

# Exploiting formyl peptide receptor 2 to promote microglial resolution: a new approach to Alzheimer's disease treatment

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Alzheimer's disease and dementia are among the most significant current healthcare challenges given the rapidly growing elderly population, and the almost total lack of effective therapeutic interventions. Alzheimer's disease pathology has long been considered in terms of accumulation of amyloid beta and hyperphosphorylated tau, but the importance of neuroinflammation in driving disease has taken greater precedence over the last 15–20 years. Inflammatory activation of the primary brain immune cells, the microglia, has been implicated in Alzheimer's pathogenesis through genetic, preclinical, imaging and *postmortem* human studies, and strategies to regulate microglial activity may hold great promise for disease modification. Neuroinflammation is necessary for defence of the brain against pathogen invasion or damage but is normally self-limiting due to the engagement of endogenous pro-resolving circuitry that terminates inflammatory activity, a process that appears to fail in Alzheimer's disease. Here, we discuss the potential for a major regulator and promoter of resolution, the receptor FPR2, to restrain pro-inflammatory microglial activity, and propose that it may serve as a valuable target for therapeutic investigation in Alzheimer's disease.

## The burden of Alzheimer's disease and dementia

Dementias are a considerable global health challenge. In 2016, the number of individuals living with dementia was estimated to be 44 million, a 50% increase compared to 1990 [1]. Of the different forms of dementia, Alzheimer's disease (AD) is by far the most common, affecting approximately 4% of individuals over the age of 65 [2] with a predicted prevalence of over 78 million people by 2050. [3]. These figures represent

a significant burden on patients, their families and wider healthcare systems in society, with an estimated cost in 2015 of £27 450 per patient with moderate dementia, per year in the UK [4]. Moreover, while mortality rates due to heart disease, stroke and cancer have all steadily decreased in the last 20 years, AD-associated death continues to rise [5,6], and there are still no effective therapies to slow down or halt disease

## Abbreviations

AD, Alzheimer's disease; A $\beta$ , amyloid beta peptide; CNS, central nervous system; DAM, disease-associated microglia; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; fA $\beta$ , fibrillar A $\beta$ ; FPR1, formylpeptide receptor 1 (human); FPR2, formylpeptide receptor 2 (human); Fpr2, formylpeptide receptor 2 (murine); GWAS, genome-wide association study; oA $\beta$ , oligomeric A $\beta$ ; ROS, reactive oxygen species; SPM, specialized pro-resolving mediator; TBI, traumatic brain injury.

progression, despite AD first being identified over a century ago. Strategies to address AD are thus an urgent public health priority.

## Neuropathology of AD

Alzheimer's disease is primarily associated with two characteristic pathologies: extracellular plaques consisting of fibrillar  $\beta$ -amyloid peptides (fA $\beta$ ) and intraneuronal tangles of insoluble, hyperphosphorylated tau protein. The A $\beta$  peptide is formed by sequential digestion of the ubiquitous transmembrane amyloid precursor protein by the enzymes  $\beta$ - and  $\gamma$ -secretase [7]. This generates soluble A $\beta$  monomers, which can spontaneously aggregate into oligomers (oA $\beta$ ) and subsequent insoluble fibrils (fA $\beta$ ), which are the basis for plaque formation [8]. While plaques were initially suggested as the toxic moiety [9], opinion has shifted in recent years to focus on the detrimental actions of soluble oA $\beta$  and their potent pro-inflammatory and neurotoxic effects [10–13]. Under normal circumstances, tau protein is a regulator of microtubule formation [14], but for reasons that are still not fully understood, it undergoes hyperphosphorylation in AD, driving the formation of filamentous intraneuronal inclusions thought to impair trafficking and ultimately lead to neuronal death [15]. As with A $\beta$ , soluble tau formulations appear to be sufficient to suppress neuronal activity [16], emphasizing the complexity of the pathological relationships between these disease-associated proteins.

While much research has focused on the role played by these classical protein hallmarks in AD pathogenesis, there is increasing evidence for a role of neuroinflammation in the disease, driven by the principal brain immune cells, the macrophage-like microglia [17], aided, as we are increasingly realizing, by the actions of astrocytes [18].

## Neuroinflammation in AD

Although active brain inflammation has long been recognized as a feature of AD, for many years this was believed to be a response to dying neurons and thus a secondary phenomenon. However, more recent human imaging studies and preclinical models indicate that progressive neuroinflammation may directly contribute to AD pathology. Furthermore, with the advent of genome-wide association studies (GWAS) investigating genetic risk elements for AD, the immune response itself has now taken centre-stage in our understanding of AD pathology [19–22]. Many immune system-related genetic loci have been identified as disease risk

factors, and both human imaging studies [23,24] and preclinical models [25,26] have identified progressively increasing inflammatory activity in AD. It is worth examining the links suggesting inflammation may drive AD pathology, as this may offer new clues for intervention points and perhaps indicate novel targets for therapeutic development.

