Correlated Inflammatory Responses and Neurodegeneration in Peptide-Injected Animal Models of Alzheimer’s Disease

James G. McLarnon

Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, BC, Canada V6T 1W3

Correspondence should be addressed to James G. McLarnon; mclarnon@mail.ubc.ca

Received 4 December 2013; Revised 26 February 2014; Accepted 27 February 2014; Published 13 April 2014

Copyright © 2014 James G. McLarnon. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Animal models of Alzheimer’s disease (AD) which emphasize activation of microglia may have particular utility in correlating proinflammatory activity with neurodegeneration. This paper reviews injection of amyloid-\(\beta\) (A\(\beta\)) into rat brain as an alternative AD animal model to the use of transgenic animals. In particular, intrahippocampal injection of A\(\beta\)1-42 peptide demonstrates prominent microglial mobilization and activation accompanied by a significant loss of granule cell neurons. Furthermore, pharmacological inhibition of inflammatory reactivity is demonstrated by a broad spectrum of drugs with a common endpoint in conferring neuroprotection in peptide-injected animals. Peptide-injection models provide a focus on glial cell responses to direct peptide injection in rat brain and offer advantages in the study of the mechanisms underlying neuroinflammation in AD brain.

1. Introduction

Chronic inflammation is a characteristic feature of Alzheimer’s disease (AD) brain [1, 2]. However, evidence supporting contributory roles of chronic inflammation as a causative factor in mediating neuronal damage in neurodegenerative diseases remains elusive. Indeed, a balanced perspective on roles of inflammation in AD suggests that cellular inflammatory responses can yield both beneficial and detrimental outcomes depending on a complexity of factors [3–5]. At present, the use of anti-inflammatory therapies for AD patients has demonstrated limited or minimal clinical utility. It is noteworthy that nonsteroidal anti-inflammatory drugs (NSAIDs) treatment, which primarily targets COX-2 enzymatic activity, has shown some efficacy in clinical studies [6–8]. The overall lack of benefits for anti-inflammatory-based therapies in AD may reflect the activation of multiple and complex signaling pathways mediating inflammatory reactivity.

A number of review articles are available which have considered the characteristic properties, including roles of inflammatory responses, demonstrated by a host of transgenic animal models [9–11]. In general, commonly used transgenic mouse models such as Tg2576 exhibit relatively subtle and modest degrees of chronic inflammation in the progression of disease [12]. In addition, transgenic animal models also do not generally manifest extensive amounts of neuronal damage and loss [13, 14]. A caveat, however, is that some transgenic models have been developed which exhibit significant neurodegeneration in aged mice [15–18]. A relevant point is that transgenic animal models are often not examined for neurodegeneration but instead more routinely tested for changes in cognitive performance in aged animals. One possible underlying reason for the benign levels of chronic inflammation in transgenic mouse AD models is the relatively slow and progressive accumulation in amyloid-\(\beta\) (A\(\beta\)) plaques in aged animals. It also can be considered that animal models using rats rather than mice may offer particular advantages in testing AD pathology due to closer similarities between the animal species and human tissue [19].

The injection of A\(\beta\) into rat brain serves as a stimulus for eliciting proinflammatory reactivity. Although the specific mechanisms by which A\(\beta\) deposition contributes to disease pathology are not well understood [20], deposits of peptide are noteworthy as chemotactic and activating stimuli for microglia, resident effector cells which mediate immune responses in brain [21, 22]. Considerable evidence has demonstrated that microglial activation serves as a critical transduction process contributing to inflammatory...
reactivity in AD brain. Once activated, microglial cells express and produce a plethora of inflammatory factors which in assemblage can be toxic to bystander cells including neurons. Results from in vitro studies have demonstrated that exposure of microglia to various forms of Aβ yields a milieu of inflammatory products with potential neurotoxic consequences [23–26].

2. Inflammatory Responses and Neuronal Damage/Impaired Behavior in Animals Receiving Injection of Aβ

Relatively few studies have examined effects of Aβ injection as a stimulus for inflammation-induced neurodegeneration in animals with early work focused on impairment in behavioral response. A number of criteria can be noted which reflect the validity in using peptide injection animal models. Firstly, Aβ should be injected at relatively low levels (low nM range) to approximate conditions in AD brain. The type of Aβ is also relevant since soluble species of peptide applied in vivo may be less effective compared with aggregated peptide [27, 28]. Secondly, peptide injection should be made into a well-defined brain region separate from the area of analysis for neuron viability. This procedure is required to minimize possible direct neuronal damage from the effects of injected peptide. The needle track can be used as a marker for specific placement of the injection system (see below). Thirdly, the extents of gliosis in response to peptide injection should be measured in proximity to neuronal expression to allow the possibility in correlating glial and neuronal responses.

