CCL21 in endangered neurons: a possible explanation for microglia activation remote from a primary lesion," *Journal of Neuroscience*, vol. 25, no. 33, pp. 7548–7557, 2005.

[31] K. Färber and H. Kettenmann, "Physiology of microglial cells," *Brain Research Reviews*, vol. 48, no. 2, pp. 133–143, 2005.

[32] M. Noda, H. Nakanishi, J. Nabekura, and N. Akaike, "AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia," *Journal of Neuroscience*, vol. 20, no. 1, pp. 251–258, 2000.

[33] D. L. Taylor, L. T. Diemel, M. L. Cuzner, and J. M. Pocock, "Activation of group II metabotropic glutamate receptors underlies microglial reactivity and neurotoxicity following stimulation with chromogranin A, a peptide up-regulated in Alzheimer's disease," *Journal of Neurochemistry*, vol. 82, no. 5, pp. 1179–1191, 2002.

[34] T. M. Piers, S. J. Heales, and J. M. Pocock, "Positive allosteric modulation of metabotropic glutamate receptor 5 down-regulates fibrinogen-activated microglia providing neuronal protection," *Neuroscience Letters*, vol. 505, no. 2, pp. 140–145, 2011.

[35] L. Lyras, N. J. Cairns, A. Jenner, P. Jenner, and B. Halliwell, "An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease," *Journal of Neurochemistry*, vol. 68, no. 5, pp. 2061–2069, 1997.

[36] S. Shimohama, H. Tanino, N. Kawakami et al., "Activation of NADPH oxidase in Alzheimer's disease brains," *Biochemical and Biophysical Research Communications*, vol. 273, no. 1, pp. 5–9, 2000.

[37] J. El Khoury, S. E. Hickman, C. A. Thomas, L. Cao, S. C. Silverstein, and J. D. Loike, "Scavenger receptor-mediated adhesion of microglia to β-amyloid fibrils," *Nature*, vol. 382, no. 6593, pp. 716–719, 1996.

[38] D. R. McDonald, K. R. Brunden, and G. E. Landreth, "Amyloid fibrils activate tyrosine kinase-dependent signaling and superoxide production in microglia," *Journal of Neuroscience*, vol. 17, no. 7, pp. 2284–2294, 1997.

[39] M. L. Block, "NADPH oxidase as a therapeutic target in Alzheimer's disease," *BMC Neuroscience*, vol. 9, supplement 2, article S8, 2008.

[40] M. Manczak, T. S. Anekonda, E. Henson, B. S. Park, J. Quinn, and P. H. Reddy, "Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: Implications for free radical generation and oxidative damage in disease progression," *Human Molecular Genetics*, vol. 15, no. 9, pp. 1437–1449, 2006.

[41] P. H. Reddy and M. F. Beal, "Amyloid β, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease," *Trends in Molecular Medicine*, vol. 14, no. 2, pp. 45–53, 2008.

[42] T. Alkam, A. Nitta, H. Mizoguchi, A. Itoh, and T. Nabeshima, "A natural scavenger of peroxynitrites, rosmarinic acid, protects against impairment of memory induced by Aβ25-35," *Behavioural Brain Research*, vol. 180, no. 2, pp. 139–145, 2007.

[43] R. H. Milton, R. Abeti, S. Averaimo et al., "CLIC1 function is required for β-amyloid-induced generation of reactive oxygen species by microglia," *Journal of Neuroscience*, vol. 28, no. 45, pp. 11488–11499, 2008.

[44] M. D. Maines, "The heme oxygenase system: a regulator of second messenger gases," *Annual Review of Pharmacology and Toxicology*, vol. 37, pp. 517–554, 1997.

[45] H. Atamna and W. H. Frey II, "A role for heme in Alzheimer's disease: heme binds amyloid β and has altered metabolism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 30, pp. 11153–11158, 2004.

[46] M. Takahashi, S. Doré, C. D. Ferris et al., "Amyloid precursor proteins inhibit heme oxygenase activity and augment neurotoxicity in Alzheimer's disease," *Neuron*, vol. 28, no. 2, pp. 461–473, 2000.

[47] D. Piani, M. Spranger, K. Frei, A. Schaffner, and A. Fontana, "Macrophage-induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines," *European Journal of Immunology*, vol. 22, no. 9, pp. 2429–2436, 1992.

[48] S. W. Barger and A. S. Basile, "Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function," *Journal of Neurochemistry*, vol. 76, no. 3, pp. 846–854, 2001.

[49] G. E. Hardingham, Y. Fukunaga, and H. Bading, "Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways," *Nature Neuroscience*, vol. 5, no. 5, pp. 405–414, 2002.

[50] A. Ivanov, C. Pellegrino, S. Rama et al., "Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons," *Journal of Physiolgy*, vol. 572, pp. 789–798, 2006.

[51] F. le veille, F. El Gaamouch, E. Gouix et al., "Neuronal viability is controlled by a functional relation between synaptic and extrasynaptic NMDA receptors," *The FASEB Journal*, vol. 22, no. 12, pp. 4258–4271, 2008.

[52] R. I. Stanika, N. B. Pivovarova, C. A. Brantner, C. A. Watts, C. A. Winters, and S. B. Andrews, "Coupling diverse routes of calcium entry to mitochondrial dysfunction and glutamate excitotoxicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 24, pp. 9854–9859, 2009.

[53] K. Bordji, J. Becerril-Ortega, O. Nicole, and A. Buisson, "Activation of extrasynaptic, but not synaptic, NMDA receptors modifies amyloid precursor protein expression pattern and increases amyloid-β production," *Journal of Neuroscience*, vol. 30, no. 47, pp. 15927–15942, 2010.

[54] N. Hori and D. O. Carpenter, "Functional and morphological changes induced by transient *in vivo* ischemia," *Experimental Neurology*, vol. 129, no. 2, pp. 279–289, 1994.

[55] J. W. Swann, S. Al-Noori, M. Jiang, and C. L. Lee, "Spine loss and other dendritic abnormalities in epilepsy," *Hippocampus*, vol. 10, no. 5, pp. 617–625, 2000.

[56] S. Goel, S. B. Wharton, L. P. Brett, and I. R. Whittle, "Morphological changes and stress responses in neurons in cerebral cortex infiltrated by diffuse astrocytoma," *Neuropathology*, vol. 23, no. 4, pp. 262–270, 2003.

[57] T. C. Dickson, C. E. King, G. H. McCormack, and J. C. Vickers, "Neurochemical diversity of dystrophic neurites in the early and late stages of Alzheimer's disease," *Experimental Neurology*, vol. 156, no. 1, pp. 100–110, 1999.

[58] J. Liang, H. Takeuchi, Y. Doi et al., "Excitatory amino acid transporter expression by astrocytes is neuroprotective against microglial excitotoxicity," *Brain Research*, vol. 1210, pp. 11–19, 2008.

[59] I. Yawata, H. Takeuchi, Y. Doi, J. Liang, T. Mizuno, and A. Suzumura, "Macrophage-induced neurotoxicity is mediated by glutamate and attenuated by glutaminase inhibitors and gap junction inhibitors," *Life Sciences*, vol. 82, no. 21-22, pp. 1111–1116, 2008.

[60] H. Takeuchi, S. Jin, H. Suzuki et al., "Blockade of microglial glutamate release protects against ischemic brain injury," *Experimental Neurology*, vol. 214, no. 1, pp. 144–146, 2008.

[61] J. Shijie, H. Takeuchi, I. Yawata et al., "Blockade of glutamate release from microglia attenuates experimental autoimmune encephalomyelitis in mice," *Tohoku Journal of Experimental Medicine*, vol. 217, no. 2, pp. 87–92, 2009.

[62] H. Takeuchi, H. Mizoguchi, Y. Doi et al., "Blockade of gap junction hemichannel suppresses disease progression in mouse models of amyotrophic lateral sclerosis and Alzheimer's disease," *PLoS One*, vol. 6, no. 6, Article ID e21108, 2011.

[63] J. Brito-Moreira, A. C. Paula-Lima, T. R. Bomfim et al., "Aβ oligomers induce glutamate release from hippocampal neurons," *Current Alzheimer Research*, vol. 8, no. 5, pp. 552–562, 2011.

[64] J. Jiao, B. Xue, L. Zhang et al., "Triptolide inhibits amyloid-β1-42-induced TNF-α and IL-1β production in cultured rat microglia," *Journal of Neuroimmunology*, vol. 205, no. 1-2, pp. 32–36, 2008.

[65] G. Sebastiani, C. Morissette, C. Lagacé et al., "The cAMP-specific phosphodiesterase 4B mediates Aβ-induced microglial activation," *Neurobiology of Aging*, vol. 27, no. 5, pp. 691–701, 2006.

[66] R. Kuno, J. Wang, J. Kawanokuchi, H. Takeuchi, T. Mizuno, and A. Suzumura, "Autocrine activation of microglia by tumor necrosis factor-α," *Journal of Neuroimmunology*, vol. 162, no. 1-2, pp. 89–96, 2005.

[67] M. Kaul, G. A. Garden, and S. A. Lipton, "Pathways to neuronal injury and apoptosis in HIV-associated dementia," *Nature*, vol. 410, no. 6831, pp. 988–994, 2001.

[68] D. L. Taylor, F. Jones, E. S. F. Chen Seho Kubota, and J. M. Pocock, "Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor α-induced neurotoxicity in concert with microglial-derived Fas ligand," *Journal of Neuroscience*, vol. 25, no. 11, pp. 2952–2964, 2005.

[69] E. A. Eugenín, D. Eckardt, M. Theis, K. Willecke, M. V. L. Bennett, and J. C. Sáez, "Microglia at brain stab wounds express connexin 43 and *in vitro* form functional gap junctions after treatment with interferon-γ and tumor necrosis factor-α," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 7, pp. 4190–4195, 2001.

[70] Z. Yu, G. Cheng, X. Wen, G. D. Wu, W. T. Lee, and D. Pleasure, "Tumor necrosis factor α increases neuronal vulnerability to excitotoxic necrosis by inducing expression of the AMPA-glutamate receptor subunit GluR1 via an acid sphingomyelinase-and NF-κB-dependent mechanism," *Neurobiology of Disease*, vol. 11, no. 1, pp. 199–213, 2002.