## Neuroinflammation in AD: evidence from human genetic analysis

While the strongest genetic risk factor for sporadic AD is the  $\epsilon$ 4 allelic variation of the apolipoprotein gene *APOE* [27], over 20 other genes have been associated with sporadic AD, many of which encode microglial proteins linked to inflammation (Table 1). Of

**Table 1.** Microglial genes with risk variants for sporadic AD. Functions listed refer to potential roles in AD progression and do not necessarily encompass all biological functions that these genes are associated with.

Gene	Function	Source
<i>CD33</i>	Immune regulator can reduce phagocytosis and clearance of A $\beta$	[29,248,249]
<i>CR1</i>	Complement cascade regulator. Receptor for complement components C3b and C4b	[20,250]
<i>TREM2</i>	Phagocytosis, cell differentiation and proliferation	[35,114]
<i>ABI3</i>	Innate immunity through interferon-associated signalling	[114,251]
<i>MS4A gene cluster</i>	Modulates cerebrospinal fluid levels of soluble TREM2	[19,28]
<i>HLA-DRB1</i>	Antigen presentation	[19,252]
<i>INPP5D</i>	Regulator of microglial proliferation and phagocytosis of both cellular debris and A $\beta$	[20,253]
<i>PICALM</i>	Accessory protein in endocytosis. Involved in APP processing, A $\beta$ clearance and tau pathology	[250,254,255]
<i>ABCA7</i>	Phagocytosis and lipid metabolism; associated with APP processing and A $\beta$ clearance	[19,256,257]
<i>SPI1</i>	Transcription factor and phagocytosis regulator	[258]
<i>CLU</i>	Extracellular chaperone linked to A $\beta$ clearance	[250,259]
<i>EPHA1</i>	Endocytosis. Tau toxicity modulator	[29,260]
<i>BIN1</i>	Clathrin-mediated endocytosis and recycling. APP processing and tau pathology propagation	[113,261,262]
<i>MEF2C</i>	Modulates microglial inflammatory responses	[96,112]
<i>SORL1</i>	Endocytosis receptor. Limits amyloidogenic processing of APP	[19]

these genetic risk factors, *TREM2* and *CD33* are of particular interest given the important roles they have been shown to play in microglial handling of A $\beta$  [28,29]. *TREM2* acts to control inflammatory responses, including suppressing pro-inflammatory cytokine production, stimulating phagocytosis, promoting biosynthetic metabolism and sustaining cellular energetics [30–33]. Importantly, allelic variants of *TREM2* have been shown to increase AD risk by up to threefold [34,35] and have been detected in a range of different human populations [19,20,36,37], underlining its role as a global risk variant for sporadic AD. The receptor *CD33* has been implicated in a range of anti-inflammatory microglial functions including inhibition of cytokine release [38], regulation of inflammatory Toll-like receptor 4 signalling [39] and suppression of A $\beta$  phagocytotic clearance [40]. Notably, individuals with variants resulting in high *CD33* expression experience significantly greater AD-related cognitive decline [41].

Interestingly, there appears to be a degree of overlap between the risk variants associated with sporadic AD and those with other inflammatory diseases. A GWAS accumulating data from more than 100 000 individuals in patient vs. control cohorts identified eight single nucleotide polymorphisms (SNPs) which were associated with both AD and immune-related conditions such as Crohn's disease and psoriasis [42]. Overall, the study of myeloid cell genomics has revealed significant over-representation of microglia in the populations of cells bearing AD risk genes [43], with notable AD risk allele enrichment in enhancers of microglial, macrophage and monocyte activity [44], strongly indicating an important role for microglial activity in AD development.

### Influences of other inflammatory diseases upon AD risk

Many conditions known to drive neuroinflammation have been associated with AD, most notably in the case of traumatic brain injury (TBI) [45,46]. In particular, severe or repeated incidents of TBI have been directly linked to increased AD risk [47]: TBI increases soluble A $\beta$  production in cortical tissue [48], and A $\beta$  deposits and tau neurofibrillary tangles have been found at higher rates in TBI patients than controls [49].

Similarly, a wide range of chronic peripheral diseases have been linked with increased AD risk, including conditions as common as periodontitis [50], hypertension [51] and type 2 diabetes [52,53]. While the mechanistic links underlying these associations are

often poorly understood, it is notable that they share increased peripheral inflammatory activity as a common feature. More directly, increased plasma levels of the inflammatory markers lipopolysaccharide binding protein [54] and C-reactive protein [55] have been associated with an increased risk of AD development, again suggestive of a link between inflammation and AD.