In an early study, no significant behavioral impairment was reported in rats subjected to long-term hippocampal injection of Aβ1-42 [29]. Although concomitant neuronal viability was not measured, the lack of impaired behavior was suggested to reflect minimal loss of neurons in relevant areas of brain. The associated changes in microglial and astroglial activity were not determined in this work. A subsequent work concluded activation of glial cells underlay abnormal behavioral responses in rats receiving Aβ1-42 injected into the CA3 region of hippocampus [28]. The suggestion of glial responses as a factor in impaired behavior was based on findings of delayed impairment in behavior response which showed similar time course to gliosis. Another study reported increased astrogliaisis and IL-1β immunoreactivity in microglia and neurons in animals receiving injections (into amygdala) of shorter length peptide, Aβ25-35 [30]. Although early histopathological effects of peptide injection were evident, the overall results indicated no severe impairment in behavior for injected animals.

A critical study using specific markers for microglia, astrocytes, and neurons was designed to examine roles of gliosis in animals receiving peptide injection [31]. Importantly, direct neuronal loss was determined in the work rather than recording abnormalities in behavior. Gliosis and viability of neurons were measured in rats injected with fibrillar Aβ1-40 (fAB) into striatum (forebrain area of CNS affected in AD). The results showed long-term effects (30 d after injection) of fAB were marked increases in microgliosis and astrogliosis with both cell types exhibiting enhanced iNOS expression. Although fAB induced considerable gliosis, differences in glial responses were noted in terms of spatial and morphological responses to peptide. Importantly, loss of striatal neurons was observed with fAB relative to controls (vehicle and soluble peptide) and correlated with glial responses. The authors concluded that inflammatory factors derived from peptide-stimulated glia could contribute to neuronal degeneration.

As noted above, some evidence suggests limited beneficial actions of NSAIDs in AD. One such compound, ibuprofen, was tested as an inhibitor of plaques in a transgenic animal model of AD [32]. Ibuprofen was found effective in inhibiting microglial activation and astrogliosis and plaque development. The positive effects of ibuprofen in the transgenic model prompted study of the compound in peptide-injected rat brain. Full-length Aβ1-42 was injected into the CA3 region of hippocampus and a battery of behavioral tests conducted after injection [33]. Behavioral abnormalities were measured after 30 d of peptide injection with ibuprofen administration found effective in restoring normal patterns of behavior. Furthermore, withdrawal of the compound from Aβ1-42-injected rats resulted in a progressive decline in behavior responses. Considerable astrogliosis accompanied long-term peptide injection with gliosis inhibited in animals administered ibuprofen. An increase in astrogliosis with ibuprofen withdrawal suggested the possibility of inflammatory response contribution to impaired behavior in the peptide injection animal model; however, concomitant effects of ibuprofen on microglial responses were not studied.

Inflammatory responses mediated by activated astrocytes and microglia were correlated with neuron damage following cerebroventricular infusion of Aβ1-42 in mouse brain [34]. This work also examined extents of gliosis and hippocampal levels of the proinflammatory factors IL-1β, TNF-α, and S100B. The administration of an aminopyridazine compound diminished numbers of activated glial cells and expression of a host of proinflammatory cytokines. Drug treatment was found effective in attenuating neuronal damage for CA1 neurons and maintaining levels of synaptophysin (synaptic vesicle protein). The authors concluded that inhibition of glial responses provided neuroprotection in the neuroinflammation animal model.

Recent work has examined behavioral performance in rats receiving bilateral intrahippocampal injection of aggregated Aβ1-42 [35]. Impaired learning and memory tasks were demonstrated in peptide-injected animals relative to controls. Oral administration of an anti-inflammatory pyrimidine derivative was found to improve behavioral responses over long-term (90 d) treatment periods. Interestingly, the pyrimidine compound demonstrated enhanced efficacy in protecting behavior compared to NSAID treatment. This work did not assess neuronal viability or microgliosis in vivo but reported enhanced astrogliosis following intrahippocampal Aβ1-42 injection which was reduced in animals receiving drug administration. Accompanying in vitro experiments showed that Aβ1-42-induced macrophage (microglia) release of a host of proinflammatory factors was significantly reduced with administration of the pyrimidine compound. The overall results suggested inhibition of neuroinflammation as
a mechanism for improving learning and memory in peptide-injected rat brain.