[71] L. Bernardino, S. Xapelli, A. P. Silva et al., "Modulator effects of interleukin-1β and tumor necrosis factor-α on AMPA-induced excitotoxicity in mouse organotypic hippocampal slice cultures," *Journal of Neuroscience*, vol. 25, no. 29, pp. 6734–6744, 2005.

[72] A. M. Floden, S. Li, and C. K. Combs, "β-Amyloid-stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor α and NMDA receptors," *Journal of Neuroscience*, vol. 25, no. 10, pp. 2566–2575, 2005.

[73] A. H. Moore and M. K. O'Banion, "Neuroinflammation and anti-inflammatory therapy for Alzheimer's disease," *Advanced Drug Delivery Reviews*, vol. 54, no. 12, pp. 1627–1656, 2002.

[74] M. A. Lynch, "Age-related impairment in long-term potentiation in hippocampus: A role for the cytokine, interleukin-1β?" *Progress in Neurobiology*, vol. 56, no. 5, pp. 571–589, 1998.

[75] A. Halle, V. Hornung, G. C. Petzold et al., "The NALP3 inflammasome is involved in the innate immune response to amyloid-β," *Nature Immunology*, vol. 9, no. 8, pp. 857–865, 2008.

[76] E. Torres, M. D. Gutierrez-Lopez, A. Mayado, A. Rubio, E. O'Shea, and M. I. Colado, "Changes in interleukin-1 signal modulators induced by 3,4-methylenedioxymethamphetamine (MDMA): regulation by CB2 receptors and implications for neurotoxicity," *Journal of Neuroinflammation*, vol. 8, p. 53, 2011.

[77] W. Cerpa, G. G. Farías, J. A. Godoy, M. Fuenzalida, C. Bonansco, and N. C. Inestrosa, "Wnt-5a occludes Aβ oligomer-induced depression of glutamatergic transmission in hippocampal neurons," *Molecular Neurodegeneration*, vol. 5, no. 1, p. 1, 2010.

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

[79] M. Miyanishi, K. Tada, M. Koike, Y. Uchiyama, T. Kitamura, and S. Nagata, "Identification of Tim4 as a phosphatidylserine receptor," *Nature*, vol. 450, no. 7168, pp. 435–439, 2007.

[80] R. Hanayama, M. Tanaka, K. Miwa, A. Shinohara, A. Iwamatsu, and S. Nagata, "Identification of a factor that links apoptotic cells to phagocytes," *Nature*, vol. 417, no. 6885, pp. 182–187, 2002.

[81] F. Leonardi-Essmann, M. Emig, Y. Kitamura, R. Spanagel, and P. J. Gebicke-Haerter, "Fractalkine-upregulated milk-fat globule EGF factor-8 protein in cultured rat microglia," *Journal of Neuroimmunology*, vol. 160, no. 1-2, pp. 92–101, 2005.

[82] P. Iribarren, K. Chen, J. Hu et al., "CpG-containing oligodeoxynucleotide promotes microglial cell uptake of amyloid β 1-42 peptide by up-regulating the expression of the G-protein-coupled receptor mFPR2," *The FASEB Journal*, vol. 19, no. 14, pp. 2032–2034, 2005.

[83] K. Chen, P. Iribarren, J. Hu et al., "Activation of toll-like receptor 2 on microglia promotes cell uptake of Alzheimer disease-associated amyloid β peptide," *Journal of Biological Chemistry*, vol. 281, no. 6, pp. 3651–3659, 2006.

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

[85] K. Tahara, H. D. Kim, J. J. Jin, J. A. Maxwell, L. Li, and K. I. Fukuchi, "Role of toll-like receptor signalling in Aβ uptake and clearance," *Brain*, vol. 129, pp. 3006–3019, 2006.

[86] P. B. Tran and R. J. Miller, "Chemokine receptors: signposts to brain development and disease," *Nature Reviews Neuroscience*, vol. 4, no. 6, pp. 444–455, 2003.

[87] M. Noda, Y. Doi, J. Liang et al., "Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression," *Journal of Biological Chemistry*, vol. 286, no. 3, pp. 2308–2319, 2011.

[88] D. Morse, L. Lin, A. M. K. Choi, and S. W. Ryter, "Heme oxygenase-1, a critical arbitrator of cell death pathways in lung injury and disease," *Free Radical Biology and Medicine*, vol. 47, no. 1, pp. 1–12, 2009.

[89] P. J. Syapin, "Regulation of haeme oxygenase-1 for treatment of neuroinflammation and brain disorders," *British Journal of Pharmacology*, vol. 155, no. 5, pp. 623–640, 2008.

[90] T. Mizuno, J. Kawanokuchi, K. Numata, and A. Suzumura, "Production and neuroprotective functions of fractalkine in the central nervous system," *Brain Research*, vol. 979, no. 1-2, pp. 65–70, 2003.

[91] S. H. Cho, B. Sun, Y. Zhou et al., "CX3CR1 protein signaling modulates microglial activation and protects against plaque-independent cognitive deficits in a mouse model of Alzheimer disease," *Journal of Biological Chemistry*, vol. 286, no. 37, pp. 32713–32722, 2011.

[92] J. T. Rogers, J. M. Morganti, A. D. Bachstetter et al., "CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity," *Journal of Neuroscience*, vol. 31, no. 45, pp. 16241–16250, 2011.

[93] S. Lee, N. H. Varvel, M. E. Konerth et al., "CX3CR1 deficiency alters microglial activation and reduces β-amyloid deposition in two Alzheimer's disease mouse models," *American Journal of Pathology*, vol. 177, no. 5, pp. 2549–2562, 2010.

[94] H. Lin, E. Lee, K. Hestir et al., "Discovery of a cytokine and its receptor by functional screening of the extracellular proteome," *Science*, vol. 320, no. 5877, pp. 807–811, 2008.

[95] T. Mizuno, Y. Doi, H. Mizoguchi et al., "Interleukin-34 selectively enhances the neuroprotective effects of microglia to attenuate oligomeric amyloid-β neurotoxicity," *The American Journal of Pathology*, vol. 179, no. 4, pp. 2016–2027, 2011.

[96] Z. Zhao, Z. Xiang, V. Haroutunian, J. D. Buxbaum, B. Stetka, and G. M. Pasinetti, "Insulin degrading enzyme activity selectively decreases in the hippocampal formation of cases at high risk to develop Alzheimer's disease," *Neurobiology of Aging*, vol. 28, no. 6, pp. 824–830, 2007.

[97] M. A. Leissring, W. Farris, A. Y. Chang et al., "Enhanced proteolysis of β-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death," *Neuron*, vol. 40, no. 6, pp. 1087–1093, 2003.

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

[99] V. Boissonneault, M. Filali, M. Lessard, J. Relton, G. Wong, and S. Rivest, "Powerful beneficial effects of macrophage colony-stimulating factor on β-amyloid deposition and cognitive impairment in Alzheimer's disease," *Brain*, vol. 132, no. 4, pp. 1078–1092, 2009.

[100] T. Chihara, S. Suzu, R. Hassan et al., "IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation," *Cell Death and Differentiation*, vol. 17, no. 12, pp. 1917–1927, 2010.

[101] A. H. Dalpke, M. K. H. Schäfer, M. Frey et al., "Immunostimulatory CpG-DNA activates murine microglia," *Journal of Immunology*, vol. 168, no. 10, pp. 4854–4863, 2002.

[102] A. I. Iliev, A. K. Stringaris, R. Nau, and H. Neumann, "Neuronal injury mediated via stimulation of microglial toll-like receptor-9 (TLR9)," *The FASEB Journal*, vol. 18, no. 2, pp. 412–414, 2004.

[103] Y. J. Surh, J. K. Kundu, M. H. Li, H. K. Na, and Y. N. Cha, "Role of Nrf2-mediated heme oxygenase-1 upregulation in adaptive survival response to nitrosative stress," *Archives of Pharmacal Research*, vol. 32, no. 8, pp. 1163–1176, 2009.

[104] T. W. Kensler, N. Wakabayashi, and S. Biswal, "Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway," *Annual Review of Pharmacology and Toxicology*, vol. 47, pp. 89–116, 2007.

[105] K. Kanninen, R. Heikkinen, T. Malm et al., "Intrahippocampal injection of a lentiviral vector expressing Nrf2 improves spatial learning in a mouse model of Alzheimer's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 38, pp. 16505–16510, 2009.

Hindawi Publishing Corporation
International Journal of Alzheimer's Disease
Volume 2012, Article ID 685739, 11 pages
doi:10.1155/2012/685739

Research Article

Microglial Amyloid-β1-40 Phagocytosis Dysfunction Is Caused by High-Mobility Group Box Protein-1: Implications for the Pathological Progression of Alzheimer's Disease

Kazuyuki Takata,[1] Tetsuya Takada,[1] Aina Ito,[1] Mayo Asai,[1] Manami Tawa,[1] Yuki Saito,[1] Eishi Ashihara,[1,2] Hidekazu Tomimoto,[3] Yoshihisa Kitamura,[1] and Shun Shimohama[4]

[1] *Department of Clinical and Translational Physiology, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan*
[2] *Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan*
[3] *Department of Neurology, Mie University, Graduate School of Medicine, Tsu 514-8507, Japan*
[4] *Department of Neurology, School of Medicine, Sapporo Medical University, S1W16, Chuo-ku, Sapporo 060-8543, Japan*

Correspondence should be addressed to Kazuyuki Takata, kaz@mb.kyoto-phu.ac.jp

Received 30 November 2011; Accepted 24 February 2012

Academic Editor: Akio Suzumura

In Alzheimer disease (AD) patient brains, the accumulation of amyloid-β (Aβ) peptides is associated with activated microglia. Aβ is derived from the amyloid precursor protein; two major forms of Aβ, that is, Aβ1-40 (Aβ40) and Aβ1-42 (Aβ42), exist. We previously reported that rat microglia phagocytose Aβ42, and high mobility group box protein 1 (HMGB1), a chromosomal protein, inhibits phagocytosis. In the present study, we investigated the effects of exogenous HMGB1 on rat microglial Aβ40 phagocytosis. In the presence of exogenous HMGB1, Aβ40 markedly increased in microglial cytoplasm, and the reduction of extracellular Aβ40 was inhibited. During this period, HMGB1 was colocalized with Aβ40 in the cytoplasm. Furthermore, exogenous HMGB1 inhibited the degradation of Aβ40 induced by the rat microglial cytosolic fraction. Thus, extracellular HMGB1 may internalize with Aβ40 in the microglial cytoplasm and inhibit Aβ40 degradation by microglia. This may subsequently delay Aβ40 clearance. We further confirmed that in AD brains, the parts of senile plaques surrounded by activated microglia are composed of Aβ40, and extracellular HMGB1 is deposited on these plaques. Taken together, microglial Aβ phagocytosis dysfunction may be caused by HMGB1 that accumulates extracellularly on Aβ plaques, and it may be critically involved in the pathological progression of AD.