While there is little evidence that AD has a direct infectious aetiology, several studies have indicated that microbes or microbial components may act to drive A $\beta$  pathology. A $\beta$  peptides have similar properties to the antimicrobial peptides of the peripheral innate immune response, in particular the human cathelicidin LL-37. Both peptides can activate the inflammation-associated receptor formyl peptide receptor 2 (FPR2) [56] and share an ability to bind and kill multiple different microbial species *in vitro* [57,58]. Moreover, transgenic mice overexpressing A $\beta$  are relatively protected against experimental bacterial meningitis, while animals lacking the amyloid precursor protein are more susceptible to this infection [59]. These actions of A $\beta$  may be relevant to human disease as several studies have found microbes in the brains of AD patients *postmortem* [60–63]. While caution must be taken with such findings, as techniques for detection of microbes in the brain are highly susceptible to environmental artefact, the idea that microbial actors or their components could trigger A $\beta$  production is of interest, and at the least provides further circumstantial evidence for an involvement of the immune response in AD pathogenesis.

### Neuroinflammation in AD: Evidence from preclinical studies

Beyond the associative data obtained from human population studies, preclinical models have provided a second source of information suggesting a role for (neuro)inflammation in AD pathogenesis. Notably, single cell transcriptomic analysis of murine AD models have identified a significant population of so-called disease-associated microglia (DAMs) [64], a finding that has since been replicated in humans [65], although it is important to note that significant differences exist between DAMs across the two species. In general, however, DAMs are characterized by reduced expression of a variety of regulatory genes known to maintain microglial homeostasis, for example the fractalkine receptor *CX<sub>3</sub>CRI*, alongside increased expression of genes associated with lipid metabolism and phagocytosis, notably including *TREM2*. Interestingly, DAMs were also reported for murine models of

other neurodegenerative conditions [64,66], suggesting that the pro-inflammatory phenotype shift they represent may be a common response to disease processes.

Inflammatory pathways themselves appear intrinsically tied to the development and worsening of AD pathology. For example, while components of the complement pathway are required for synapse refinement by microglia during development [67], accumulation of the same proteins has been linked to both amyloid and tau pathology in the murine PS2APP and TauP301S models, respectively [68–70]. Notably, microglial-derived C1q is sufficient to activate reactive astrocytes, reducing their ability to promote neuronal survival and instead promote neuronal death [71]. Microglia to astrocyte communication is not unidirectional, however, as astrocytes have been shown to drive microglial phenotype in return. For example, using APP/PS1 mice, Stat3-driven astrogliosis has been shown to associate with increased A $\beta$  levels and plaque burden, suppression of the microglial A $\beta$  clearance proteins CD33 and neprilysin, and increased production of pro-inflammatory cytokines, leading to impaired spatial learning and memory [72]. The relationship between astrocytes and AD progression is complex and incompletely understood, however, as attenuation of astrocyte activity both enhanced A $\beta$  plaque burden and dystrophic neurite number in APP/PS1 mice [73], and increased levels of pro-inflammatory cytokines and soluble A $\beta$  in APP23 animals [74]. It seems likely that the role astrocytes play in AD will change according to the stage of disease progression, and is likely to be linked to different microglial activation states [71], but a detailed consideration of their function lies beyond the scope of this review.

Overall, neuroinflammation has been reported in at least seven independent animal models of AD and associated disease, including both A $\beta$  and tau transgenics [75–80], and reducing inflammation through use of anti-inflammatory agents has successfully restored memory function in many of these models [76,80,81]. While the involvement of neuroinflammation in AD models is clear, further research is required to decipher the role of different glial (sub)-populations in disease, enabling us to fully understand the dynamic and complex roles of microglia and astrocytes during disease development, and to permit their translation to human disease.

## The role(s) of microglia in AD

Inflammation in the central nervous system (CNS) differs markedly from that in the periphery with a relatively limited role for circulating leukocytes, their place

largely being taken by central cells including astrocytes [71,82], perivascular macrophages [83] and probably most importantly the microglia [84,85]. Microglia are cells of myeloid origin that migrate into the brain tissue early in development and remain there, monitoring and maintaining the CNS environment [86]. These cells play a number of critical roles in tissue maintenance and response to injury [87], pathogen defence [88], removing damaged tissue [89] and synaptic plasticity, learning and memory [90,91]. For reasons that are still unclear however, microglia tend to become hyper-reactive with increasing age, exhibiting stronger responses to stimuli and failing to return completely to their baseline status after threat elimination [92].