The mechanisms by which microglia (or astrocytes) respond to injected forms of Aβ in the animal model studies are not well understood. However, a diversity of cell receptors responsive to different forms of peptide have been implicated in mediating glial cellular responses. Putative receptors in microglia include scavenger receptor [36], scavenger receptor complex [37], formyl peptide receptor [38], and receptor for advanced glycation end products (RAGE) [39]. A complexity in transduction processes, including both calcium-dependent and -independent pathways, couples receptor activation to cellular functional responses. Products of activated microglia include superoxide [40], proinflammatory cytokines such as tumor necrosis factor-α [41] and interleukins IL-1β [42] and IL-6 [43] and excitatory amino acids including glutamate [44]. In essence an elevated milieu of inflammatory factors can be produced from Aβ-stimulated microglia resulting in localized brain microenvironments which are potentially toxic to bystander neurons. Thus an assemblage of inflammatory agents could act in concert to alter synaptic signaling and damage hippocampal and cortical neurons in the ongoing progression of AD pathology. It is important to note that activated microglia can also mediate anti-inflammatory activity, a point discussed below.

Work from this laboratory has systematically examined intrahippocampal injection of full-length peptide Aβ1-42 as an animal model of AD [45]. A focus of study is correlational changes between inflammatory responses and neuronal viability and the effects of pharmacological intervention in peptide-injected brain. Overall, a diversity of drugs including thalidomide [41], pyrazole compound 2-MBAPA [46], angiostatin [47], or antibody treatments including anti-VEGF [48] and anti-MAC-1 antibody for antigen CD11b [49] have been found efficacious in reducing microgliosis in the peptide-injection animal model of AD. The common aspect of drug action from this disparate group of compounds is generalized anti-inflammatory activity in the AD animal model. In all studies peptide-injected animals administered drugs or receiving antibody treatment demonstrated modest, but significant, increases in neuron viability.

We consider that the common finding in reduction of microgliosis could involve drug effects to reduce microglial chemotactic responses mediated by a host of chemokines and inhibition of subsequent cell activation mediated by a host of receptors (noted above). The finding that all treatment strategies lead to diminished microgliosis and associated decreases in numbers of GCL neurons suggests correlation between inhibition of microgliosis and enhanced neuronal viability. It can be noted that, with the different drug administrations, levels of microglial immunoreactivity are still significantly higher compared to those observed in untreated control animals. In addition, astroglialosis is also increased in peptide-injected rat brain compared with controls. However, unlike microglial responses, astroglialosis appears minimally affected by any of the drug treatments noted above.

3. Practicalities and Experimental Example Using Aβ1-42 Intrahippocampal Injection as an AD Animal Model

3.1. Background. This model determines neuronal viability in the granule cell layer (GCL) and glial inflammatory responses (and changes in microvasculature) in the adjacent molecular layer (ML). The essential intent of the Aβ1-42 injection model is to initiate a chemotactic inflammatory response from resident microglia and astrocytes. A diversity of chemokines are upregulated in the animal model and implicated in enhancing microglial mobility to localize cells in proximity to amyloid deposition. Examples of increased chemokines include monocyte chemotactic protein, macrophage inflammatory peptide-α, and interleukin IL-8. The chemotactic response to intrahippocampal injection of Aβ1-42 rapidly progresses to glial activation mediated by a host of cellular receptors (noted above) stimulated by peptide. In order to examine association of neuronal viability with inflammation, immunohistochemical staining with specific neuronal, microglial, and astrocytic markers is employed. Quantification of staining density can be done using specifically designed programs which measure the area density of cell markers. Typical cellular markers include NeuN (for neurons), Iba-1 (for microglia), and GFAP (for astrocytes). Changes in microvasculature expression can also be examined using antibodies for RECA-1 (rat endothelial cell antigen), laminin, or vWF (von Willebrand factor).

An important aspect of AD animal model study is to validate findings in comparison to results obtained in human AD brain (see below). In this case animal model data for specific cellular responses and functional processes can be utilized and compared with marker expression in samples of hippocampal and cortical human brain tissue obtained from nondemented (ND) controls and AD individuals. A number of brain banks serve critical roles for supply of human brain tissue.

3.2. Example of Data Recorded Using Aβ1-42 Injection Animal Model. In practice [46–49] for detailed methodology), stereotaxic injection of peptide (Aβ1-42 at 1 or 2 nM) is made into the CA1 region of rat hippocampus. The specific location of peptide injection is well-defined (established coordinates from bregma) by the needle track. Peptide injection evokes a prominent inflammatory response which is assessed at a distance from the injection site and located in the molecular layer (ML) of dentate gyrus. The viability of neurons is determined in the granule cell layer (GCL) adjacent to ML. The proximity in analytic regions allows assessment of correlative information between glial and neuronal responses. In particular extents of microgliosis, astrogliosis and neuronal loss can be determined. Furthermore changes in properties of microvasculature including abnormalities in the morphology of microcapillaries, changes in the density of microvessels, and increased leakiness of blood-brain barrier (BBB) can also be examined.