1. Introduction

Alzheimer's disease (AD) is characterized by the deposition of amyloid-β (Aβ) plaques, accumulation of neurofibrillary tangles (NFTs), and loss of synapses and neurons in particular brain areas [1]. Experimental studies using transgenic AD mouse models have demonstrated that Aβ accelerates NFT formation [2, 3] and is closely associated with synaptic damage [4]. In contrast, Aβ reduction in the brain by Aβ immunization restores cognitive functions in transgenic AD mouse models [5–9] and also appears to slow cognitive decline in human AD patients [10]. Thus, the accumulation of Aβ may play a key role in the pathogenesis of AD [11].

Aβ is derived from the sequential proteolysis of amyloid precursor protein (APP) by β- and γ-secretases and is composed of 37–43 amino acid residues because γ-secretase, which is a protein complex including presenilin (PS), generates the C-terminal of Aβ with different lengths [12]. Among the variations in Aβ, Aβ1-40 (Aβ40) and Aβ1-42 (Aβ42) are the major species found in AD brains. The most predominant species deposited in Aβ plaques is Aβ42 [13], which is prone to aggregation [14] and indicates increased neurotoxicity [15]. On the other hand, Aβ40 is the major

soluble species; its secretion is 10-fold more than that of Aβ42 in normal brains. A previous study demonstrated that the deposition of Aβ40 in AD brains is particularly correlated with synaptic and neuronal loss [16]. Thus, lowering the concentration of Aβ40 and Aβ42 in the brain may serve as a disease-modifying therapy for AD patients.

Activated microglia accumulate on Aβ plaques in AD brains. Although microglial accumulation was initially believed to be involved in the formation of Aβ plaques [17], experimental studies later demonstrated the ability of microglia to phagocytose Aβ peptides [18, 19]. In addition, we demonstrated microglial contribution in Aβ42 clearance using primary cultured rat microglia [20–25]. However, it has been reported that microglial dystrophy occurs in aging human brains [26], and the age-related disability of microglial Aβ phagocytosis has been demonstrated experimentally [27]. Thus, the dysfunction of microglial Aβ phagocytosis appears to be closely involved in the progression of AD pathology.

High-mobility group box protein 1 (HMGB1) is an abundant nonhistone chromosomal protein that is released from cells undergoing necrosis [28, 29]. The released HMGB1 behaves like an inflammatory mediator by acting on receptor for advanced glycation end products (RAGE) and Toll-like receptors (TLRs) 2 and 4 [30, 31]. We have previously reported that HMGB1 is extracellularly associated with Aβ plaques in AD brain and is involved in the pathogenesis of AD as an inhibitory factor against microglial Aβ42 phagocytosis by interfering with uptake [32, 33]. However, the effect of extracellular HMGB1 on the microglial phagocytosis of Aβ40, but not Aβ42, has not been elucidated. Therefore, in the present study, we analyzed rat microglial Aβ40 phagocytosis in the presence and absence of exogenous HMGB1.

2. Materials and Methods

2.1. Primary Culture of Rat Microglia and Drug Treatment. The primary culture experimental procedure was reviewed and approved by the Committee for Animal Research at Kyoto Pharmaceutical University. Primary cultured microglia (over 97% pure) were prepared, as described previously [32]. Briefly, brain tissues were isolated from newborn Wistar rats, minced, and gently dissociated by trituration in Dulbecco's modified Eagle medium (DMEM). The tissue suspension was filtered through a 50 μm diameter nylon mesh into 50 mL tubes, and cells were collected by centrifugation at 200 $\times$g for 10 min. Cells were resuspended in DMEM with 10% fetal calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin; they were then plated onto 100 mm diameter dishes at 37°C in humidified 5% CO_2/95% air. We then harvested the floating microglia from mixed glial cultures. Microglia were transferred to 24-well plates (3.0×10^5 cells/well) and were allowed to adhere at 37°C overnight; they were then treated with sterilized phosphate-buffered saline (PBS) as the vehicle or synthetic human Aβs (Aβ40 or Aβ42; Anaspec, San Jose, CA) in the presence or absence of calf thymus-purified HMGB1 (WAKO Chemicals, Osaka, Japan). We previously demonstrated that 1 μM Aβ42 for 12 h markedly phagocytosed by rat microglia [20], and 0.3 μM HMGB1 inhibits the phagocytosis [32, 33]. When Aβ40 at 1–3 μM were added into the culture, we could detect Aβ40 phagocytosed by rat microglia by Western blot analysis [25]. Therefore, in the present study, we adopted the concentrations at 1 μM and 0.3 μM for the treatments with Aβs and HMGB1, respectively. To make the experimental conditions more accurate, we dissolved the lyophilized human Aβ peptides in distilled and sterilized water at a high concentration, and small aliquots were kept at −80°C until use. Subsequently, Aβ stock solutions were diluted using sterilized PBS, and once Aβ was thawed, no Aβ was refrozen to eliminate variance due to repeated freezing and thawing.

2.2. Immunocytochemistry. Twelve hours after Aβ treatment, microglia were gently rinsed three times with PBS and then fixed with 4% paraformaldehyde in 100 mM phosphate buffer (PB) for 30 min. Cells were then incubated with a mouse monoclonal antibody against Aβ (clone 6E10, 1 : 1000; Chemicon, Temecula, CA) and a rabbit polyclonal antibody against HMGB1 (1 : 1000; BD Pharmingen, San Diego, CA). The primary antibodies were followed by application of a rhodamine-labeled anti-mouse immunoglobulin (Ig)G antibody and fluorescein isothiocyanate-labeled anti-rabbit IgG antibody (each diluted 1 : 500; Molecular Probes, Eugene, OR). Furthermore, cells were incubated with Hoechst 33258 (1 : 5000; Molecular Probes) to visualize microglial nuclei. Labeled fluorescence was detected using a laser scanning confocal microscope LSM 510 (Carl Zeiss, Jena, Germany).

2.3. Aβ Phagocytosis and Clearance Assay by Western Blot Analysis. Twelve hours after Aβ treatment, microglia and culture media were collected and lysed with Laemmli's sample buffer and then analyzed by Western blot analysis. Briefly, samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 20% polyacrylamide gels in Tricine buffer). Proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA) by electroelution and then incubated with a mouse monoclonal antibody against Aβ (clone 6E10, 1 : 2000; Chemicon), followed by a horseradish peroxidase-linked secondary antibody against mouse IgG (1 : 1,000; Amersham, Buckinghamshire, UK). Subsequently, protein bands were detected on radiographic films (Kodak, Rochester, NY) using a chemiluminescence kit (ECL kit; Amersham). For semi-quantitative analysis, radiographic films were scanned with a CCD color scanner (DuoScan, AGFA, Mortsel, Belgium) and then analyzed densitometrically using the public domain US National Institutes of Health image 1.56 program.

2.4. Aβ Degradation Assay. Microglia were harvested and resuspended in 100 mM Tris-HCl buffer (pH 7.5) containing 10 mM KCl, 1.5 mM $MgCl_2$, and 1 mM DTT and then homogenized. After centrifugation (50,000 $\times$g) for 20 min at 4°C, the protein concentration of the supernatant was measured and used as the microglial cytosolic fraction.

The Aβ peptide (3 μM Aβ40 or Aβ42) was incubated with the microglial cytosolic fraction (final concentration of 1 mg/mL) in the presence or absence of 0.3 μM HMGB1. At the time points of 0, 6, and 12 h after incubation, Laemmli's sample buffer was added, and samples were boiled at 100°C for 5 min to stop Aβ degradation. Subsequently, samples were analyzed using the antibody against Aβ (clone 6E10, 1 : 2000; Chemicon) by Western blot analysis, as described previously.

2.5. Immunoprecipitation. HMGB1 (1.5 μg, 2.6 μM) was mixed with 3 μg of synthetic Aβ40 (37.5 μM) in PBS. Twenty-four hours after incubation, the antibody (10 μg of IgG) against HMGB1 (BD Pharmingen) or Aβ (clone 6E10; Chemicon) was added to the mixture and further incubated for 2 h at 4°C. Protein A-Sepharose (50 μL of a 50% slurry) was then added, and the mixture was incubated overnight at 4°C. After centrifugation, immunoprecipitates were resuspended in Laemmli's sample buffer. Subsequently, samples were analyzed using the antibody against HMGB1 (1 : 1000; BD Pharmingen) or Aβ (clone 6E10, 1 : 2000; Chemicon) by Western blot analysis, as described previously.

2.6. Immunohistochemical Study Using Human AD Brain Sections. All experiments using human samples were performed in accordance with the guidelines of the ethical committees of Kyoto Pharmaceutical University. Informed consent was obtained from all subjects. For histological examination, frontal cortex tissue from a patient who was clinically and histopathologically diagnosed as human AD (age, 67 years) was used. Neuropathological assessment of AD was conducted in accordance with the criteria of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Dissected tissue blocks were fixed in 10% formalin and transferred to a 15% sucrose solution in 100 mM PB containing 0.1% sodium azide at 4°C. The cryoprotected brain blocks were cut into 20 μm sections on a cryostat, and the collected sections were stored in PBS containing 0.3% Triton X-100 (PBS-T) and 0.1% sodium azide at 4°C until use.