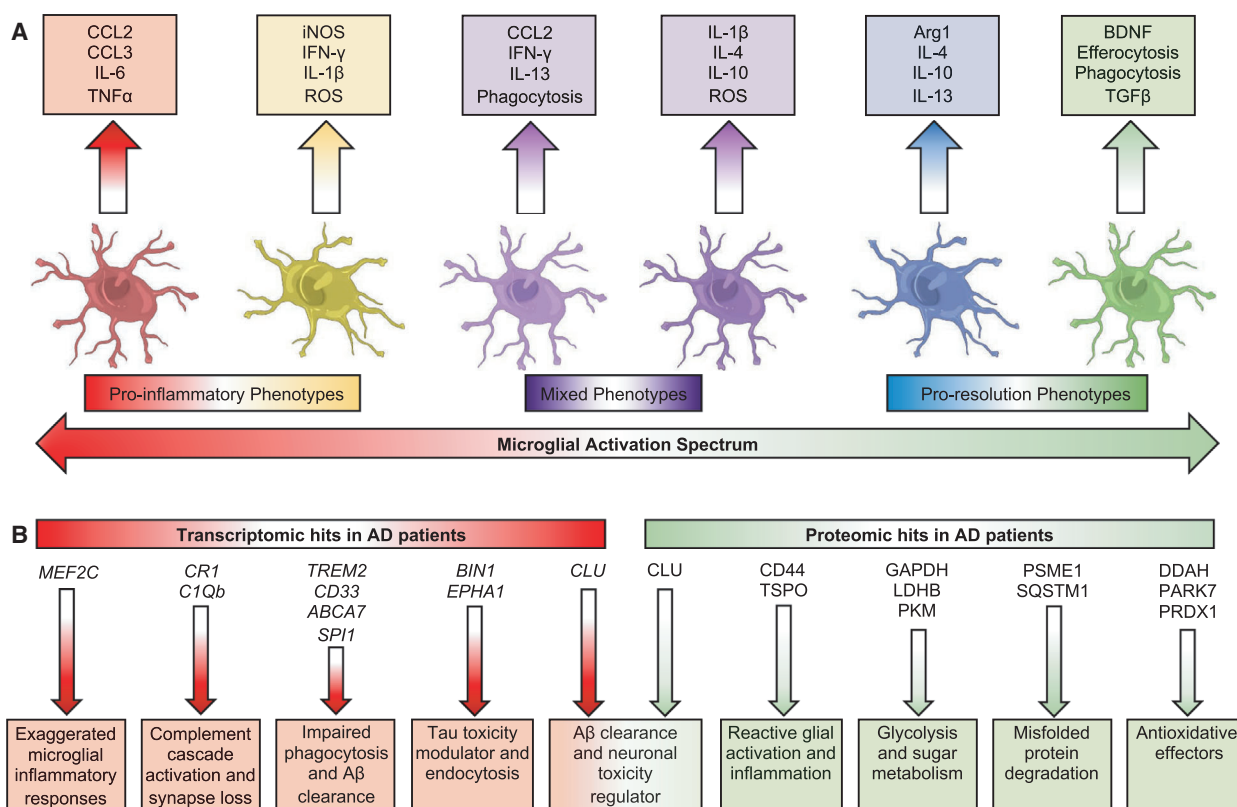
As suggested above, several cell types have been implicated in driving the neuroinflammatory response, including astrocytes [71,82] and perivascular macrophages [83], but the primary actors are thought to be microglia [84,85]. These cells play critical roles in tissue maintenance and response to injury [87], alongside pathogen defence [88], synaptic plasticity and consequently learning and memory [90], and are thus in a position to exert beneficial or detrimental effects on all of these AD-associated processes.

Depending on brain region, microglia compose approximately 5–20% of the total cells in the brain [93,94]. Their complexity and dynamicity are evidenced by transcriptional analysis of murine microglia, wherein at least nine distinct cell states were identified [95]. Greatest diversity was seen in prenatal and infant mice, with cells gradually becoming more uniform across the brain into adulthood. This uniformity declined upon advancing age and following induction of multiple sclerosis-like injury [95]. In human AD and control patients, single-nucleus RNA-Seq (snRNA-Seq) was able to identify four subclusters of microglia based on their transcriptomes, with at least one being over-represented in AD brain samples [96]. Notably, and unlike the previous murine studies, the presence of AD-associated cell populations was greater in women than men, possibly aligning with the increased incidence of AD in this sex [96]. Interestingly, of the 229 DAM genes previously identified to be upregulated in 5XFAD mice [64], only 28 were observed in the upregulated microglial cluster, including *APOE* and *SPPI* [96]. A further 49 genes upregulated in this human cohort had not previously been identified in mouse models, including *CD14* and the complement receptor *CIQB* [96]. Thus, differential microglial phenotypes likely exist between human patients and mouse models, often shifting with disease progression, which needs to be taken into consideration in translational work.