In effect the intrahippocampal injection of Aβ1-42 initiates an acute microglial response which transitions to a chronic inflammatory process within a short duration
following peptide injection. The usual timeframe for analysis of inflammatory response is one week after injection. In practice, at 3 d following $\text{A}\beta_{1-42}$ injection, microglial cells are localized in the vicinity of peptide and exhibit characteristic properties of an activated phenotype. Several controls are employed in the study including intrahippocampal injection of vehicle (phosphate buffer solution (PBS)) and reverse peptide ($\text{A}\beta_{42-1}$), an inactive form of peptide. The former serves as a measure for quantification of the effects of peptide injection ($\text{A}\beta_{1-42}$ versus PBS) and the latter as a measure of peptide activity ($\text{A}\beta_{1-42}$ versus $\text{A}\beta_{42-1}$). It can be noted that the glial, neuronal, and vasculature properties for the two controls exhibit very similar patterns of response.

Representative responses from the different animal groups treated in the peptide injection model are presented below (all data obtained at 7 d after peptide injection). Control animals receiving PBS injection exhibit low expression of Iba-1 indicating relatively small extents of microgliosis in ML (Figure 1(a), left panel). A similar pattern of staining is found in animals receiving injection of reverse peptide $\text{A}\beta_{42-1}$ (Figure 1(a), middle panel). For both control animal groups, morphologies of cells appear ramified with elaborated cell processes suggesting cells may be in a quiescent and low level of activation. A very different profile of Iba-1 expression is found in rats injected with $\text{A}\beta_{1-42}$. In this case a considerably enhanced level of microgliosis is observed (Figure 1(a), right panel). Previous work [49] has shown Iba-1 is increased fourfold in $\text{A}\beta_{1-42}$ versus PBS-injected rat hippocampus (time point of 7 d after injection). Furthermore cells exhibit an altered morphology from controls including evidence for ameboid morphology (some retraction of processes and a trend to larger cell bodies). Examples for clustering of microglia, commonly involving a grouping of several cells, are also demonstrated in peptide-injected animals. Clustered and activated microglia, in proximity to deposits of peptide, are reported as a characteristic feature of AD inflammatory reactivity in animal models of AD [50, 51] and in AD brain tissue [52].

Representative patterns of astrocytic staining are presented in Figure 1(b). For controls, rats injected with either PBS or reverse peptide $\text{A}\beta_{42-1}$ show modest levels of GFAP immunoreactivity (ir) in the ML of hippocampus (left and middle panels of Figure 1(b)). A considerably elevated GFAP expression is evident in animals injected with $\text{A}\beta_{1-42}$ (right panel, Figure 1(b)). The increase in GFAP staining in peptide-injected rats indicates astrogliosis in the AD animal model. Previous work has demonstrated that GFAP ir is approximately doubled for $\text{A}\beta_{1-42}$ versus PBS at a time point of 7 d after injection [41]. The morphology of astrocytes is similar between control and peptide-injected animals with cells showing extended processes.

The viability of neurons is evaluated in the GCL of dentate gyrus, adjacent to ML, using NeuN as a cell-type specific marker. Representative staining for granule cell neurons in control animals is shown in segments of GCL in Figure 2. PBS and reverse peptide-injected rats exhibit an intact GCL for NeuN expression; the results (Figure 2, left and middle panels) indicate that about 8 cells span the width of the GCL. Similar results are obtained using other markers for neuronal viability in control groups, for example, the protein calbindin [47]. An altered pattern of NeuN staining for rats injected with $\text{A}\beta_{1-42}$ is demonstrated in Figure 2 (right panel).
Figure 2: Viability of GCL neurons in animal model. Representative neuronal expression (NeuN marker) for the same animal groups as in Figure 1. Left and middle panels show NeuN for respective PBS and reverse peptide controls. Right panel is marker staining for Aβ1-42 injection.

Figure 3: Microglial and neuronal responses in angiostatin-treated rats. (a) Representative microgliosis is shown for Aβ1-42-injected animals (left panel) and for peptide-injected animals receiving angiostatin treatment (right panel). (b) Typical expression of GCL neurons for untreated peptide-injected (left panel) and angiostatin-treated (right panel) animals.

with GCL considerably reduced in width. The loss of viable neurons in GCL is a characteristic feature of the Aβ1-42-injected rat hippocampus.