Immunohistochemical study was essentially performed as described previously [34]. Free-floating human brain sections were treated with 0.1% hydrogen peroxide for 30 min to quench endogenous peroxidase activity; they were then incubated with 1% goat serum to block nonspecific binding in PBS. Sections were then incubated with a mouse monoclonal antibody against Aβ40 (1 : 1000; nanoTools, Teningen, Germany) and rabbit polyclonal antibody against Aβ42 (1 : 1000; IBL, Gunma, Japan), a rabbit polyclonal antibody against Aβ40 (1 : 1000; IBL) and mouse monoclonal antibody against human leukemia antigen (HLA)-DR (1 : 50; Dako, Glostrup, Denmark), or a rabbit polyclonal antibody against Aβ42 (1 : 1000; IBL) and mouse monoclonal antibody against HLA-DR (1 : 50; Dako) in PBS-T with 0.1% sodium azide for 4 days at 4°C. After washing with PBS-T, the sections were incubated with biotinylated anti-rabbit IgG antibody (1 : 2000; Vector Laboratories, Burlingame, CA) for 2 h at room temperature. The sections were then incubated with avidin peroxidase (1 : 4000; ABC Elite Kit; Vector Laboratories) for 1 h at room temperature. Subsequently, labeling was visualized by incubation with 50 mM Tris-HCl buffer (pH 7.6) containing 0.02% 3,3′-diaminobenzidine (DAB) and 0.0045% hydrogen peroxide with nickel enhancement using 0.6% nickel ammonium sulfate, which yielded a dark blue color. In the second cycle, sections were incubated with a biotinylated anti-mouse IgG antibody (1 : 2000; Vector Laboratories) for 2 h at room temperature. The sections were then incubated with avidin peroxidase (1 : 4000; ABC Elite Kit; Vector Laboratories) for 1 h at room temperature. Subsequently, the DAB reaction was performed without nickel ammonium sulfate, which yielded a brown color.

In laser confocal microscopic analysis, human AD brain sections were treated with 1% goat serum to block nonspecific binding in PBS. Sections were then coincubated with a rabbit polyclonal antibody against Aβ40 (1 : 1000; IBL) and a mouse monoclonal antibody against HLA-DR (1 : 50; Dako) or a mouse monoclonal antibody against Aβ40 (1 : 1000; nanoTools) and a rabbit polyclonal antibody against HMGB1 (1 : 1000; BD Pharmingen) in PBS-T with 0.1% sodium azide for 4 days at 4°C. The primary antibodies were probed with Alexa Fluor 546-labeled anti-rabbit IgG antibody and Alexia Fluor 488-labeled anti-mouse IgG antibody or Alexa Fluor 546-labeled anti-mouse IgG antibody and Alexia Fluor 488-labeled anti-rabbit IgG antibody (each diluted 1 : 500; Molecular Probes). Subsequently, fluorescence was observed using a laser scanning confocal microscope LSM 510 (Carl Zeiss).

2.7. Statistical Evaluation. Results of the densitometric analysis are given as the mean ± standard error of mean. The statistical significance of differences was determined by analysis of variance. Further statistical analysis for *post hoc* comparisons was conducted using the Bonferroni/Dunn test (StatView, Abacus Concepts, Berkeley, CA).

3. Results

3.1. Binding of HMGB1 with Aβ40. In our previous study, we found that HMGB1 is extracellularly accumulated on Aβ plaques in AD brains and further demonstrated that HMGB1 binds to Aβ42 in *in vitro* cell-free study [33]. In the present cell-free study, we first examined the binding affinity of HMGB1 for Aβ40. Following incubation of the HMGB1 peptide alone, a 29 kDa band of HMGB1 and its high-molecular-weight aggregates was detected, while an approximately 33 kDa band (arrow in Figure 1(a)), which is believed to be a complex of HMGB1 and Aβ40, appeared as an upper band 6 h after incubation of HMGB1 and Aβ40 peptides (Figure 1(a)). Following incubation with Aβ40 (Figure 1(b)), monomers and oligomers of Aβ40 were the major components present in the absence of HMGB1 at each time point. Predictably, the 33 kDa band, which seemed to be a complex of HMGB1 and Aβ40, was detected by the addition of the HMGB1 peptide (arrow in Figure 1(b)).

To confirm the binding affinity between HMGB1 and Aβ40, we further examined immunoprecipitation using the

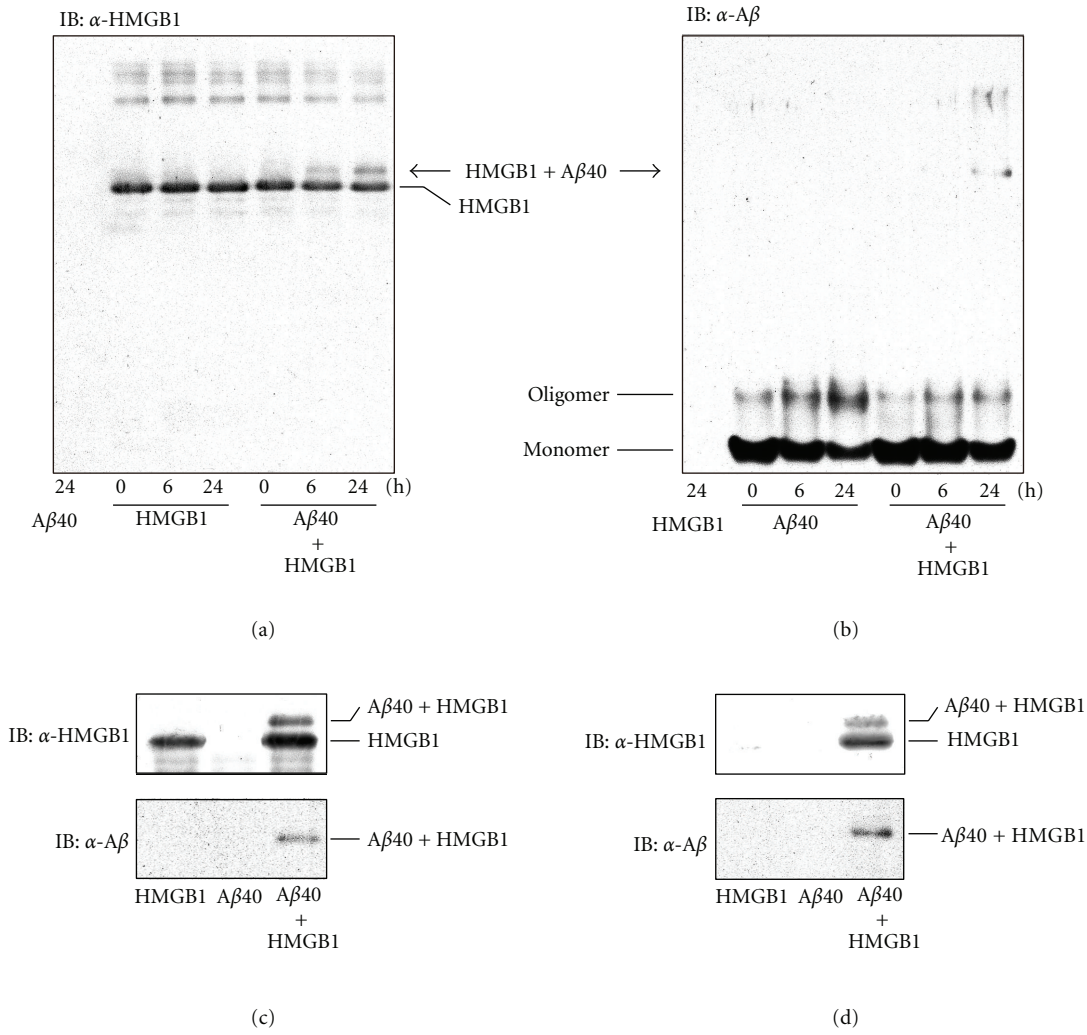


Figure 1: Binding affinity of HMGB1 for Aβ40. (a, b) After incubation of HMGB1, Aβ40, and the mixture of HMGB1 with Aβ40 for 0, 6, and 24 h, samples were analyzed by Western blot analysis using the anti-HMGB1 antibody (a) or anti-Aβ antibody (b). (c, d) After incubation of HMGB1, Aβ40, and the mixture of HMGB with Aβ40 for 24 h, samples were immunoprecipitated using the anti-HMGB1 antibody (c) or anti-Aβ antibody (d). The precipitates were then analyzed by Western blot analysis using the anti-HMGB1 antibody and anti-Aβ antibody.

anti-HMGB1 antibody (Figure 1(c)) or anti-Aβ antibody (Figure 1(d)). As a result, in the mixture of HMGB1 and Aβ, the complex of HMGB1 and Aβ40 (approximately 33 kDa) was immunoprecipitated with A-Sepharose-linked antibodies against HMGB1 (Figure 1(c)) or Aβ (Figure 1(d)). These results demonstrated that HMGB1 had a binding affinity for Aβ40.

3.2. Microglial Aβ Phagocytosis and Effect of Exogenous HMGB1. We previously demonstrated that microglia markedly phagocytose Aβ42 [25], and extracellular HMGB1 inhibits phagocytosis on the cell surface [32, 33]. In the present study, we analyzed the microglial Aβ40 phagocytosis and the effects of extracellular HMGB1 on phagocytosis using laser confocal microscopy (Figure 2). Endogenous HMGB1 was detected in the nuclei of primary cultured rat microglia (Figures 2(a)–2(f), cyan). When treated with the vehicle (Figure 2(a)) or HMGB1 alone (Figure 2(b)), no Aβ immunoreactivity was detected. Consistent with previous studies, in the presence of Aβ42, microglia phagocytosed Aβ42 (Figure 2(c), red), exogenous HMGB1 was colocalized with Aβ42 on the microglial cell surface, and Aβ internalization was inhibited (Figure 2(d), yellow). When treated with Aβ40, the immunoreactivity of Aβ40 was barely detected in the microglial cytoplasm (Figure 2(e), red). Interestingly, in the presence of exogenous HMGB1, small vesicle-like immunoreactivities of Aβ40 (Figure 2(f), red) and HMGB1 (Figure 2(f), green) were markedly increased in the microglial cytoplasm, and parts of them were colocalized with each other (Figure 2(f), yellow).

