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Inflammatory Cyclooxygenase Activity and PGE_2 Signaling in Models of Alzheimer's Disease

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Abstract: The inflammatory response is a fundamental driving force in the pathogenesis of Alzheimer's disease (AD). In the setting of accumulating immunogenic Aß peptide assemblies, microglia, the innate immune cells of the brain, generate a non-resolving immune response and fail to adequately clear accumulating Aß peptides, accelerating neuronal and synaptic injury. Pathological, biomarker, and imaging studies point to a prominent role of the innate immune response in AD development, and the molecular components of this response are beginning to be unraveled. The inflammatory cyclooxygenase-PGE_2 pathway is implicated in pre-clinical development of AD, both in epidemiology of normal aging populations and in transgenic mouse models of Familial AD. The cyclooxygenase-PGE_2 pathway modulates the inflammatory response to accumulating Aß peptides through actions of specific E-prostanoid G-protein coupled receptors.

Keywords: Alzheimer's disease, microglia, amyloid beta, inflammation, cyclooxgenases, prostaglandin E_2, EP2 receptor, EP3 receptor, EP4 receptor.

INTRODUCTION

Currently, 1/9 people older than 65 years of age have Alzheimer's disease (AD), and 1/3 of people older than 85 are diagnosed with AD. As the aging population expands, the projected number of AD cases is expected to triple by 2050 [1], with economic costs escalating from $214 billion in 2014 to $1.2 trillion in 2050 (Alzheimer's Association Facts 2014). This represents an enormous economic and societal challenge, particularly given the limited efficacy of currently available AD therapeutics. In terms of AD prevention, attention to diet, exercise, and cardiovascular health are likely to help reduce the risk of developing AD. However, identification of specific molecular pathways that could be targeted for prevention in aging and at-risk populations is an urgently needed strategy to help stem this dementia epidemic. Given that the prevalence of AD doubles every 5 years in persons above the age of 65 and given the average human longevity of ~80 years, preventive strategies that could delay the onset of cognitive decline by only 5 years may reduce disease burden by half. Recent data indicate that cyclooxygenase/PGE_2/EP receptor signaling may play important roles in preclinical development of AD in both human epidemiology and in mouse models of AD.

NEUROINFLAMMATION IN AD

Pathological changes in AD consist of amyloid ß accumulation, tau phosphorylation, and synaptic and neuronal loss. These pathological hallmarks develop in the context of a potent and chronic inflammatory response characterized by glial activation, generation of cytokines and chemokines, complement proteins, inflammatory enzymes, and oxidative stress [2, 3]. This chronic inflammatory response is not only injurious to synapses, neurons, and circuits, but is persistent and non-resolving.

Recent studies indicate that the pre-clinical development of AD begins years to decades before initial diagnosis [4]. While the initiating pathological events are not well defined, they are likely to involve synergistic interactions between Aß oligomer-mediated synaptic injury and dysregulated inflammatory responses. Amyloid ß peptide generation and accumulation is an initiating event, and precedes onset of symptoms by years to decades. However, amyloid PET imaging studies suggest that Aß peptide accumulation can occur in subjects who do not have evidence of cognitive decline, leading to the hypothesis that Aß peptide accumulation is "necessary but not sufficient" for progression to AD [5]. One or more additional factors may be necessary. In that regard, recent GWAS studies of sporadic late-onset AD have identified genes involved in the innate immune response that are expressed in microglia, the resident myeloid cells of the central nervous system (CNS). These microglial genes include the sialic acid-binding immunoglobulin-like lectin *CD33* [6-8] and *TREM2* [9, 10] which regulate phagocytosis and Aß peptide clearance [11-13], and the complement receptor *CR1* [14, 15]. These findings indicate that the microglial immune response may play a pathogenic role in the development of AD.

Microglia have embryonic origins [16, 17] and genetic signatures [18-20] that distinguish them from monocytes and

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other tissue macrophages. Like other tissue macrophages, microglia maintain local homeostasis by clearing toxic proteins and noxious substances and regulating inflammation. However, because microglia reside in the brain, they have additional and unique functions, including the establishment, maintenance, and pruning of synapses that are dynamically regulated by synaptic activity [21-24]. Microglia can also regulate synaptic activity through mechanisms that are beginning to be identified, including secretion of neuroactive and neurotrophic factors [25]. A role for microglia in the development of AD may therefore involve not only perpetuation of dysfunctional innate immune responses to accumulating Aß peptides and dying synapses and neurons, as the current literature supports, but may also involve direct effects of microglia on synapse integrity and function.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) AND PGE_2 IN AD DEVELOPMENT