Microglia are notably plastic in their phenotypic expression and have the capacity to shift their functions and behaviour during the course of an inflammatory response (Fig. 1A), with different phenotypes often corresponding to distinct *in vivo* states [97]. Following injury or infection, cells become polarized towards inflammatory phenotypes, releasing a battery of pro-inflammatory mediators and reactive oxygen species (ROS), which may contribute to neurodegeneration if uncontrolled [98,99]. Many of the processes and mediators involved in driving this pro-inflammatory phase are also potent pro-resolving signals [100], driving microglia to a more reparative phenotype involving the phagocytosis and removal of apoptotic cells, cellular debris and protein aggregates, all of which accumulate during neurological damage [101] and ageing [102,103]. The pro-resolving mediators released at this point are thought to have a number of

beneficial effects, concurrently initiating a negative feedback control of pro-inflammatory cytokine production and stimulating the release of pro-resolving factors such as IL-10 and TGF $\beta$  [104–106]. Thus, the change in microglial phenotype seen in the progression of an inflammatory reaction is fundamental in both limiting the acute inflammatory response and inducing tissue repair, thereby restoring neural homeostasis [107].

Changes to microglial phenotype have been reported in multiple *postmortem* AD analyses, with increased expression of the activation markers MHC II and CD68 seen repeatedly in different groups of AD patients [108]. In addition, both familial and sporadic AD patients have been shown to bear higher expression of neuroinflammatory pathological markers in the brain areas most affected by the disease, including the entorhinal and temporal cortices and the dentate gyrus



**Fig. 1.** Microglial phenotypes are diverse, wide ranging and modifiable. (A) With nine transcriptionally unique microglial subsets identified in mice, it is important to emphasize the complexity of microglia, whereby ageing, pathogen exposure and disease progression are all thought to modify microglial phenotype. Moreover, despite often being classified as ‘pro-inflammatory’ or ‘anti-inflammatory’, a more mixed phenotype is often the case, with cells displaying characteristic features of each immune state, or a combination of both. (B) Transcriptomic and proteomic studies have identified key changes in microglial phenotype in AD, highlighting the diversity of microglial actions in the brain, and their potential for involvement in key disease-related processes. Intriguingly, pathways identified by transcriptomic and proteomic analyses do not always overlap, highlighting the scope for further investigation that still lies in this field.

of the hippocampus [109]. These findings are further supported by *in vivo* imaging studies of AD patients, where a clear correlation has been found between binding of ligands to the microglial activation marker translocator protein 18 kDa (TSPO) and progression of the disease [110,111]. The advent of detailed transcriptomic [112–114] and proteomic [115] analyses of AD patients has provided further insight into the complexity of microglial phenotypic changes during disease progression, although interestingly these two sources of information do not always concur (Fig. 1B).

### Microglia, neuroinflammation and neurodegeneration in AD

In healthy individuals, microglial phenotype remains relatively homeostatic, wherein these phagocytes remove dead cells and debris, preventing their accumulation in the brain [88]. However, microglia are long-lived cells, renewing by sometimes imperfect clonal expansion [116], and as they age, their degradative capacity becomes less efficient [117], leading to the build-up of unwanted cellular debris and aggregation of both A $\beta$  and tau [118,119]. The relationship between microglial behaviour and AD pathology is complex. Microglia actively phagocytose A $\beta$  plaques and dead/dying cells in what is thought to be an attempt to clear pathology [120], but A $\beta$ , particularly in its oligomeric form, potently activates microglia and can directly contribute to neuronal damage and loss [10,121–123]. Moreover, several AD risk genes are associated with microglial phagocytosis, including *APOE* [124] and *TREM2* [32], suggesting risk variants may compound the defective effects of ageing on microglial function.

Beyond these deleterious interactions with ageing, there are also indications that aberrant microglial phagocytosis may be implicated in early-stage disease. Several AD risk genes are associated with complement [125], a system that interacts with microglia to mediate phagocytic synapse removal in animal models challenged with oA $\beta$  [68]. Microglia expressing the complement component C1q are responsible for the proliferation of neurotoxic reactive astrocytes in mice [71]. However, depletion of the complement component C3 can stimulate plaque accumulation and increase neurodegeneration in the APP<sub>SW</sub> mouse model of AD [126], indicating a complex interaction between microglial function, ageing and other immune parameters in disease. This complexity is further underlined by the neuroprotective roles microglia often display in prodromal disease states. Using the 5xFAD model, microglia were shown to phagocytose A $\beta$  via

TREM2 without triggering evident neuroinflammation [64]. The role played by TREM2 and microglia appears to change with disease progression, however, with TREM2-expressing microglia exhibiting a strong inflammatory phenotype following interaction with tau aggregates, exacerbating neurodegeneration [127].

Microglia can therefore either promote extensive neuroinflammation upon amyloid or tau reaction or assist the clearance of age-associated amyloid accumulation, apparently depending on the stage of disease progression. Inhibiting the more neuroprotective functions of microglia in AD patients may therefore be detrimental, with blunt microglial inhibition strategies potentially causing more harm than good. Greater understanding of microglial behaviour and particularly of how to manipulate the phenotypic changes exhibited by these highly plastic cells will be crucial in permitting development of microglial-targeting therapies for AD.