3.3. Amounts of Aβ40 inside and outside Microglia and Effect of Exogenous HMGB1. Twelve hours after Aβ40 treatment, microglial cell lysate and conditioned medium were collected and subjected to Western blot analysis; semiquantitative analysis was then examined to measure the concentration

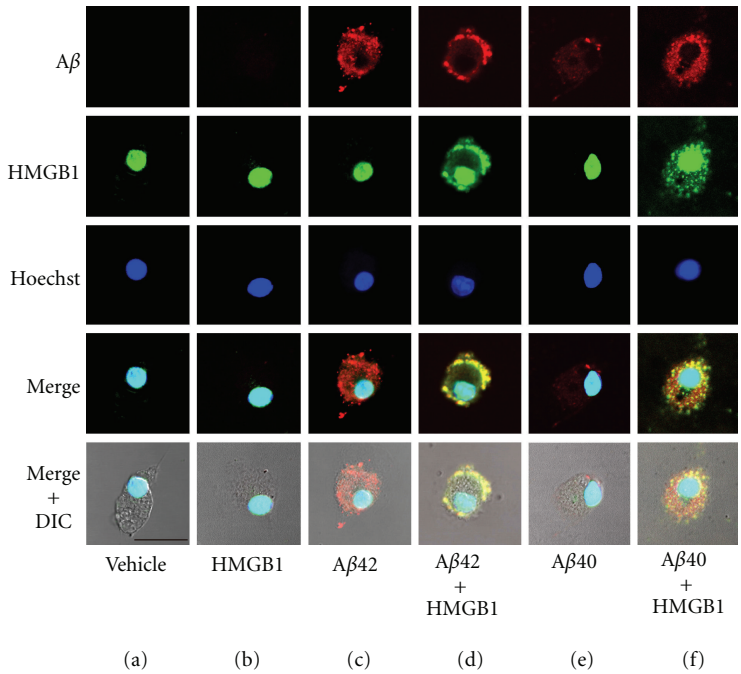


FIGURE 2: Effect of exogenous HMGB1 on microglial Aβ phagocytosis analyzed by laser confocal microscopy. Rat microglia were incubated with the vehicle (a), HMGB1 (b), Aβ42 (c), Aβ42 and HMGB1 (d), Aβ40 (e), or Aβ40 and HMGB1 (f) for 12 h. Fixed cells were further incubated with the anti-Aβ antibody (red), anti-HMGB1 antibody (green), and Hoechst 33258 (dye for nuclei; blue); they were analyzed using a laser scanning confocal microscope. DIC: differential interference contrast. Scale bar = 20 μm for all panels.

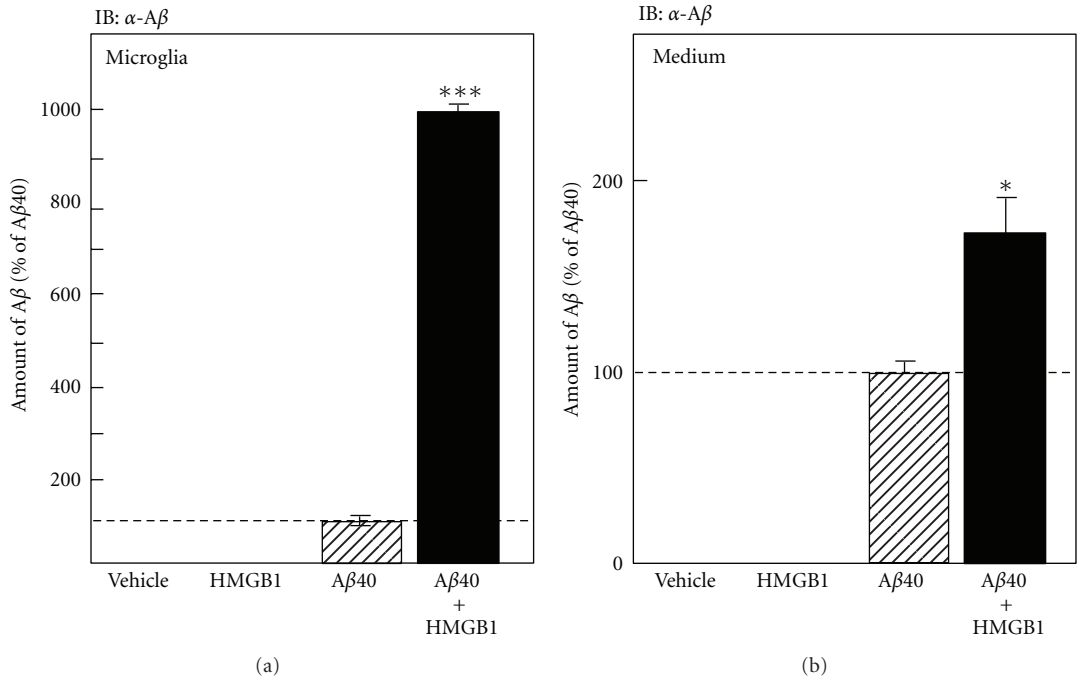


FIGURE 3: Effect of exogenous HMGB1 on microglial Aβ phagocytosis analyzed by Western blot. Rat microglia were incubated with the vehicle, HMGB1, Aβ40, or Aβ40 and HMGB1 for 12 h. Microglial cell lysate (a) and conditioned medium (b) were then subjected to Western blot analysis using the anti-Aβ antibody, and then the amounts of Aβ40 inside (a) and outside microglia (b) were semiquantitatively measured. $^{*}P < 0.05$, $^{***}P < 0.001$ versus treatment with Aβ40 alone.

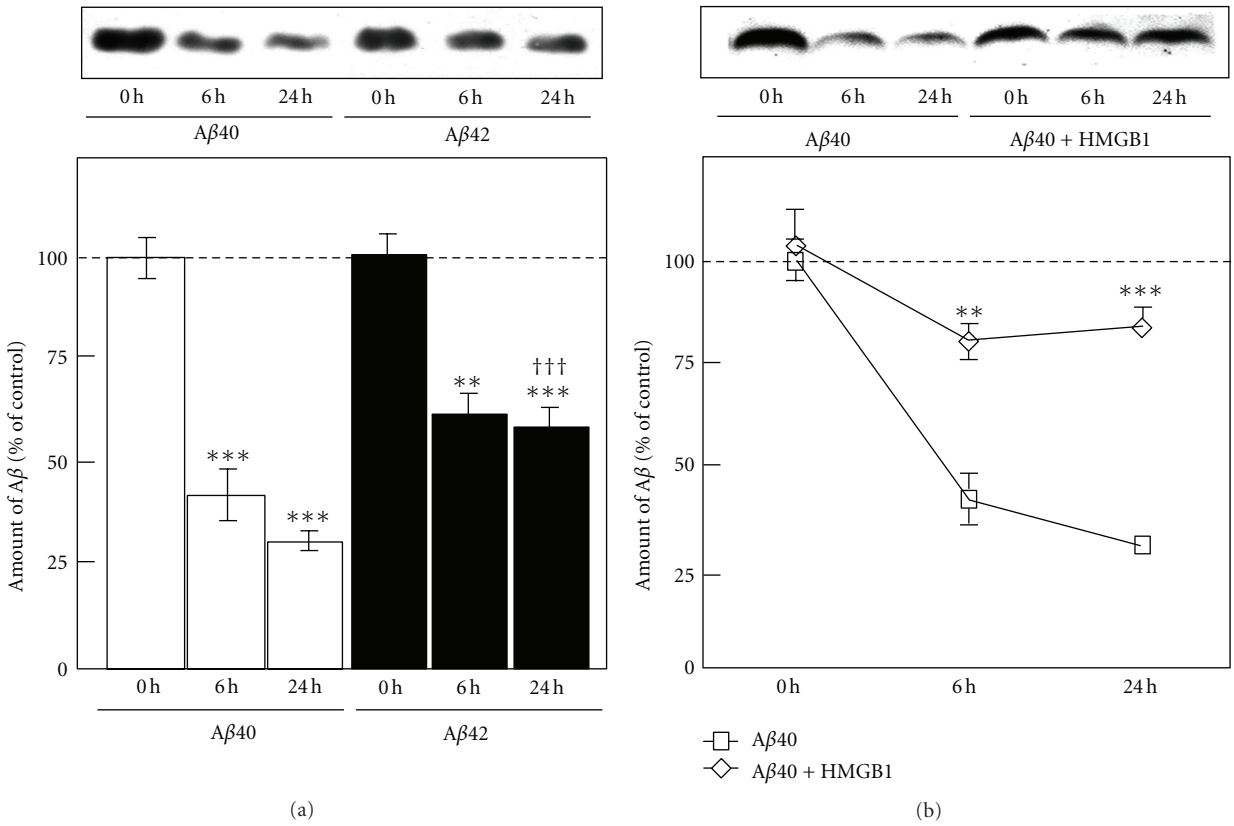


Figure 4: Aβ degradation with the microglial cytosolic fraction. (a) Aβ40 and Aβ42 were incubated with the rat microglial cytosolic fraction for 0, 6, and 24 h. After incubation, samples were subjected to Western blot analysis using the anti-Aβ antibody, and the amount of Aβ was semiquantitatively measured. $^{**}P < 0.01$, $^{***}P < 0.001$ versus time point 0 h. $^{\dagger\dagger\dagger}P < 0.001$ versus Aβ40 at time point 24 h. (b) Aβ40 and Aβ40 with HMGB1 were incubated with rat microglial cytosolic fractions for 0, 6, and 24 h. After incubation, the samples were subjected to Western blot analysis using the anti-Aβ antibody, and the amount of Aβ was semiquantitatively measured. $^{**}P < 0.01$, $^{***}P < 0.001$ versus Aβ40 alone at each time point.

of Aβ40 inside (Figure 3(a)) and outside microglia (Figure 3(b)). When microglia were treated with the vehicle or exogenous HMGB1 alone, no Aβ40 immunoreactivity was detected inside them (Figure 3(a)). After treatment with Aβ40, a small amount of Aβ40 was detected inside microglia (Aβ40 phagocytosed by microglia), and this amount increased dramatically by simultaneous treatment with exogenous HMGB1 (Figure 3(a)). This result raises two possibilities: (i) extracellular HMGB1 increases microglial Aβ40 uptake, and (ii) HMGB1 inhibits the degradation of Aβ40 in the microglial cytoplasm after uptake. To address these possibilities, we measured the amount of Aβ40 in the culture medium (Aβ40 remaining outside microglia) (Figure 3(b)). After treatment with the vehicle or exogenous HMGB1 alone, no Aβ was detected in the culture medium. Twelve hours after Aβ40 treatment, the amount of Aβ40 in the culture medium significantly increased by simultaneous treatment with exogenous HMGB1. Thus, in the presence of exogenous HMGB1, the amount of Aβ40 both inside and outside microglia was much higher than that when treated with Aβ40 alone. These results suggest that exogenous HMGB1 phagocytosed by microglia inhibits the degradation of Aβ40 in the microglial cytoplasm and subsequently delays Aβ clearance by microglia.