The primary action of NSAIDs is the enzymatic inhibition of the cyclooxygenases COX-1 and COX-2, cytosolic enzymes that generate PGH_2 from membrane stores of arachidonic acid. PGH_2 is the precursor of the prostaglandins PGE_2, PGD_2, PGI_2, and PGF_{2a}, and the thromboxane TXA_2. In epidemiologic studies of cognitively normal aging populations, NSAIDs prevent and delay development of AD [26-31]. Although selected NSAIDs may have cyclooxygenase-independent effects, structurally distinct NSAIDs, including ibuprofen, naproxen, and sulindac all reduce risk of developing AD in large epidemiologic studies [27, 28, 30] suggesting that inflammatory prostaglandin signaling plays an important role in pre-clinical development of AD. Interestingly, the beneficial effects of NSAIDs are restricted to the pre-clinical asymptomatic phase, as NSAIDs or selective COX-2 inhibitors do not help patients with mild cognitive impairment (MCI) or AD [31-36]. NSAIDs however are not a good choice for large scale AD prevention because both toxic as well as beneficial downstream prostaglandin signaling pathways are inhibited and lead to adverse effects, including renal and gastric toxicity and increased risk for vascular disease [37]. Recent studies have identified beneficial prostaglandin signaling pathways downstream of NSAID action, including the vasodilatory prostacyclin PGI_2 receptor and the neuroprotective, anti-inflammatory, and vasodilatory PGE_2 EP4 receptor [38-42]. This major limitation may help explain why in MCI and AD, NSAIDs show no benefit, either because protective downstream prostaglandin pathways are inhibited or disease progression is already too advanced.

Inhibition of COX enzymatic activity by NSAIDs therefore has different consequences depending on timing of AD development, and inhibition of COX-1/COX-2 by non-selective NSAIDs is beneficial in preventing disease in healthy aging individuals but ineffectual once symptoms begin [31, 43]. Given the expanding population of aging individuals and the anticipated rise in AD cases, understanding the molecular mechanisms by which NSAIDs prevent AD has taken on significant urgency. Targeting of toxic inflammatory prostaglandin signaling downstream of COX may potentially slow or prevent progression to AD.

PGE_2, PGD_2, PGI_2, and PGF_{2a}, and the thromboxane TXA_2 are lipid signaling molecules that bind and activate specific G-protein-coupled receptors designated EP (for E-prostanoid receptor), DP, IP, FP, and TP, respectively [44]. PGE_2 in particular has generated interest as a potential inflammatory agent in pre-clinical AD, as it was found to be increased 5-fold in cerebrospinal fluid (CSF) of patients with early or probable AD [45, 46], but then declined with disease progression [46]. In parallel, levels of the breakdown product of prostacyclin (PGI_2), widely considered to be anti-inflammatory, were significantly decreased in CSF of probable AD subjects [45]. PGE_2 binds four G-protein coupled receptors (GPCRs) termed E-prostanoid receptors (EP1-4) that have distinct downstream signaling cascades and cellular distributions in brain. *In vivo*, all four EP receptors are expressed in neurons; microglial expression of EP2, EP3, and EP4 receptors has been confirmed in mouse brain [41, 47, 48]. Activation of EP receptors leads to changes in the production of cAMP and/or phosphoinositol turnover and Ca^{2+} mobilization. EP2 and EP4 receptors couple positively to G_s to increase cAMP formation whereas EP3 couples negatively to cAMP through G_i; EP1 couples to G_q, and activation results in increased intracellular calcium concentrations.

MOUSE MODELING OF PREVENTIVE EFFECTS OF NSAIDS

To better understand the inflammatory PGE_2 signaling pathway in the context of Aß peptide accumulation, investigators have studied mouse models of Familial AD, where transgenic mice express mutant forms of the amyloid precursor protein (APP) and/or presenilin 1 (PS1) genes. Microglial activation and elaboration of inflammatory and oxidative stress are well documented in these models, particularly as these mutant APP mice age and accumulate $Aß_{42}$ and $Aß_{40}$ peptides. Mutant APP models display either loss of synapses or loss of synaptic proteins that are associated with spatial memory deficits, and these have been linked to effects of Aß oligomers, which are directly toxic to synapses [49], and to effects of inflammatory mediators like IL1ß [50] or TNFα [51]. However, because mutant APP models do not develop significant neuronal loss and tau pathology, two hallmarks of MCI and AD in human subjects, these models are believed to be more reflective of the pre-clinical or asymptomatic phases of human AD [52]. If considered in this way, the beneficial effects of NSAIDs have been validated in these mutant APP models, where a correlation between NSAID administration and reduction of brain inflammation, Aβ deposition, and rescue of learning and memory deficits has been established [53-56].

ROLES OF MICROGLIAL EP RECEPTORS IN MODELS OF AD: OPPOSING EFFECTS OF MICROGLIAL EP2 AND EP4 IN MOUSE AD MODELS

Early studies examining *in vivo* effects of EP2 signaling in innate immunity demonstrated a significant reduction in lipid peroxidation following intracerebroventricular (ICV) administration of lipopolysaccharide (LPS) [57], a canonical inducer of the innate immune response. LPS-dependent

increases in F2-isoprostanes, which are free radical-generated isomers of prostaglandin PGF_{2a}, and F4-neuroprostanes (isoPs), which are products of neuronal docosohexanoic acid (DHA) oxidation, were significantly suppressed in cerebral cortex of EP2-/- mice [57]. *In vitro* studies parsing out the cellular specificity of the EP2 oxidative effect demonstrated that microglial EP2 elicited paracrine neurotoxicity in co-cultures of neurons and LPS-primed microglia, and this effect was dependent on increased inducible nitric oxide synthase (iNOS) and COX-2 activity [58]. *In vivo*, in the APPSwe-PS1ΔE9 model of AD (APP-PS1), global deletion of EP2 also led to significant decreases in lipid peroxidation in aging APP-PS1 mice [59], suggesting a toxic inflammatory role for the EP2 receptor. Additional *in vivo* studies demonstrated that global deletion of EP2 reduced expression of oxidative enzymes iNOS and components of the NADPH oxidase complex in the APP-PS1 model of AD, and reduced expression of COX-1, COX-2, iNOS, and many components of the NADPH oxidase complex in a related model of familial amyotrophic lateral sclerosis (ALS) [60].