This laboratory has used a diversity of animal treatments to target microgliosis in the peptide animal model. The underlying hypothesis is that inhibition of inflammatory reactivity could serve as a rationale strategy to confer neuroprotection. As an example, recent work has employed the antiangiogenic agent, angiostatin, as a novel putative anti-inflammatory factor [47]. Control animals (injected with vehicle, PBS) exhibit minimal extents of microgliosis (data not shown). Representative microgliosis is presented in animals receiving intrahippocampal injection of Aβ1-42 (Figure 3(a), left panel) and in peptide-injected animals receiving angiostatin (Figure 3(a), right panel). Angiostatin treatment of rats was associated with a marked reduction in
levels of microgliosis. The patterns of Iba-1 immunostaining indicated a reduced cellular activation in drug-treated animals.

The corresponding neuronal staining in GCL layer is presented in Figure 3(b). Animals receiving the administration of angiostatin with Aβ1-42 injection exhibited increased numbers of GCL neurons compared with untreated Aβ1-42-injected animals. Indeed, the patterns of NeuN immunoreactivity and intactness of GCL were similar between peptide-injected rats receiving angiostatin and control animals (data not shown). The expression of neuronal viability, using calbindin as a marker, is also demonstrated for the different animal groups [47]. Thus angiostatin confers a modest, but significant, degree of neuroprotection in the AD animal model. The partial neuroprotection may reflect the abundance and complexity of inflammatory processes initiated by peptide injection. Interestingly, the extents of astroglia were not significantly reduced with angiostatin treatment of animals receiving Aβ1-42. A similar finding has been obtained in other studies [41, 49] whereby enhanced astroglia in peptide-injected rat hippocampus is not significantly modified by administration of anti-inflammatory compounds.

4. Establishing Animal Model

Validity by Comparison with Data from AD Brain Tissue

An important aspect in using animal models is to examine and test the validity of data in comparison to conditions in AD brain. The comparison can be facilitated by using cortical and hippocampal tissue isolated from controls (ND nondemented) and AD individuals. Specific guidelines are available to distinguish between ND and AD brain tissue including criteria based on extents of plaque deposition and tau formation [53, 54]. In our case human tissue is available from the Kinsmen laboratory brain bank located at the University of British Columbia. Independent analysis from trained pathologists has classified AD and ND cases and provided cortical and hippocampal tissue sections from the Kinsmen laboratory.

Our findings are consistent with previous work [2] in showing sections from AD brain tissue exhibit marked increases in microgliosis and astrogliosis compared with ND tissue [52, 55]. Overall, staining of gliosis activity in AD and ND tissue demonstrates similar patterns of immunoreactivity to those found between Aβ1-42 and PBS injection in the intrahippocampal Aβ1-42 injection animal model. Furthermore clusters of microglia in proximity to peptide are characteristic features in cortical and hippocampal sections isolated from human AD tissue [52]. A spectrum of similarities in morphological perturbations and abnormalities in properties of microvasculature are also evident between peptide-injected rat brain and tissue obtained from AD individuals [55].

5. Future Directions and Consideration in Using Injection of Aβ as an Animal Model of AD

Neuroinflammation is a critical component of Alzheimer’s disease brain [5, 56, 57]. Animal models using injected Aβ as a stimulus for induction of inflammatory reactivity will have utility in characterization of processes contributing to neurodegeneration in disease. The results described above using the Aβ1-42-injected rat model represent correlated data between extents of microgliosis and viability of neurons. As such, the findings are not readily interpretable as to inflammatory reactivity as a contributing causative process for neurodegeneration. To examine the latter process in detail, studies are required which are designed to examine time-dependent changes in microglial responses and neuronal viability over long-term durations following intrahippocampal peptide injection. Such experiments will be useful to determine if microgliosis precedes neuronal loss, examining the mechanisms involved which link inflammatory reactivity with neuron viability and the nature of the neurodegenerative processes. In addition, AD animal models including intrahippocampal injection of peptide and numerous transgenic mouse models have not been extensively studied for abnormalities in synaptic function. However, perturbations in synaptic transmission could constitute an early and sensitive measure of neuronal damage and cognitive impairment in AD brain.

It should be emphasized that the intrahippocampal injection of Aβ1-42 represents an AD animal model which amplifies proinflammatory microenvironments and understates anti-inflammatory responses in AD animal brain. This particular animal model is most suitable for investigating effects and mechanisms of actions of a host of compounds which demonstrate anti-inflammatory activity. As noted above, studies of inflammatory responses in peptide-injected rat hippocampus offer some advantages as an alternative to the much more commonly used transgenic mouse models in relation to inflamed human brain.

Another noteworthy point is that although considerable evidence for putative detrimental actions of microglial reactivity is available [2], activation of microglial cells in AD can have positive effects. For example, increased levels of a number of anti-inflammatory factors such as TGF-β1 can be produced by activated glial cells in transgenic AD mice and in AD brain [58, 59]. The beneficial responses of activated microglia in disease have been considered [3, 5, 60–62]. Although chronic inflammation may tilt the balance towards proinflammatory reactivity in AD brain, activation of microglia can lead to functional cell responses which confer neuroprotection.