### Interactions of microglia with AD hallmarks

Aggregation of large quantities of fA $\beta$  is a classical hallmark of AD, most probably linked with degradative enzymatic dysfunction [128,129], and has been shown to correlate with changes in the microglial proteome [130,131], although fA $\beta$  is unlikely to be the main toxic form of A $\beta$  [13]. Rather, oA $\beta$  species appear to directly contribute to neuroinflammation in AD [132]. Microglial pattern recognition receptors, including several Toll-like receptors and the scavenger receptors RAGE and CD36, recognize A $\beta$  and trigger a pro-inflammatory response [133] that impairs learning and memory [134,135]. Murine age-related cognitive impairment is also directly associated with chronic neuroinflammation [136], wherein microglia may damage and remove healthy neurones, further contributing to pathology [137]. Direct interaction of microglia with oA $\beta$  also appears to cause the release of a battery of pro-inflammatory cytokines, chemokines and ROS [10,138,139]. Moreover, microglial phagocytic ability appears to be hindered following A $\beta$ -elicited activation [140], suggesting that A $\beta$  may therefore promote its own accumulation, manifesting a self-propagating inflammatory cycle [141].

Evidence from animal models ties microglia to amyloid plaque production, although the exact role these cells play is complex and, to an extent, dependent on disease stage. Depletion studies have revealed both positive and negative effects of microglia with respect to plaque development and consequent behavioural phenotypes. Microglial removal using the CSF1R inhibitor PLX5622 in early stages of the 5xFAD mouse

model prevented plaque formation but aggravated behavioural deficits [142], whereas the same group found that removal at later time points did not affect plaque density but did prevent neuronal loss and improve behavioural measures [143]. In contrast, use of a different CSF1R inhibitor PLX3397 to remove microglia in early stages of the 5xFAD model decreased plaque burden but improved cognitive behaviour [144]. Similarly, microglial removal using PLX3397 abolished the protective effects of soluble TREM2 administration upon plaque load and cognitive deficits in the 5xFAD model [145]. These discrepancies emphasize both the complexity of microglia-A $\beta$  interactions and our paucity of understanding, although it should be borne in mind that microglial depletion strategies commonly have off-target effects, both within and without the CNS [146], as is highlighted by the finding that the nondepleting CSF1R inhibitor GW2580 prevented synapse loss without modulating A $\beta$  plaque number [147], and the discovery that an alternative viral-mediated microglial removal strategy had no effect on plaque formation or neuritic dystrophy in either APP23 or APP/PS1 animals [148].

While this interstudy variation is important to consider in evaluating murine model data and may reflect the artificial nature of these systems to an extent, a key observation in human disease is that microglial activation correlates with AD pathology, appearing to occur primarily after plaque development but before tau tangle deposition [101]. This is supported by positron emission tomography studies in patients, which show microglial activation to occur well before clinical AD symptoms are apparent [149,150]. Furthermore, microglial activation correlates well with A $\beta$  deposition in AD patients [151], and brain inflammation has been shown to accompany A $\beta$  deposition in the majority of patients with mild cognitive impairment that progress to having AD [152]. Thus, while the relationship between A $\beta$  pathology and microglial activation is complex, it seems likely that these cells are important in the development of the clinical symptoms associated with A $\beta$  accumulation.

Although the progression of A $\beta$  pathology correlates poorly with clinical symptom development [153], both neuroinflammation and tau pathology correlate well with AD symptom onset and disease severity in humans [23,151,154–156]. In line with this association, microglia may contribute to the pathological seeding of tau, with evidence suggesting that cultured microglia from the tauopathy-associated rTg4510 mouse can release tau seeds that act as foci of aggregation [157]. This argument is supported by the demonstration that transfer of purified microglia from hTauCx3cr1<sup>-/-</sup>

transgenic mice into wild-type animals induced host tau hyperphosphorylation [156] and that depletion of microglia and macrophages by treatment with clodronate liposomes significantly suppressed tau propagation [158]. Microglia do not appear to be merely passive distributors of tau seeds, however, with evidence indicating a direct role for the NLRP3 inflammasome, a major initiator of inflammatory activity through its role in producing IL-1 $\beta$  and IL-18. Specifically, intracerebral injection of brain homogenate from APP/PS1 mice into Tau22 mutant animals effectively induced tau hyperphosphorylation, a response not seen in two different lines of Tau22 mutant and NLRP3 null mice [159].

Thus, microglia appear to be intimately involved in the propagation of AD pathological hallmarks, and to correlate with worsening clinical symptoms and neurodegeneration. As both A $\beta$  and tau pathology appear to be linked to pro-inflammatory microglial behaviour, controlling and reversing microglial activation may be therapeutically beneficial. Exploiting the pathways governing inflammatory resolution which naturally control microglial activity may thus have significant potential in the treatment of AD.