3.4. Aβ Degradation with the Microglial Cytosol Fraction and Effect of Exogenous HMGB1. To confirm whether exogenous HMGB1 inhibits Aβ40 degradation in microglial cytoplasm, we prepared cytosolic fractions from rat microglia and mixed them with Aβ. Degradation of Aβ40 and Aβ42 by microglial cytosol fractions was compared (Figure 4(a)). Aβ40 and Aβ42 were gradually degraded by the addition of the microglial cytosolic fraction in a time-dependent manner. Aβ40 was degraded earlier than Aβ42 (Figure 4(a)). We next examined the effect of exogenous HMGB1 on the Aβ40 degradation induced by the microglial cytosolic fraction (Figure 4(b)). At 6 and 24 h after incubation, the degradation of Aβ40 was significantly delayed by the addition of exogenous HMGB1. Thus, this result suggests that exogenous HMGB1 phagocytosed by microglia inhibits the degradation of Aβ40 in the microglial cytoplasm.

3.5. Accumulation of Aβ40, Aβ42, and Microglia in AD Brains. We further investigated the localization of Aβ40 and Aβ42 in AD brains using specific antibodies (Figures 5(a) and 5(b)) and microglial accumulation on the plaques composed of Aβ40 (Figures 5(c) and 5(d)) and Aβ42 (Figures 5(e) and

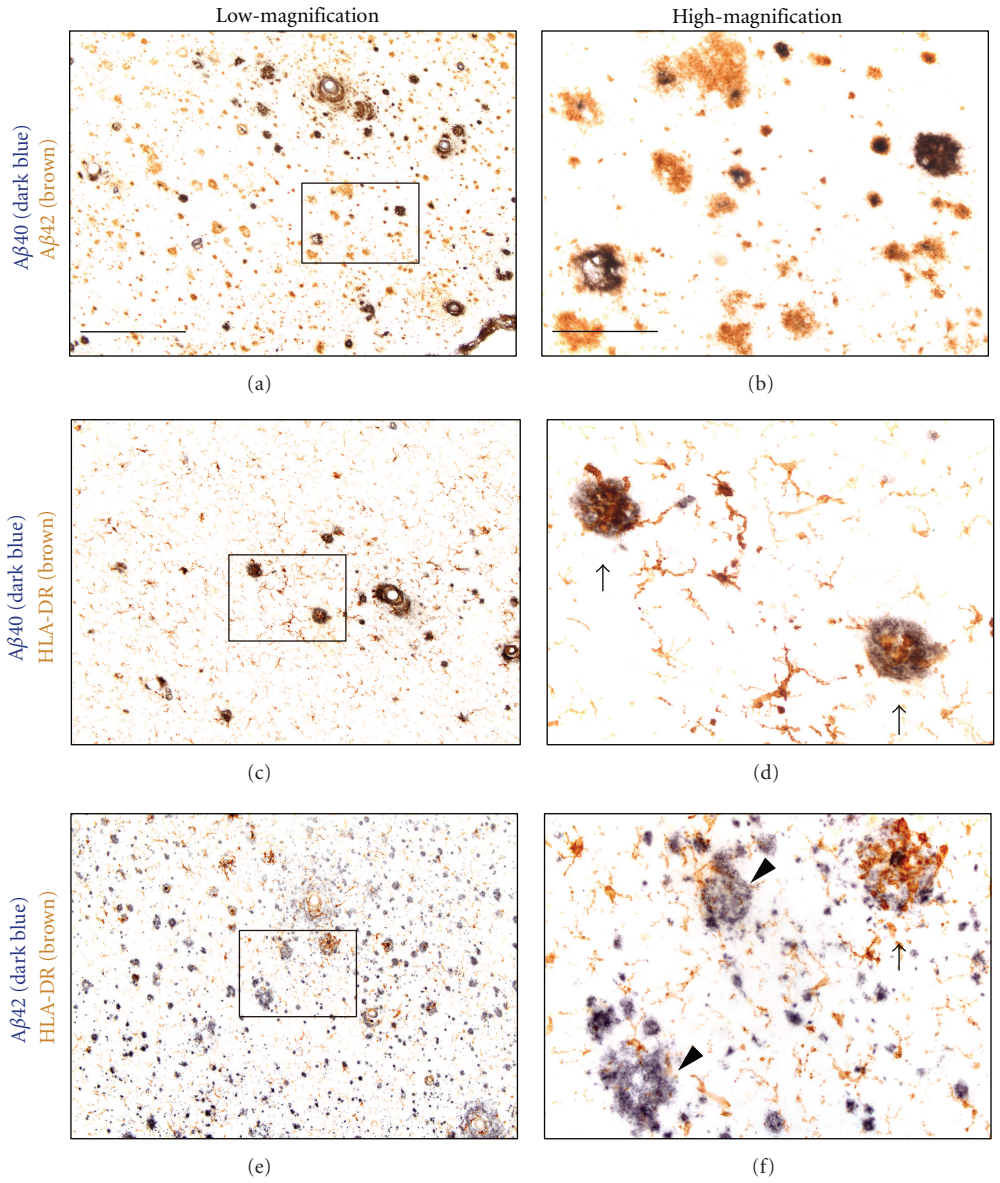


Figure 5: Immunohistochemical study of microglial accumulation on Aβ plaques in human AD brains. Free-floating human AD brain sections were incubated with the anti-Aβ40 specific antibody (dark blue) and anti-Aβ42 specific antibody (brown) (a, b), anti-Aβ40 specific antibody (dark blue) and anti-HLA-DR antibody (for microglial staining; brown) (c, d), and anti-Aβ42 specific antibody (dark blue) and anti-HLA-DR antibody (for microglial staining; brown). Arrows and arrow heads show marked and poor microglial accumulations, respectively. (b), (d), and (f) show high-magnification views of squared area in (a), (c), and (e), respectively. Scale bar in (a) equals 400 μm for (a), (c), and (e). Scale bar in (b) equals 100 μm for (b), (d), and (f).

5(f)). The number of Aβ40 plaques (dark blue deposits in Figure 5(a)) was lesser than that of Aβ42 plaques (brown deposits in Figure 5(a)). High-magnification photographs revealed that Aβ40 accumulated on Aβ42 plaques (Figure 5(b)). Regarding microglial accumulations (Figures 5(c)–5(f)), almost all Aβ40 plaques were markedly surrounded by activated microglia (Figure 5(c) and arrows in Figure 5(d)). Although some Aβ42 plaques were markedly accumulated by microglia (Figure 5(e) and arrow in Figure 5(f)), others were moderately or poorly surrounded by microglia (arrowheads in Figure 5(f)).

3.6. Accumulation of HMGB1 and Microglia on Aβ40 Plaques in AD Brains. We previously demonstrated that extracellular HMGB1 accumulates on Aβ plaques, as detected using an anti-Aβ antibody that reacts with a broad spectrum of Aβ species [33]. Therefore, in the present study, we investigated the colocalization of extracellular HMGB1 on Aβ40 plaques in AD brains using a specific anti-Aβ40 antibody. Consistent with the immunohistochemical study (Figures 5(c) and 5(d)), microglia (Figure 6(b)) markedly accumulated on Aβ40 plaques (Figure 6(a)) in AD brains (Figure 6(c)). We further demonstrated that extracellular HMGB1

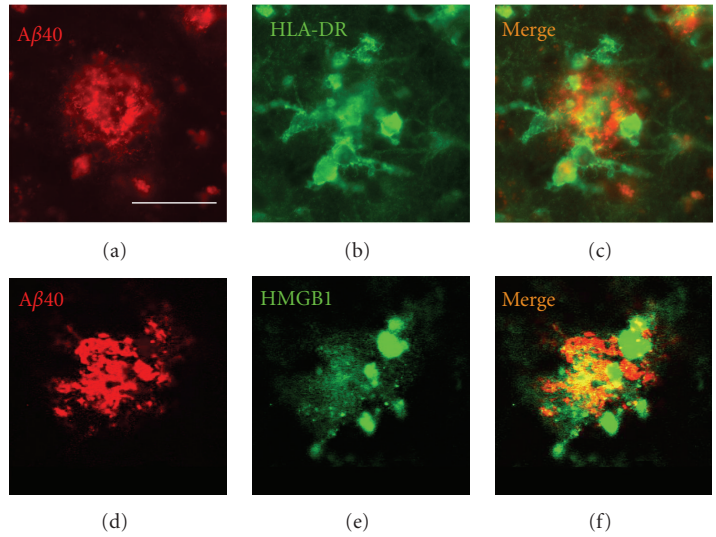


Figure 6: Laser confocal microscopic study on the accumulation of HMGB1 and microglia on Aβ40 plaques in human AD brains. (a–c) Free-floating human AD brain sections were incubated with the anti-Aβ40 specific antibody ((a) red) and anti-HLA-DR antibody ((b) for microglial staining, green). The merged image is indicated in (c). (d–f) Free-floating human AD brain sections were incubated with the anti-Aβ40 specific antibody ((a); red) and anti-HMGB1 antibody ((b); green). The merged image is indicated in (f). Scale bar in (a) equals 50 μm for all panels.