6. Conclusions

In summary, animal models using injection of peptide into animal brain offer alternative approaches to the more thoroughly studied transgenic mouse models. Peptide injection models commonly manifest considerable gliosis which, in
some studies, has been linked to neuronal damage. In particular, injection of Aβ1-42 peptide into rat hippocampus exacerbates inflammatory reactivity in an AD animal model. The amplified proinflammatory responses in peptide-injected rat brain are associated with neurodegenerative processes with drug inhibition of microglial reactivity conferring partial neuroprotection. Importantly, the peptide-injection model appears to replicate the changes in cellular properties and brain microenvironments evident in inflamed brain in the progression of AD pathology.

Conflict of Interests
The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments
Grants from Pacific Alzheimer’s Research Foundation and Canadian Institute for Health Research have supported this work. Dr. Jae K. Ryu has actively participated in experimental work using the peptide-intrahippocampal injection animal model.

References
[1] P. Eikelenboom, R. Veerhuis, W. Scheper, A. J. M. Rozemuller, W. A. Van Gool, and J. J. M. Hoozemans, “The significance of neuroinflammation in understanding Alzheimer's disease,” Journal of Neural Transmission, vol. 113, no. 11, pp. 1685–1695, 2006.
[2] H. Akiyama, S. Barger, S. Barnum et al., “Inflammation and Alzheimer's disease,” Neurobiology of Aging, vol. 21, no. 3, pp. 383–421, 2000.
[3] T. Wyss-Coray, “Inflammation in Alzheimer disease: driving force, bystander or beneficial response?” Nature Medicine, vol. 12, no. 9, pp. 1005–1015, 2006.
[4] E. G. McGeer and P. L. McGeer, “Inflammatory processes in Alzheimer's disease,” Progress in Neuro-Psychopharmacology and Biological Psychiatry, vol. 27, no. 5, pp. 741–749, 2003.
[5] G. Cappellano, M. Carecchio, T. Fleetwood et al., “Immunity and inflammation in neurodegenerative diseases,” American Journal of Neurodegenerative Disease, vol. 2, pp. 89–107, 2013.
[6] J. C. S. Breitner, B. A. Gau, K. A. Welsh et al., “Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study,” Neurology, vol. 44, no. 2, pp. 227–232, 1994.
[7] M. Sastre and S. M. Gentleman, “NSAIDs: how they work and their prospects as therapeutics in Alzheimer's disease,” Frontiers in Aging Neuroscience, vol. 2, article 20, 2010.
[8] P. L. McGeer and E. G. McGeer, “NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies,” Neurobiology of Aging, vol. 28, no. 6, pp. 639–647, 2007.
[9] R. M. Petters and J. R. Sommer, “Transgenic animals as models for human disease,” Transgenic Research, vol. 9, no. 4-5, pp. 347–351, 2000.
[10] J. Götz, J. R. Streffer, D. David et al., “Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy,” Molecular Psychiatry, vol. 9, no. 7, pp. 664–683, 2004.
[11] T. L. Spires and B. T. Hyman, “Transgenic models of Alzheimer's disease: learning from animals,” NeuroRx, vol. 2, no. 3, pp. 423–437, 2005.
[12] G. Münch, J. Apelt, R. Kientsch-Engel, P. Stahl, H. J. Lüth, and R. Schliebs, “Advanced glycation endproducts and pro-inflammatory cytokines in transgenic Tg2576 mice with amyloid plaque pathology,” Journal of Neurochemistry, vol. 86, no. 2, pp. 283–289, 2003.
[13] J. Götz, J. R. Streffer, D. David et al., “Transgenic animal models of Alzheimer’s disease and related disorders: histopathology, behavior and therapy,” Molecular Psychiatry, vol. 9, no. 7, pp. 664–683, 2004.
[14] M. C. Irizarry, M. McNamara, K. Fedorchak, K. Hsiiao, and B. T. Hyman, “APP(Sw) transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1,” Journal of Neuropathology and Experimental Neurology, vol. 56, no. 9, pp. 965–973, 1997.
[15] 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.
[16] R. F. Mervis, J. McKeon, T. Pindell et al., “Quantitative assessment of neuronal damage in a transgenic murine model of Alzheimer's disease,” CNS Drug Reviews, vol. 5, no. 1, pp. 20–21, 1999.
[17] M. E. Calhoun, K.-H. Wiederhold, D. Abramowski et al., “Neuron loss in APP transgenic mice,” Nature, vol. 395, no. 6704, pp. 755–756, 1998.
[18] C. A. Colton, M. P. Vitek, D. A. Wink et al., “NO synthase 2 (NOS2) deletion promotes multiple pathologies in a mouse model of Alzheimer's disease,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 34, pp. 12867–12872, 2006.
[19] S. D. Carma and A. C. Cuello, “Modelling Alzheimer’s disease in transgenic rats,” Molecular Neurodegeneration, vol. 8, article 37, 2013.
[20] 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.
[21] L.-F. Lue, R. Rydel, E. F. Brigham et al., “Inflammatory repertoire of Alzheimer's disease and non-demented elderly microglia in vitro,” Glia, vol. 35, no. 1, pp. 72–79, 2001.
[22] 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.
[23] C. K. Combs, J. Colleen Karlo, S.-C. Kao, and G. E. Landreth, “β-amyloid stimulation of microglia and monocytes results in TNFα-dependent expression of inducible nitric oxide synthase and neuronal apoptosis,” Journal of Neuroscience, vol. 21, no. 4, pp. 1179–1188, 2001.
[24] 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.
[25] D. G. Walker, L.-F. Lue, and T. G. Beach, “Gene expression profiling of amyloid beta peptide-stimulated human post-mortem brain microglia,” Neurobiology of Aging, vol. 22, no. 6, pp. 957–966, 2001.
[26] S. Franciosi, J. K. Ryu, H. B. Choi, L. Radow, S. U. Kim, and J. G. McLarnon, “Broad-spectrum effects of 4-aminopyridine to modulate amyloid β1-42-induced cell signaling and functional
responses in human microglia,” *Journal of Neuroscience*, vol. 26, no. 45, pp. 11652–11664, 2006.