A major target of the innate immune response in the CNS is the clearance of toxic and misfolded proteins. The accumulation of misfolded or aggregated proteins is a common feature of several chronic neurodegenerative diseases, including AD, Parkinson's disease, Huntington's disease, and ALS. Microglia play a crucial role in the clearance of these toxic protein assemblies [61]. However, with progression of disease, notably in AD models, the healthy phagocytic response of microglia to Aß peptides falters, either because microglia become ineffective or because they are overwhelmed by levels of accumulating Aß peptides. In parallel with progression of pathology in AD model mice, microglia also develop a more toxic inflammatory phenotype [62]. This leads to a damaging feed-forward cycle, with increasing accumulation of toxic Aß peptide assemblies along with increased elaboration of toxic cytokines. In AD model mice, Aβ signaling through Toll-like receptors 2 and 4 (TLR2 and TLR4) drives downstream activation of NF-κB transcription factors as a central inflammatory pathway in AD [63].

A role for microglial EP2 in inhibiting phagocytosis of Aß fibrils was demonstrated in an ex vivo acute preparation using AD brain sections coated with EP2-/- microglia [64]. Microglia lacking EP2 receptor cleared human Aß peptides more effectively than wild type microglia, and EP2-/- microglia were associated with lower paracrine neurotoxicity. *In vivo*, in the APP-PS1 model, deletion of EP2 resulted in significant reductions in total Aß40 and Aß42 levels and amyloid plaque deposition, an outcome likely reflecting both a more benign inflammatory milieu and an enhanced clearance of Aß peptides [65]. In chimeric APP-PS1 mice subjected to whole body irradiation followed by transplantation of wild type or EP2-/- bone marrow, levels of amyloid plaque were reduced in APP-PS1 mice receiving wild type bone marrow, however EP2-/- bone marrow elicited even larger decreases in cerebral cortical plaque load [66]. A role for EP2 signaling in phagocytosis has been shown in non-CNS models, where myeloid EP2 suppresses phagocytosis of latex beads [67, 68] and bacteria [69-72].

Recent *in vivo* studies using conditional knockout strategies have further defined the critical toxicity of microglial EP2 signaling in models of AD. Conditional knock down of myeloid EP2 receptor using the Cd11bCre recombinase line, where levels of myeloid EP2 are reduced ~50% [47], had multiple beneficial effects and restored healthy microglial responses to Aß peptides [73]. Cell-specific knockout of microglial EP2 increased microglial clearance of Aß peptides and suppressed toxic inflammatory gene expression. In addition, unbiased genomic studies of microglia isolated from brain revealed that knockdown of microglial EP2 receptor increased generation and local signaling of insulin-like growth factor 1 (IGF1) in hippocampus in response to Aß peptide stimulation [73]. IGF1 promotes synaptogenesis, neurogenesis, and neuroprotection through the PI3K/Akt pathway in brain [74]. Deletion of microglial EP2 also increased expression of members of the PPAR signaling pathway, including RXRγ which along with its binding partner PPARγ reduces proinflammatory gene expression [75] and enhances clearance of Aß peptides [76]. RXRγ is also the target of the FDA-approved RXR agonist bexarotene (Targretin) that has been shown in some studies to lower interstitial levels of soluble Aß peptides and to prevent memory deficits in AD model mice [77, 78]. Additional genes suppressed by EP2 signaling in microglia but intimately related to Aß peptide clearance included the cholesterol transporter ABCA1 [79] and apolipoprotein E (ApoE) [80], proteins that enhance proteolytic degradation of soluble Aß peptides [81, 82]. In addition, lipoprotein lipase (lpl), which binds Aß peptide [83], was also upregulated in hippocampus with EP2 microglial deletion; interestingly, an intronic polymorphism in Lpl is associated with reduced Lpl mRNA, increased amyloid and neurofibrillary tangle densities, and increased prevalence of AD [84]. The upregulation of these genes in microglial EP2 knockout mice suggests that EP2-deficient microglia respond to $Aß_{42}$ peptides *in vivo* by inducing anti-inflammatory and Aß-clearing nuclear hormone receptor signaling genes. Consistent with this beneficial effect of reduced EP2 signaling, conditional deletion of microglial EP2 in the APP-PS1 model prevented synaptic injury and spatial memory deficits [73].

The toxic effects of microglial EP2 contrast significantly with the beneficial anti-inflammatory effects of microglial EP4 *in vivo*. Previous *in vivo* studies of innate immune responses to LPS had identified a pronounced anti-inflammatory effect of microglial EP4 activation that was associated with reduced nuclear translocation of NF-κB subunits p65 and p50 [41] in myeloid cells. Conversely, following LPS stimulation *in vivo*, conditional deletion of the EP4 receptor in myeloid cells led to an increase in brain pro-inflammatory gene expression and lipid peroxidation, suggesting that the function of myeloid EP4 is to attenuate and/or terminate innate immune responses. Subsequent studies of Aß42-mediated inflammatory responses confirmed the anti-inflammatory nature of microglial EP4 signaling, where in primary microglial cells, EP4 stimulation attenuated levels of $Aβ_{42}$-induced inflammatory factors and potentiated phagocytosis of $Aβ_{42}$ [41]. Unbiased genomic studies showed that EP4 receptor activation broadly opposed $Aβ_{42}$-driven gene expression changes in microglia, with