(Figure 6(e)) colocalized with Aβ40 plaques (Figure 6(d)) in AD brains (Figure 6(f)).

4. Discussion

In studies on familial AD, mutations in the *APP*, *PS1*, and *PS2* genes have been detected, and transgenic mice models carrying these familial AD-linked mutations show enhanced Aβ production in their brains. In particular, transgenic mice carrying the *APP* mutation display characteristics that closely resemble AD, such as Aβ deposition and memory dysfunction [35, 36], and introduction of the double mutations of *PS/APP* exhibits the early onset of these pathologies [37]. Thus, all mutations are involved in Aβ generation, and the accumulation of Aβ in the brain has been strongly suggested to be the primary event driving the pathogenesis of AD. However, familial AD accounts for less than 1% of all AD cases [38]; most cases develop sporadically. Although the etiology of sporadic AD remains much more elusive than that of familial cases, neurological and pathological events in sporadic AD are essentially indistinguishable from those in familial cases. In sporadic AD, a decreased Aβ clearance rate has been reported [39].

One proposed mechanism of Aβ clearance is microglial Aβ phagocytosis [40, 41]. Reports on AD patients treated with Aβ immunization also indicate microglial contribution to Aβ clearance in human AD brains [42, 43]. However, it has been suggested that the ability of microglia to clear Aβ decreases with age and progression of AD pathology [26, 27], and it may, at least in part, account for the dysregulation of Aβ clearance in sporadic AD.

HMGB1 inhibits microglial Aβ42 phagocytosis by interfering with Aβ42 internalization [32, 33]. In the present study, we further showed that exogenous HMGB1 inhibits the degradation of Aβ40 in rat microglial cytoplasm and subsequently delays Aβ40 clearance. We demonstrated the binding affinities of HMGB1 for Aβ40 and Aβ42 [33]. Aβ contains an amino acid sequence (^{18}VFFA^{21}) that has been identified to be essential for aggregation and fibril formation [44]. Interestingly, HMGB1 contains a homologous motif (^{16}AFFV^{19}), and this sequence is thought to be critically involved in the interactions of Aβ with HMGB1 [33, 45]. Among the many peptidases that have been proposed as Aβ-degrading enzymes [46], insulin-degrading enzyme, cathepsin D, and neprilysin are the principle enzymes involved in microglia-mediated Aβ degradation [25, 47, 48]. Many cleavage sites that are the targets of microglial Aβ-degrading enzymes are located on and in the vicinity of the amino acid sequence (^{18}VFFA^{21}) of Aβ [46]. Therefore, we speculate that the cleavage sites of Aβ are masked by the binding of HMGB1; subsequently, the degradation of Aβ40 may be inhibited in the microglial cytoplasm. In case of Aβ42, Aβ42 itself forms high-molecular-weight fibrils during incubation [33]. Therefore, the binding of HMGB1 may stabilize Aβ42 fibril formation, and high-molecular-weight complex of HMGB1 and Aβ42 fibril may interrupt the uptake of Aβ42 by microglia. Thus, extracellular HMGB1 may serve as a chaperone protein for Aβ and inhibit microglial Aβ clearance by interrupting Aβ40 degradation and Aβ42 internalization by microglia. On the other hand, RAGE, TLR2, and TLR4 are receptors for HMGB1 [30, 31]; they are also involved in microglial Aβ phagocytosis [49, 50]. Therefore, there is a possibility that the interactions of HMGB1 with these receptors on microglia may be related to the inhibitory events on Aβ.

Consistent with a previous study [13], plaques containing Aβ42 predominantly existed in AD brains, and

Aβ40 accumulated on parts of Aβ42 plaques. Despite the restricted distribution of Aβ40, almost all plaques containing Aβ40 were markedly surrounded by activated microglia. We previously reported that small oligomers formed by Aβ40 strongly induce rat microglial reactions such as cytokine production [51]. Thus, Aβ40 may play an important role in microglial activation and/or recruitment on Aβ plaques. However, we have found that the level of HMGB1 was significantly increased in AD brains [33], and extracellular HMGB1 accumulated on Aβ plaques. Therefore, in AD brains, microglial degradation of Aβ40 and uptake of Aβ42 may be inhibited by extracellular HMGB1 despite the marked accumulation of reactive microglia on Aβ plaques. Moreover, in the present study, we demonstrated that Aβ40 is more readily degraded by the microglial cytosolic fraction than Aβ42. However, in the presence of exogenous HMGB1, the degradation of Aβ40 by microglia is inhibited, and a lot of Aβ40 granules are existed in the cytoplasm of rat microglia as shown in Figure 2(f). Interestingly, numerous microglia containing Aβ40 granules, but not Aβ42, have also been detected in AD brains [52]. Thus, this event in AD brain is well replicated by the treatment with Aβ40 in the presence of extracellular HMGB1 in primary-cultured rat microglia. Results suggest that our findings in the effect of HMGB1 on rat microglia may reflect on the pathological event induced in AD brain and are expected the critical implication of extracellular HMGB1 in the progression of AD pathologies. In addition, we have postulated that the origin of extracellular HMGB1 is leakage from dead neurons during the progression of AD [32], like ischemic neurodegeneration [53]. Extracellular HMGB1 leaked from dead neurons may then accumulate on Aβ plaques through its binding affinity for Aβ in AD brains.

It has been reported that the released HMGB1 is involved in the pathologies of various inflammatory-related disease [54]. In ischemic stroke [55] and intracerebral hemorrhage especially [56], extracellular HMGB1 is suggested to exacerbate brain insult through the disruption of the blood-brain barrier (BBB), overfacilitation of microglia, and intense production of proinflammatory molecules. These studies also demonstrated that a neutralizing anti-HMGB1 monoclonal antibody and glycyrrhizin which bind to and inhibit cytokine-like activity of HMGB1 attenuate the brain insult induced by transient ischemia and intracerebral hemorrhage in rat, respectively. Therefore, there is a possibility that the neutralizing anti-HMGB1 monoclonal antibody and glycyrrhizin may bind to the extracellular HMGB1 accumulated on the Aβ plaques in the AD brain, cancel the inhibitory effects of HMGB1 on microglial Aβ phagocytosis, and then may provide novel therapeutic options for the AD treatment.

In conclusion, in the present study, we found that HMGB1 extracellularly accumulates on Aβ plaques containing Aβ40 in AD brains. We further demonstrated that HMGB1 has a binding affinity for Aβ40, and exogenous HMGB1 is internalized into rat microglial cytoplasm with Aβ40 and inhibits Aβ40 degradation. Subsequently, exogenous HMGB1 delays Aβ40 clearance in the culture medium. Thus, these results suggest that extracellular HMGB1 attenuates microglial Aβ clearance and is possibly involved in the progression of AD pathology.

Acknowledgments

The authors thank Toshiyuki Kawasaki for his technical assistance. This study was supported by the Frontier Research Programs of the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grants-in-Aid from the Japan Society for the Promotion of Science; and Kyoto Pharmaceutical University Fund for the Promotion of Scientific Research.

References

[1] D. J. Selkoe, "Alzheimer's disease is a synaptic failure," *Science*, vol. 298, no. 5594, pp. 789–791, 2002.

[2] J. Götz, F. Chen, J. Van Dorpe, and R. M. Nitsch, "Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Aβ42 fibrils," *Science*, vol. 293, no. 5534, pp. 1491–1495, 2001.

[3] J. Lewis, D. W. Dickson, W. L. Lin et al., "Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP," *Science*, vol. 293, no. 5534, pp. 1487–1491, 2001.

[4] K. Takata, Y. Kitamura, Y. Nakata et al., "Involvement of WAVE accumulation in Aβ/APP pathology-dependent tangle modification in Alzheimer's disease," *American Journal of Pathology*, vol. 175, no. 1, pp. 17–24, 2009.

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

[6] J. C. Dodart, K. R. Bales, K. S. Gannon et al., "Immunization reverses memory deficits without reducing brain Aβ burden in Alzheimer's disease model," *Nature Neuroscience*, vol. 5, no. 5, pp. 452–457, 2002.

[7] C. Janus, J. Pearson, J. McLaurin et al., "Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease," *Nature*, vol. 408, no. 6815, pp. 979–982, 2000.

[8] D. Morgan, D. M. Diamond, P. E. Gottschall et al., "A β peptide vaccination prevents memory loss in an animal model of Alzheimer's disease," *Nature*, vol. 408, no. 6815, pp. 982–985, 2000.

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

[10] C. Hock, U. Konietzko, J. R. Streffer et al., "Antibodies against β-amyloid slow cognitive decline in Alzheimer's disease," *Neuron*, vol. 38, no. 4, pp. 547–554, 2003.

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

[12] J. Wiltfang, H. Esselmann, M. Bibl et al., "Highly conserved and disease-specific patterns of carboxyterminally truncated Aβ peptides 1-37/38/39 in addition to 1-40/42 in Alzheimer's disease and in patients with chronic neuroinflammation," *Journal of Neurochemistry*, vol. 81, no. 3, pp. 481–496, 2002.

[13] T. Iwatsubo, A. Odaka, N. Suzuki, H. Mizusawa, N. Nukina, and Y. Ihara, "Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an

initially deposited species is Aβ42(43)," *Neuron*, vol. 13, no. 1, pp. 45–53, 1994.

[14] J. T. Jarrett, E. P. Berger, and P. T. Lansbury Jr., "The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease," *Biochemistry*, vol. 32, no. 18, pp. 4693–4697, 1993.

[15] K. N. Dahlgren, A. M. Manelli, W. Blaine Stine, L. K. Baker, G. A. Krafft, and M. J. Ladu, "Oligomeric and fibrillar species of amyloid-β peptides differentially affect neuronal viability," *Journal of Biological Chemistry*, vol. 277, no. 35, pp. 32046–32053, 2002.