[27] S. A. Frautschy, F. Yang, L. Calderón, and G. M. Cole, “Rodent models of Alzheimer’s disease: rat Aβ infusion approaches to amyloid deposits,” *Neurobiology of Aging*, vol. 17, no. 2, pp. 311–321, 1996.

[28] E. O’Hare, D. T. Weldon, P. W. Mantyh et al., “Delayed behavioral effects following intrahippocampal injection of aggregated Aβ<sub>1–42</sub>,” *Brain Research*, vol. 815, no. 1, pp. 1–10, 1999.

[29] J. Winkler, D. J. Connor, S. A. Frautschy et al., “Lack of long-term effects after β-amyloid protein injections in rat brain,” *Neurobiology of Aging*, vol. 15, no. 5, pp. 601–607, 1994.

[30] E. M. Sigurdsson, J. M. Lee, X.-W. Dong, M. J. Hejna, and S. A. Lorens, “Bilateral injections of amyloid-β 25–35 into the amygdala of young Fischer rats: behavioral, neurochemical, and time dependent histopathological effects,” *Neurobiology of Aging*, vol. 18, no. 6, pp. 591–608, 1997.

[31] D. T. Weldon, S. D. Rogers, J. R. Ghilardi et al., “Fibrillar β-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo,” *Journal of Neuroscience*, vol. 18, no. 6, pp. 2161–2173, 1998.

[32] G. P. 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.

[33] R. L. Richardson, E.-M. Kim, R. A. Shephard, T. Gardiner, J. Cleary, and E. O’Hare, “Behavioural and histopathological analyses of ibuprofen treatment on the effect of aggregated Aβ<sub>1–42</sub> injections in the rat,” *Brain Research*, vol. 954, no. 1, pp. 1–10, 2002.

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

[35] E. O’Hare, D. I. C. Scopes, J. M. Treherne et al., “Novel anti-inflammatory compound SEN1176 alleviates behavioral deficits induced following bilateral intrahippocampal injection of aggregated amyloid-β<sub>1–42</sub>,” *Journal of Alzheimer’s Disease*, vol. 25, no. 2, pp. 219–229, 2011.

[36] 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.

[37] 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.

[38] D. Lorton, J. Schaller, A. Lala, and E. De Nardin, “Chemotactic-like receptors and Aβ peptide induced responses in Alzheimer’s disease,” *Neurobiology of Aging*, vol. 21, no. 3, pp. 463–473, 2000.

[39] 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.

[40] 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.

[41] J. K. Ryu and J. G. McLarnon, “Thalidomide inhibition of perturbed vasculature and glial-derived tumor necrosis factor-α in an animal model of inflamed Alzheimer’s disease brain,” *Neurobiology of Disease*, vol. 29, no. 2, pp. 254–266, 2008.

[42] D. Lorton, J. -M. Kocsis, L. King, K. Madden, and K. R. Brunden, “β-amyloid induces increased release of interleukin-1β from lipopolysaccharide-activated human monocyte,” *Journal of Neuroimmunology*, vol. 67, no. 1, pp. 21–29, 1996.