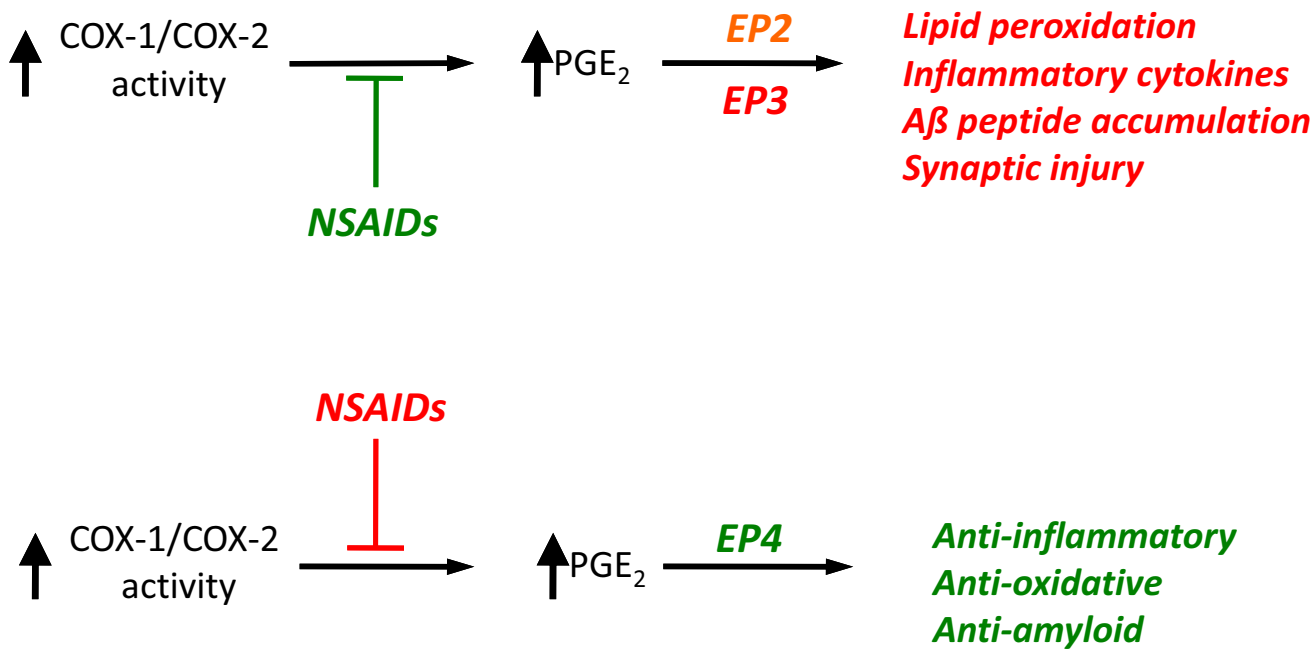

Fig. (1). Summary of inflammatory effects of the COX/PGE$_2$/EP receptor signaling pathways in mouse mutant APP models. Modeling of EP2 and EP3 (top) and EP4 (bottom) inflammatory signaling in mouse models of AD indicates that EP2 and EP3 receptors enhance inflammatory oxidative stress, pro-inflammatory gene expression and are pro-amyloidogenic. In contrast, EP4 signaling in the setting of Aß-mediated innate immune responses is anti-inflammatory and enhances Aß phagocytosis. In preclinical AD, use of NSAIDs is preventive only in normal cognitive aging populations. Later symptomatic stages do not respond, potentially because beneficial PGE$_2$ signaling pathways such as the EP4 receptor, as well as others including the prostacyclin (IP) receptor, are inhibited along with the toxic EP2 and EP3 pathways.

significant enrichment of targets of IRF1, IRF7, and NF-κB transcription factors [41]. *In vivo*, in APP-PS1 mice deficient for microglial EP4, inflammatory gene expression, oxidative protein modification, and Aβ deposition in brain were significantly increased at early stages of pathology, but not at later stages, suggesting an early anti-inflammatory function of microglial EP4 signaling in the APP-PS1 model. Thus, although both the EP2 and EP4 receptors are positively coupled to cAMP, they appear to have divergent inflammatory functions in models of Aß peptide mediated neuroinflammation and neurodegeneration. The carboxy terminal cytoplasmic tail of the EP4 receptor is significantly longer than that of the EP2 receptor and can recruit distinct signaling molecules [85], a difference likely to influence downstream signaling of the EP4 versus EP2 receptors [86].

FUNCTION OF THE EP3 AND EP1 RECEPTORS IN THE INFLAMMATORY RESPONSE IN AD

The EP3 receptor is a central component in the regulation of the febrile response [87, 88]. In classical models of innate immunity, the function of inflammatory EP3 has been explored in a model of bacterial lung infection, where deletion of EP3 resulted in a marked increase in clearance of bacteria, reduced neutrophil ingress, and improved survival [89], a phenotype reminiscent of the pro-phagocytic and anti-inflammatory effects of EP2 deletion, as discussed above. In the brain, EP3 has mainly been localized to neurons, however immunocytochemical induction of EP3 expression has been observed in striatal microglia in the setting of an excitotoxic lesion induced by injection of quinolinic acid, a potent ligand at the glutamatergic N-methyl-D-Aspartate (NMDA) receptor [48]. This suggests that although EP3 may not be expressed in microglia under physiological conditions, in the appropriate inflammatory or injury contexts, EP3 might be induced and contribute to innate immune responses.