## Inflammatory resolution in AD

Over the last 15–20 years, we have come to realize that inflammation is naturally a self-limiting response and that many of the endogenous mediators and receptors activated in the course of an inflammatory response have roles in the termination of that same response, the process termed inflammatory resolution [100]. Resolution of inflammation is a distinct, active process associated with the catabolism of pro-inflammatory mediators, removal of cell debris and tissue repair [160], with its failure often resulting in chronic inflammation, tissue damage and disease [161]. Whereas conventional anti-inflammatory therapies are usually designed as antagonists or inhibitors of specific factors and receptors which drive the inflammatory response, resolution-based therapeutics aim to specifically target and upregulate endogenous signalling pathways which reduce the cardinal signs of inflammation, but also actively promote tissue repair and promote a return to homeostasis [162]. This shift in approach could be crucial for neuroinflammatory disease, as events present early in the inflammatory cascade likely engage this co-ordinated endogenous resolution process [100], indicating that blockade of inflammation through the use of traditional anti-inflammatories may also stall the resolution response. Indeed, this effect may have contributed to the disappointing results of clinical trials

investigating NSAID use in treating AD [163] – such suppressive drugs may have halted inflammatory processes, but they will also have impaired resolution and thus initiating stimuli are unlikely to have been fully removed. Moreover, for patients with AD, given that it is above all an age-related disorder, avoiding direct inhibition of the inflammatory pathways may be particularly beneficial as this could avoid compromising immunity and aggravation of the pre-existing enhanced risk the elderly have of infection associated mortality [164].

Many endogenous specialized pro-resolving mediators (SPMs) have been identified [165,166], with a remarkable degree of molecular diversity, ranging from gases such as hydrogen sulphide and carbon monoxide [167], through the lipid mediators lipoxins, maresins, protectins and resolvins [168,169], to proteins such as annexin A1 [170]. The benefit of utilizing SPMs for disease was first identified over a decade ago, stemming from reports that both lipoxin A4 and annexin A1 could stimulate production of known anti-inflammatory mediators, such as IL-10 [171,172]. These mediators also appear to stimulate the endogenous production of other SPMs, as was observed for resolvin E1, which upregulated lipoxin A4 expression in mice [173].

Preclinical models and patient analysis indicate that endogenous resolution pathways may be dysfunctional in AD. In particular, attention has focused on lipid pro-resolving mediators such as the maresins, protectins and resolvins derived from the omega-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [174]. Notably, studies of AD patients revealed reduced levels of maresin 1, protectin D1 and resolvin D5 in the entorhinal cortex [175] and reduced levels of lipoxin A4 in the hippocampus and cerebrospinal fluid [176], and expression of the leukotriene B4 receptor BLT1 and the chemerin 1 receptor, both of which bind resolvin E1, associates with higher Braak stages in human AD [177], suggesting a failure in engagement of resolving circuitry. Moreover, exogenous administration of lipid mediators has been shown to improve symptoms in a number of murine AD models, suggesting that activation of resolution circuitry may have therapeutic benefit. Namely, DHA modulates glial responses in AD [178], bilateral hippocampal administration of maresin 1 significantly reduced microglial and astrocyte activation and cognitive decline following injection of A $\beta$  aggregates to mice [179], and lipoxin A4 improves cognitive function and reduces signs of AD including synaptotoxicity, A $\beta$  deposition and tau phosphorylation in both 3xTg-AD [180] and Tg2576 [181] mouse AD models. Lipid

mediators themselves are readily oxidized in air and have poor half-lives and complex storage requirements [182], but synthetic agonists targeting their receptors may be of greater utility.

In particular, the G protein-coupled receptor FPR2 (also termed the lipoxin A4 receptor, ALX) has received substantial interest. This receptor plays an important role in governing the behaviour of innate immune cells such as neutrophils, monocytes and macrophages [183], mediating conversion from pro- to anti-inflammatory behaviour and driving inflammatory resolution. Expression of FPR2 has been reported in a number of different CNS cell types, with the strongest evidence being for presence within microglia [176,184], particularly following activation by pro-inflammatory stimuli [185], and in endothelial cells [186,187]. There is some evidence for FPR2 expression within neurons, with reports indicating the receptor is present within hippocampal and cortical projection cells, primarily in the neuropil rather than cell bodies, and in the cerebellum [188], and there is evidence for FPR2 in neural stem cells [189] and in neuroblastoma lines [190]. Whether FPR2 is found in other brain cell types is more controversial, with conflicting reports both indicating [191,192] and refuting [188,193] expression within astrocytes; currently, there are no reports of FPR2 expression within oligodendrocytes or other central glial subtypes. Intriguingly, there is compelling evidence to suggest targeting FPR2 may be a viable approach to restraining inflammatory activity in AD.