[43] S. Franciosi, H. B. Choi, S. U. Kim, and J. G. McLarnon, “IL-8 enhancement of amyloid-beta (Aβ1-42)-induced expression and production of pro-inflammatory cytokines and COX-2 in cultured human microglia,” *Journal of Neuroimmunology*, vol. 159, no. 1-2, pp. 66–74, 2005.

[44] M. Noda, H. Nakanishi, and N. Akaike, “Glutamate release from microglia via glutamate transporter is enhanced by amyloid-beta peptide,” *Neuroscience*, vol. 92, no. 4, pp. 1465–1474, 1999.

[45] J. G. McLarnon and J. K. Ryu, “Relevance of Aβ<sub>1–42</sub> intrahippocampal injection as an animal model of inflamed Alzheimer’s disease brain,” *Current Alzheimer Research*, vol. 5, no. 5, pp. 475–480, 2008.

[46] S. Hashioka, J. G. McLarnon, J. K. Ryu et al., “Pyrazole compound 2-MBAPA as a novel inhibitor of microglial activation and neurotoxicity in vitro and in vivo,” *Journal of Alzheimer’s Disease*, vol. 27, no. 3, pp. 531–541, 2011.

[47] J. K. Ryu, J. P. Little, A. Kugleris, N. Jantararatni, and J. G. McLarnon, “Actions of the anti-angiogenic compound angiotatin in an animal model of Alzheimer’s disease,” *Current Alzheimer Research*, vol. 10, no. 3, pp. 252–260, 2013.

[48] J. K. Ryu, T. Cho, H. B. Choi, Y. T. Wang, and J. G. McLarnon, “Microglial VEGF receptor response is an integral chemotactic component in Alzheimer’s disease pathology,” *Journal of Neuroscience*, vol. 29, no. 1, pp. 3–13, 2009.

[49] 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. 9A, pp. 2911–2925, 2009.

[50] S. A. Frautschy, A. Baird, and G. M. Cole, “Effects of injected Alzheimer β-amyloid cores in rat brain,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 19, pp. 8362–8366, 1991.

[51] M. Stalder, A. Phinney, M. Staufenbiel, and M. Jucker, “Association of microglia with amyloid plaques in brains of APP23 transgenic mice,” *The American Journal of Pathology*, vol. 154, no. 6, pp. 1673–1684, 1999.

[52] N. Jantararatni, C. Schwab, J. K. Ryu, P. L. McGeer, and J. G. McLarnon, “Converging perturbed microvasculature and microglial clusters characterize Alzheimer disease brain,” *Current Alzheimer research*, vol. 7, no. 7, pp. 625–636, 2010.

[53] H. Braak and E. Braak, “Staging of Alzheimer's disease-related neurofibrillary changes,” *Neurobiology of Aging*, vol. 16, no. 3, pp. 271–278, 1995.

[54] “Consensus recommendations for the postmortem diagnosis of Alzheimer’s disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer’s Disease,” vol. 18, no. 4, pp. SI–S2, 1997.

[55] N. Jantararatni, J. K. Ryu, C. Schwab, P. L. McGeer, and J. G. McLarnon, “Comparison of vascular perturbations in an Aβ-injected animal model and in AD brain,” *International Journal of Alzheimer’s Disease*, vol. 2011, Article ID 918280, 8 pages, 2011.

[56] B. V. Zlokovic, “The blood-brain barrier in health and chronic neurodegenerative disorders,” *Neuron*, vol. 57, no. 2, pp. 178–200, 2008.

[57] P. Grammas, “Neurovascular dysfunction, inflammation and endothelial activation: implications for the pathogenesis of...
Alzheimer's disease," *Journal of Neuroinflammation*, vol. 8, article 26, 2011.

[58] 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.

[59] T. Wyss-Coray, E. Masliah, M. Mallory et al., "Amyloidogenic role of cytokine TGF-β1 in transgenic mice and in Alzheimer's disease," *Nature*, vol. 389, no. 6651, pp. 603–606, 1997.

[60] W. J. Streit, "Microglia as neuroprotective, immunocompetent cells of the CNS," *Glia*, vol. 40, no. 2, pp. 133–139, 2002.

[61] W. J. Streit, "Microglia and neuroprotection: implications for Alzheimer's disease," *Brain Research Reviews*, vol. 48, no. 2, pp. 234–239, 2005.

[62] A. R. Simard and S. Rivest, "Neuroprotective properties of the innate immune system and bone marrow stem cells in Alzheimer's disease," *Molecular Psychiatry*, vol. 11, no. 4, pp. 327–335, 2006.