The function of EP3 signaling has been examined in two models relevant to AD, namely the ICV Aß injection model, which elicits a potent and long lasting inflammatory response to Aß peptides [90, 91] and in the APP-PS1 model. In a recent study [92], the induction of pro-inflammatory gene expression, cytokine generation, and lipid peroxidation following ICV Aß$_{42}$ was significantly abrogated in EP3-/- mice, suggesting that microglial EP3 signaling engaged a toxic inflammatory response to Aß peptides. In the APP-PS1 model, deletion of EP3 significantly blunted the induction of proteins capable of increasing oxidative injury, including iNOS, components of the NADPH oxidase complex, and COX-2. Moreover, suggestive of a role in Aß clearance, APP-PS1 mice lacking either one or both EP3 alleles had significantly lower amyloid accumulation. This effect is consistent with the lung infection studies mentioned above, where deletion of EP3 led to enhanced clearance of bacteria [89]. However, it is also possible EP3 deletion impacted on the generation of Aß peptide from APP, as prior studies have correlated increased oxidative stress and inflammation with increased expression and activity of ß-secretase [93, 94], the first enzyme to cleave APP in the formation of the Aß42 peptide. Indeed, loss of EP3 receptor in the APP-PS1 background resulted in decreased ß-secretase expression and

activity. This would suggest that in the APP-PS1 model, EP3 signaling may both suppress Aß clearance, and by increasing inflammatory oxidative stress, increase the generation of Aß peptide. Interestingly, loss of just one allele of EP3 in the APP-PS1 background had significant effects on Aß peptide levels.

Of the four EP receptors, the role of inflammatory EP1 in AD is less clear. EP1 is highly expressed in neurons [95, 96] and functions in neuronal survival. In models of neuronal injury, including NMDA excitotoxicity and cerebral ischemia, pharmacologic or genetic deletion of EP1 reduces cerebral injury [96-98]. However, EP1 expression is not found in microglia after hypoxia-ischemia, nor does microglial activation induce neurotoxicity in hippocampal slices treated with LPS and IFNγ[96]. A lack of effect of inflammatory EP1 would suggest that the toxic function of this receptor is primarily neuronal. Supporting this conclusion are findings that inflammation sensitive neural progenitor cells (NPC) [99] in the subgranular zone of the dentate gyrus are vulnerable to microglial EP2 signaling and NPC EP1 signaling [100].

CONCLUSION

Aß peptides are highly immunogenic, and generate toxic inflammatory responses that injure synapses. Pre-clinical development of AD begins decades prior to diagnosis, and NSAIDs act during this time period to delay onset and progression to AD. The early accumulation of Aß42 peptides, beginning years to decades before cognitive symptoms arise, triggers microglial inflammatory responses that are initially robust, but falter as disease progresses. A summary of findings relevant to inflammatory actions of the PGE2 EP2, EP3, and EP4 receptors in the APP-PS1 model are diagrammed in Fig. (**1**). The opposing actions of the EP2/EP3 and EP4 receptors highlight the importance of targeting selected EP receptors downstream of COX-1/COX-2, as upstream inhibition of COX-1/COX-2 activity may inhibit beneficial as well as toxic PGE_2 EP receptor signaling. This is a potential future indication, as we await the identification and validation of biomarkers that can reliably predict subjects at risk for AD.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Hebert LE, Scherr PA, Bienias JL, *et al*. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 2003; 60(8): 1119-22.

[2] Akiyama H, Barger S, Barnum S, *et al*. Inflammation and Alzheimer's disease. Neurobiol Aging 2000; 21(3): 383-421.

[3] Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. J Neuroimmunol 2007; 184(1-2): 69-91.

[4] Bateman RJ, Xiong C, Benzinger TL, *et al*. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012; 367(9): 795-804.

[5] Sperling RA, Aisen PS, Beckett LA, *et al*. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7(3): 280-92.

[6] Naj AC, Jun G, Beecham GW, *et al*. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 2011; 43(5): 436-41.

[7] Bertram L, Lange C, Mullin K, *et al*. Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. Am J Hum Genet 2008; 83(5): 623-32.

[8] Hollingworth P, Harold D, Sims R, *et al*. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 2011; 43(5): 429-35.

[9] Guerreiro R, Wojtas A, Bras J, *et al*. TREM2 variants in Alzheimer's disease. N Engl J Med 2013; 368(2): 117-27.

[10] Jonsson T, Stefansson H, Steinberg S, *et al*. Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 2013; 368(2): 107-16.

[11] Griciuc A, Serrano-Pozo A, Parrado AR, *et al*. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 2013; 78(4): 631-43.

[12] Malik M, Simpson JF, Parikh I, *et al*. CD33 Alzheimer's risk-altering polymorphism, CD33 expression, and exon 2 splicing. J Neurosci 2013; 33(33): 13320-5.

[13] Kleinberger G, Yamanishi Y, Suarez-Calvet M, *et al*. TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. Sci Transl Med 2014; 6(243): 243ra86.

[14] Lambert JC, Ibrahim-Verbaas CA, Harold D, *et al*. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 2013; 45(12): 1452-8.

[15] Lambert JC, Heath S, Even G, *et al*. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009; 41(10): 1094-9.