[16] L. F. Lue, Y. M. Kuo, A. E. Roher et al., "Soluble amyloid β peptide concentration as a predictor of synaptic change in Alzheimer's disease," *American Journal of Pathology*, vol. 155, no. 3, pp. 853–862, 1999.

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

[18] D. M. Paresce, R. N. Ghosh, and F. R. Maxfield, "MicroGlial cells internalize aggregates of the Alzheimer's disease amyloid β-protein via a scavenger receptor," *Neuron*, vol. 17, no. 3, pp. 553–565, 1996.

[19] D. M. Paresce, H. Chung, and F. R. Maxfield, "Slow degradation of aggregates of the Alzheimer's disease amyloid β-protein by microGlial cells," *Journal of Biological Chemistry*, vol. 272, no. 46, pp. 29390–29397, 1997.

[20] K. Takata, Y. Kitamura, M. Saeki et al., "Galantamine-induced amyloid-β clearance mediated via stimulation of microGlial nicotinic acetylcholine receptors," *Journal of Biological Chemistry*, vol. 285, no. 51, pp. 40180–40191, 2010.

[21] K. Takata, C. Hirata-Fukae, A. G. Becker et al., "Deglycosylated anti-amyloid beta antibodies reduce microGlial phagocytosis and cytokine production while retaining the capacity to induce amyloid beta sequestration," *European Journal of Neuroscience*, vol. 26, no. 9, pp. 2458–2468, 2007.

[22] K. Takata, Y. Kitamura, D. Yanagisawa et al., "MicroGlial transplantation increases amyloid-β clearance in Alzheimer model rats," *FEBS Letters*, vol. 581, no. 3, pp. 475–478, 2007.

[23] Y. Kitamura, K. Shibagaki, K. Takata et al., "Involvement of Wiskott-Aldrich syndrome protein family verprolin-homologous protein (WAVE) and Rac1 in the phagocytosis of amyloid-β(1-42) in rat microGlia," *Journal of Pharmacological Sciences*, vol. 92, no. 2, pp. 115–123, 2003.

[24] K. Takata, Y. Kitamura, D. Tsuchiya, T. Kawasaki, T. Taniguchi, and S. Shimohama, "Heat shock protein-90-induced microGlial clearance of exogenous amyloid-β1-42 in rat hippocampus in vivo," *Neuroscience Letters*, vol. 344, no. 2, pp. 87–90, 2003.

[25] J. I. Kakimura, Y. Kitamura, K. Takata et al., "MicroGlial activation and amyloid-β clearance induced by exogenous heat-shock proteins," *FASEB Journal*, vol. 16, no. 6, pp. 601–603, 2002.

[26] W. J. Streit, N. W. Sammons, A. J. Kuhns, and D. L. Sparks, "Dystrophic MicroGlia in the Aging Human Brain," *Glia*, vol. 45, no. 2, pp. 208–212, 2004.

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

[28] S. Müller, P. Scaffidi, B. Degryse et al., "The double life of HMGB1 chromatin protein: architectural factor and extracellular signal," *EMBO Journal*, vol. 20, no. 16, pp. 4337–4340, 2001.

[29] P. Scaffidi, T. Misteli, and M. E. Bianchi, "Release of chromatin protein HMGB1 by necrotic cells triggers inflammation," *Nature*, vol. 418, no. 6894, pp. 191–195, 2002.

[30] J. S. Park, D. Svetkauskaite, Q. He et al., "Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein," *Journal of Biological Chemistry*, vol. 279, no. 9, pp. 7370–7377, 2004.

[31] R. Kokkola, A. Andersson, G. Mullins et al., "RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages," *Scandinavian Journal of Immunology*, vol. 61, no. 1, pp. 1–9, 2005.

[32] K. Takata, Y. Kitamura, D. Tsuchiya, T. Kawasaki, T. Taniguchi, and S. Shimohama, "High mobility group box protein-1 inhibits microGlial Aβ clearance and enhances Aβ neurotoxicity," *Journal of Neuroscience Research*, vol. 78, no. 6, pp. 880–891, 2004.

[33] K. Takata, Y. Kitamura, J. I. Kakimura et al., "Role of high mobility group protein-1 (HMG1) in amyloid-β homeostasis," *Biochemical and Biophysical Research Communications*, vol. 301, no. 3, pp. 699–703, 2003.

[34] Y. Kitamura, S. Shimohama, W. Kamoshima et al., "Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax, Bak, Bad, ICH-1 and CPP32, in Alzheimer's disease," *Brain Research*, vol. 780, no. 2, pp. 260–269, 1998.

[35] D. Games, D. Adams, R. Alessandrini et al., "Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein," *Nature*, vol. 373, no. 6514, pp. 523–527, 1995.

[36] K. Hsiao, P. Chapman, S. Nilsen et al., "Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice," *Science*, vol. 274, no. 5284, pp. 99–102, 1996.

[37] L. Holcomb, M. N. Gordon, E. Mcgowan et al., "Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes," *Nature Medicine*, vol. 4, no. 1, pp. 97–100, 1998.

[38] D. Campion, C. Dumanchin, D. Hannequin et al., "Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum," *American Journal of Human Genetics*, vol. 65, no. 3, pp. 664–670, 1999.

[39] K. G. Mawuenyega, W. Sigurdson, V. Ovod et al., "Decreased clearance of CNS β-amyloid in Alzheimer's disease," *Science*, vol. 330, no. 6012, p. 1774, 2010.

[40] A. R. Simard, D. Soulet, G. Gowing, J. P. Julien, and S. Rivest, "Bone marrow-derived microGlia play a critical role in restricting senile plaque formation in Alzheimer's disease," *Neuron*, vol. 49, no. 4, pp. 489–502, 2006.

[41] J. El Khoury, M. Toft, S. E. Hickman et al., "Ccr2 deficiency impairs microGlial accumulation and accelerates progression of Alzheimer-like disease," *Nature Medicine*, vol. 13, no. 4, pp. 432–438, 2007.

[42] D. Boche, N. Denham, C. Holmes, and J. A. R. Nicoll, "Neuropathology after active Aβ42 immunotherapy: implications for Alzheimer's disease pathogenesis," *Acta Neuropathologica*, vol. 120, no. 3, pp. 369–384, 2010.

[43] J. A. R. Nicolll, D. Wilkinson, C. Holmes, P. Steart, H. Markham, and R. O. Weller, "Neuropathology of human Alzheimer disease after immunization with amyloid-β peptide: a case report," *Nature Medicine*, vol. 9, no. 4, pp. 448–452, 2003.

[44] L. O. Tjernberg, D. J. E. Callaway, A. Tjernberg et al., "A molecular model of Alzheimer amyloid β-peptide fibril formation," *Journal of Biological Chemistry*, vol. 274, no. 18, pp. 12619–12625, 1999.

[45] J. Kallijärvi, M. Haltia, and M. H. Baumann, "Amphoterin includes a sequence motif which is homologous to the Alzheimer's β-amyloid peptide (Aβ), forms amyloid fibrils in vitro, and binds avidly to Aβ," *Biochemistry*, vol. 40, no. 34, pp. 10032–10037, 2001.

[46] N. Iwata, M. Higuchi, and T. C. Saido, "Metabolism of amyloid-β peptide and Alzheimer's disease," *Pharmacology and Therapeutics*, vol. 108, no. 2, pp. 129–148, 2005.

[47] W. Q. Qiu, D. M. Walsh, Z. Ye et al., "Insulin-degrading enzyme regulates extracellular levels of amyloid β- protein by degradation," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32730–32738, 1998.

[48] E. Shimizu, K. Kawahara, M. Kajizono, M. Sawada, and H. Nakayama, "IL-4-induced selective clearance of oligomeric β-amyloid peptide 1-42 by rat primary type 2 microGlia," *Journal of Immunology*, vol. 181, no. 9, pp. 6503–6513, 2008.

[49] L. F. Lue, D. G. Walker, L. Brachova et al., "Involvement of microGlial receptor for advanced glycation endproducts (RAGE)in Alzheimer's disease: identification of a cellular activation mechanism," *Experimental Neurology*, vol. 171, no. 1, pp. 29–45, 2001.

[50] K. Tahara, H. D. Kim, J. J. Jin, J. A. Maxwell, L. Li, and K. I. Fukuchi, "Role of toll-like receptor signalling in Aβ uptake and clearance," *Brain*, vol. 129, no. 11, pp. 3006–3019, 2006.

[51] K. Takata, Y. Kitamura, M. Umeki et al., "Possible involvement of small oligomers of amyloid-β peptides in 15-deoxy-Δ12,14 prostaglandin J2-sensitive microGlial activation," *Journal Pharmacological Sciences*, vol. 91, no. 4, pp. 330–333, 2003.

[52] H. Akiyama, H. Mori, T. Saido, H. Kondo, K. Ikeda, and P. L. McGeer, "Occurrence of the diffuse amyloid β-protein (Aβ) deposits with numerous Aβ-containing Glial cells in the cerebral cortex of patients with Alzheimer's disease," *Glia*, vol. 25, no. 4, pp. 324–331, 1999.

[53] G. Faraco, S. Fossati, M. E. Bianchi et al., "High mobility group box 1 protein is released by neural cells upon different stresses and worsens ischemic neurodegeneration in vitro and in vivo," *Journal of Neurochemistry*, vol. 103, no. 2, pp. 590–603, 2007.

[54] M. T. Lotze and K. J. Tracey, "High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal," *Nature Reviews Immunology*, vol. 5, no. 4, pp. 331–342, 2005.

[55] K. Liu, S. Mori, H. K. Takahashi et al., "Anti-high mobility group box 1 monoclonal antibody ameliorates brain infarction induced by transient ischemia in rats," *FASEB Journal*, vol. 21, no. 14, pp. 3904–3916, 2007.

[56] M. Ohnishi, H. Katsuki, C. Fukutomi et al., "HMGB1 inhibitor glycyrrhizin attenuates intracerebral hemorrhage-induced injury in rats," *Neuropharmacology*, vol. 61, no. 5-6, pp. 975–980, 2011.