[16] Ginhoux F, Greter M, Leboeuf M, *et al*. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 2010; 330(6005): 841-5.

[17] Ransohoff RM and Cardona AE. The myeloid cells of the central nervous system parenchyma. Nature 2010; 468(7321): 253-62.

[18] Butovsky O, Jedrychowski MP, Moore CS, *et al*. Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat Neurosci 2014; 17(1): 131-43.

[19] Hickman SE, Kingery ND, Ohsumi TK, *et al*. The microglial sensome revealed by direct RNA sequencing. Nat Neurosci 2013; 16(12): 1896-905.

[20] Gautier EL, Shay T, Miller J, *et al*. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol 2012; 13(11): 1118-28.

[21] Paolicelli RC, Bolasco G, Pagani F, *et al*. Synaptic pruning by microglia is necessary for normal brain development. Science 2011; 333(6048): 1456-8.

[22] Zhan Y, Paolicelli RC, Sforazzini F, *et al*. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 2014; 17(3): 400-6.

[23] Schafer DP, Lehrman EK, Kautzman AG, *et al*. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 2012; 74(4): 691-705.

[24] Stevens B, Allen NJ, Vazquez LE, *et al*. The classical complement cascade mediates CNS synapse elimination. Cell 2007; 131(6): 1164-78.

[25] Kettenmann H, Kirchhoff F, Verkhratsky A. Microglia: new roles for the synaptic stripper. Neuron 2013; 77(1): 10-8.

[26] Szekely CA, Thorne JE, Zandi PP, *et al*. Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review. Neuroepidemiology 2004; 23(4): 159-69.

[27] Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. Neurology 1997; 48: 626-32.

[28] in t' Veld BA, Ruitenberg A, Hofman A, *et al*. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N Engl J Med 2001; 345(21): 1515-21.

[29] McGeer PL, Schulzer M, and McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology 1996; 47(2): 425-32.

[30] Vlad SC, Miller DR, Kowall NW, *et al*. Protective effects of NSAIDs on the development of Alzheimer disease. Neurology 2008; 70(19): 1672-7.

[31] Breitner JC, Baker LD, Montine TJ, *et al*. Extended results of the Alzheimer's disease anti-inflammatory prevention trial. Alzheimers Dement 2011; 7(4): 402-11.

[32] Aisen PS, Schafer KA, Grundman M, *et al*. Effects of rofecoxib or naproxen *vs* placebo on Alzheimer disease progression: a randomized controlled trial. Jama 2003; 289(21): 2819-26.

[33] Soininen H, West C, Robbins J, *et al*. Long-term efficacy and safety of celecoxib in Alzheimer's disease. Dement Geriatr Cogn Disord 2007; 23(1): 8-21.

[34] Thal LJ, Ferris SH, Kirby L, *et al*. A randomized, double-blind, study of rofecoxib in patients with mild cognitive impairment. Neuropsychopharmacology 2005; 30(6): 1204-15.

[35] Lyketsos CG, Breitner JC, Green RC, *et al*. Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. Neurology 2007; 68(21): 1800-8.

[36] Martin BK, Szekely C, Brandt J, *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. Arch Neurol 2008; 65(7): 896-905.

[37] Andreasson K. Emerging roles of PGE2 receptors in models of neurological disease. Prostaglandins Other Lipid Mediat 2010; 91(3-4): 104-12.

[38] Egan KM, Lawson JA, Fries S, *et al*. COX-2-derived prostacyclin confers atheroprotection on female mice. Science 2004; 306(5703): 1954-7.

[39] Funk CD, FitzGerald GA. COX-2 inhibitors and cardiovascular risk. J Cardiovasc Pharmacol 2007; 50(5): 470-9.

[40] Liang X, Lin L, Wang Q, *et al*. Neuronal and vascular protection by the prostaglandin E2 EP4 receptor in a mouse model of cerebral ischemia. J Clin Invest 2011; 121(11): 4362-71.

[41] Shi J, Johansson J, Woodling NS, *et al*. The prostaglandin E2 E-prostanoid 4 receptor exerts anti-inflammatory effects in brain innate immunity. J Immunol 2010; 184(12): 7207-18.

[42] Woodling NS, Wang Q, Priyam PG, *et al*. Suppression of Alzheimer-Associated Inflammation by Microglial Prostaglandin-E2 EP4 Receptor Signaling. J Neurosci 2014; 34(17): 5882-94.

[43] Leoutsakos JM, Muthen BO, Breitner JC, *et al*. Effects of non-steroidal anti-inflammatory drug treatments on cognitive decline vary by phase of pre-clinical Alzheimer disease: findings from the randomized controlled Alzheimer's Disease Anti-inflammatory Prevention Trial. Int J Geriatr Psychiatry 2011.

[44] Breyer RM, Bagdassarian CK, Myers SA, *et al*. Prostanoid receptors: subtypes and signaling. Annu Rev Pharmacol Toxicol 2001; 41: 661-90.

[45] Montine TJ, Sidell KR, Crews BC, *et al*. Elevated CSF prostaglandin E2 levels in patients with probable AD. Neurology 1999; 53(7): 1495-8.

[46] Combrinck M, Williams J, De Berardinis MA, *et al*. Levels of CSF prostaglandin E2, cognitive decline, and survival in Alzheimer's disease. J Neurol Neurosurg Psychiatry 2006; 77(1): 85-8.

[47] Johansson JU, Pradhan, S, Lokteva LA, *et al*. Suppression of inflammation with conditional deletion of the prostaglandin E2 EP2 receptor in macrophages and brain microglia J Neurosci 2013; 33: 16012-32.

[48] Slawik H, Volk B, Fiebich B, *et al*. Microglial expression of prostaglandin EP3 receptor in excitotoxic lesions in the rat striatum. Neurochem Int 2004; 45(5): 653-60.

[49] Mucke L, Selkoe DJ. Neurotoxicity of Amyloid beta-Protein: Synaptic and Network Dysfunction. Cold Spring Harb Perspect Med 2012; 2(7): a006338.

[50] Hein AM, Stasko MR, Matousek SB, *et al*. Sustained hippocampal IL-1beta overexpression impairs contextual and spatial memory in transgenic mice. Brain Behav Immun 2010; 24(2): 243-53.

[51] Medeiros R, Prediger RD, Passos GF, *et al*. Connecting TNF-alpha signaling pathways to iNOS expression in a mouse model of Alzheimer's disease: relevance for the behavioral and synaptic deficits induced by amyloid beta protein. J Neurosci 2007; 27(20): 5394-404.

[52] Ashe KH, Zahs KR. Probing the biology of Alzheimer's disease in mice. Neuron 2010; 66(5): 631-45.

[53] Yan Q, Zhang J, Liu H, *et al*. Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer's disease. J Neurosci 2003; 23(20): 7504-9.

[54] Lim GP, Yang F, Chu T, *et al*. Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. Neurobiol Aging 2001; 22(6): 983-91.

[55] Lim GP, Yang F, Chu T, *et al*. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci 2000; 20(15): 5709-14.

[56] Kotilinek LA, Westerman MA, Wang Q, *et al*. Cyclooxygenase-2 inhibition improves amyloid-beta-mediated suppression of memory and synaptic plasticity. Brain 2008; 131(Pt 3): 651-64.

[57] Montine TJ, Milatovic D, Gupta RC, *et al*. Neuronal oxidative damage from activated innate immunity is EP2 receptor-dependent. J Neurochem 2002; 83(2): 463-70.

[58] Shie FS, Montine KS, Breyer RM, *et al*. Microglial EP2 is critical to neurotoxicity from activated cerebral innate immunity. Glia 2005; 52(1): 70-7.

[59] Liang X, Wang Q, Lokteva L, *et al*. The PGE2 PE2 receptor accelerates disease progression and inflammation in a model of amyotrophic lateral sclerosis. Soc. Neuroscience 2007; 2007(160.16).

[60] Liang X, Wang Q, Shi J, *et al*. The prostaglandin E2 EP2 receptor accelerates disease progression and inflammation in a model of amyotrophic lateral sclerosis. Ann Neurol 2008; 64(3): 304-14.

[61] Lee CY, Landreth GE. The role of microglia in amyloid clearance from the AD brain. J Neural Transm 2010; 117(8): 949-60.

[62] Hickman SE, Allison EK, and El Khoury J. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J Neurosci 2008; 28(33): 8354-60.

[63] Landreth GE, Reed-Geaghan EG. Toll-like receptors in Alzheimer's disease. Curr Top Microbiol Immunol 2009; 336: 137-53.

[64] Shie FS, Breyer RM, Montine TJ. Microglia lacking E Prostanoid Receptor subtype 2 have enhanced Abeta phagocytosis yet lack Abeta-activated neurotoxicity. Am J Pathol 2005; 166(4): 1163-72.

[65] Liang X, Wang Q, Hand T, *et al*. Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. J Neurosci 2005; 25(44): 10180-7.

[66] Keene CD, Chang RC, Lopez-Yglesias AH, *et al*. Suppressed accumulation of cerebral amyloid {beta} peptides in aged transgenic Alzheimer's disease mice by transplantation with wild-type or prostaglandin E2 receptor subtype 2-null bone marrow. Am J Pathol 2010; 177(1): 346-54.

[67] Chuang PC, Lin YJ, Wu MH, *et al*. Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. Am J Pathol 2010; 176(2): 850-60.

[68] Nagano T, Kimura SH, and Takemura M. Prostaglandin E2 reduces amyloid beta-induced phagocytosis in cultured rat microglia. Brain Res 2010; 1323: 11-7.

[69] Aronoff DM, Hao Y, Chung J, *et al*. Misoprostol impairs female reproductive tract innate immunity against Clostridium sordellii. J Immunol 2008; 180(12): 8222-30.

[70] Ballinger MN, Aronoff DM, McMillan TR, *et al*. Critical role of prostaglandin E2 overproduction in impaired pulmonary host response following bone marrow transplantation. J Immunol 2006; 177(8): 5499-508.

[71] Medeiros AI, Serezani CH, Lee SP, *et al*. Efferocytosis impairs pulmonary macrophage and lung antibacterial function *via* PGE2/EP2 signaling. J Exp Med 2009; 206(1): 61-8.

[72] Aronoff DM, Canetti C, and Peters-Golden M. Prostaglandin E2 inhibits alveolar macrophage phagocytosis through an E-prostanoid 2 receptor-mediated increase in intracellular cyclic AMP. J Immunol 2004; 173(1): 559-65.

[73] Johansson JU, Woodling NS, Wang Q, *et al*. Prostaglandin signaling suppresses beneficial microglial function in Alzheimer's disease models. J Clin Invest 2015; 125(1): 350-64.

[74] Fernandez AM and Torres-Aleman I. The many faces of insulin-like peptide signalling in the brain. Nat Rev Neurosci 2012; 13(4): 225-39.

[75] Heneka MT, Landreth GE, Hull M. Drug insight: effects mediated by peroxisome proliferator-activated receptor-gamma in CNS disorders. Nat Clin Pract Neurol 2007; 3(9): 496-504.

[76] Yamanaka M, Ishikawa T, Griep A, *et al*. PPARgamma/RXRalpha-induced and CD36-mediated microglial amyloid-beta phagocytosis results in cognitive improvement in amyloid precursor protein/presenilin 1 mice. J Neurosci 2012; 32(48): 17321-31.

[77] Cramer PE, Cirrito JR, Wesson DW, *et al*. ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models. Science 2012; 335(6075): 1503-6.

[78] Fitz NF, Cronican AA, Lefterov I, *et al*. Comment on "ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models". Science 2013; 340(6135): 924-c.

[79] Chawla A, Boisvert WA, Lee CH, *et al*. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 2001; 7(1): 161-71.

[80] Laffitte BA, Repa JJ, Joseph SB, *et al*. LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. Proc Natl Acad Sci U S A 2001; 98(2): 507-12.

[81] Jiang Q, Lee CY, Mandrekar S, *et al*. ApoE promotes the proteolytic degradation of Abeta. Neuron 2008; 58(5): 681-93.

[82] Mandrekar-Colucci S, Karlo JC, and Landreth GE. Mechanisms underlying the rapid peroxisome proliferator-activated receptor-gamma-mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. J Neurosci 2012; 32(30): 10117-28.

[83] Nishitsuji K, Hosono T, Uchimura K, *et al*. Lipoprotein lipase is a novel amyloid beta (Abeta)-binding protein that promotes glycosaminoglycan-dependent cellular uptake of Abeta in astrocytes. J Biol Chem 2011; 286(8): 6393-401.

[84] Blain JF, Aumont N, Theroux L, *et al*. A polymorphism in lipoprotein lipase affects the severity of Alzheimer's disease pathophysiology. Eur J Neurosci 2006; 24(5): 1245-51.

[85] Takayama K, Sukhova GK, Chin MT, *et al*. A novel prostaglandin E receptor 4-associated protein participates in antiinflammatory signaling. Circ Res 2006; 98(4): 499-504.

[86] Minami M, Shimizu K, Okamoto Y, *et al*. Prostaglandin E receptor type 4-associated protein interacts directly with NF-kappaB1 and attenuates macrophage activation. J Biol Chem 2008; 283(15): 9692-703.

[87] Lazarus M, Yoshida K, Coppari R, *et al*. EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. Nat Neurosci 2007; 10(9): 1131-3.

[88] Ushikubi F, Segi E, Sugimoto Y, *et al*. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature 1998; 395(6699): 281-4.

[89] Aronoff DM, Lewis C, Serezani CH, *et al*. E-prostanoid 3 receptor deletion improves pulmonary host defense and protects mice from death in severe Streptococcus pneumoniae infection. J Immunol 2009; 183(4): 2642-9.

[90] Letiembre M, Liu Y, Walter S, *et al*. Screening of innate immune receptors in neurodegenerative diseases: A similar pattern. Neurobiol Aging 2007.

[91] Walter S, Letiembre M, Liu Y, *et al*. Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. Cell Physiol Biochem 2007; 20(6): 947-56.

[92] Shi J, Wang Q, Johansson JU, *et al*. Inflammatory prostaglandin E2 signaling in a mouse model of Alzheimer disease. Ann Neurol 2012; 72(5): 788-98.

[93] Tamagno E, Bardini P, Obbili A, *et al*. Oxidative stress increases expression and activity of BACE in NT2 neurons. Neurobiol Dis 2002; 10(3): 279-88.

[94] Apelt J, Bigl M, Wunderlich P, *et al*. Aging-related increase in oxidative stress correlates with developmental pattern of beta-secretase activity and beta-amyloid plaque formation in transgenic Tg2576 mice with Alzheimer-like pathology. Int J Dev Neurosci 2004; 22(7): 475-84.

[95] Candelario-Jalil E, Slawik H, Ridelis I, *et al*. Regional distribution of the prostaglandin E2 receptor EP1 in the rat brain: accumulation in Purkinje cells of the cerebellum. J Mol Neurosci 2005; 27(3): 303-10.

[96] Kawano T, Anrather J, Zhou P, *et al*. Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. Nat Med 2006; 12(2): 225-9.

[97] Ahmad AS, Saleem S, Ahmad M, *et al*. Prostaglandin EP1 receptor contributes to excitotoxicity and focal ischemic brain damage. Toxicol Sci 2006; 89(1): 265-70.

[98] Zhen G, Kim YT, Li RC, *et al*. PGE2 EP1 receptor exacerbated neurotoxicity in a mouse model of cerebral ischemia and Alzheimer's disease. Neurobiol Aging 2012; 33(9): 2215-9.

[99] Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science 2003; 302(5651): 1760-5.

[100] Keene CD, Chang R, Stephen C, *et al*. Protection of hippocampal neurogenesis from toll-like receptor 4-dependent innate immune activation by ablation of prostaglandin E2 receptor subtype EP1 or EP2. Am J Pathol 2009; 174(6): 2300-9.

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