## Formylpeptide receptor 2

FPR2 is a member of the formylpeptide receptors, a family of both class A G protein-coupled receptors (GPCR) and pattern recognition receptors, first identified by homology-based cloning to the potently pro-inflammatory formylpeptide receptor 1 (FPR1) [194]. The biological actions of this family of receptors are complex, with three members in humans and eight identified in mice, but members have known roles in chemotaxis, host defence and inflammation [195–197]. FPR2 and its murine homologues Fpr2 and Fpr3 are unusual in having a large number of both pro-inflammatory and anti-inflammatory ligands of remarkable molecular diversity, from lipids to peptides to proteins [56].

The molecular signalling pathways triggered by FPR2 activation are complex and exhibit a significant degree of agonist bias, a feature attributable to a number of factors. Recent determination of the crystal structure of FPR2 bound to the peptide ligand WKYMVm alone [198] or in complex with Gi [199]



revealed a remarkably wide and deep ligand-binding pocket, capable of differentially accommodating and interacting with the broad variety of molecules known to have potency at this receptor. Notably, functional analysis of FPR2 structure upon binding of different pro- and anti-inflammatory ligands to this pocket revealed distinct, complex and dose-dependent conformational changes between ligands, with evidence for interligand allosteric interactions upon co-administration [200,201]. Classically, FPR2 was thought to couple to inhibitory G proteins and Ca<sup>2+</sup> mobilization, but has since been shown to engage a wider repertoire of G proteins [202], with ligand-dependent conformational changes in the receptor translating into recruitment of distinct intracellular signalling pathways [200]. A further layer of complexity to FPR2 signalling is added by its ability to both homodimerize and to heterodimerize with the pro-inflammatory FPR1, particularly upon exposure to higher ligand concentrations, thereby connecting with numerous intracellular signalling pathways [203]. The receptor is thought to constitutively homodimerize in monocytes, with pro-resolving ligands such as lipoxin A4 and annexin A1 inducing a conformational change that leads to activation of p38 MAP kinase, phosphorylation of MAPKAPK and Hsp27 and production of the anti-inflammatory cytokine interleukin 10 [203]. In contrast, the pro-inflammatory ligands of FPR2 such as serum amyloid A, LL-37, and notably in the context of AD, A $\beta_{1-42}$ , trigger phosphorylation of JNK and modulation of pro-apoptotic pathways in leukocytes [204], an effect attributed to the ability of FPR2 to form heterodimers with FPR1 [203]. While this pattern of intracellular signalling, and particularly the intricate allosteric interactions between ligands, is without doubt complex, it also offers significant opportunity for the rational design of agents able to selectively target specific downstream functions of FPR2 signalling, potentially minimizing off-target actions. In particular, there is now substantial evidence to position FPR2 as a critical actor in inflammatory resolution, helping to terminate an inflammatory response and restore homeostasis [183], raising the possibility of selective development of targeted pro-resolving therapies. The majority of evidence supports the targeting of interactions between FPR2 and microglia, but this is not necessarily an exclusive mechanism whereby FPR2 agonists could be beneficial.

Much of our knowledge of the role of FPR2 in inflammatory resolution is derived from studies of its behaviour in the peripheral system. In particular, given the close similarity in ontogeny and behaviour between microglia and monocytes/macrophages, insight into

the potential of FPR2 in regulating neuroinflammation can be obtained through analysis of its role in these cells. Studies of both human and rodent systems have identified an important role for FPR2 or its nonhuman counterparts in the recruitment of monocytes to inflammatory foci, with studies reporting positive chemotactic effects of numerous FPR2 ligands on those cells, including serum amyloid A [205–207], the cathelicidin LL-37 [208], viral and bacterial peptides [209,210], protein cleavage products [211,212] and secreted human proteins such as FAM3D [213] and annexin A1 [195]. These findings have been translated into whole organism settings, with FPR2 having been clearly identified as a key organizer of monocyte recruitment in conditions as diverse as peritonitis [195], bacterial sepsis [214], colitis [215], heart failure [216] and allergic airway inflammation [217]. The signalling pathways engaged in these processes are not fully described, but most work has been undertaken examining the role of the major pro-resolving FPR2 ligand annexin A1, which has been shown to trigger phosphorylation of both ERK1/2 to induce chemokinesis [212] and p38 MAP kinase to induce directed chemotaxis [195]. Whether similar pathways are activated in response to other known chemotactic agents that act through FPR2, such as serum amyloid A or LL-37, is unclear.

As well as recruiting monocytes to inflammatory foci, and following their conversion to macrophages, FPR2 signalling has been shown to play a major role in efferocytosis, the phagocytic removal of dead cells and debris that is essential for efficient termination of an inflammatory reaction and the restoration of tissue homeostasis [218]. FPR2 ligands including lipoxin A4, resolvin D1, annexin A1 and its peptidomimetics have been repeatedly shown to promote clearance of apoptotic cells by macrophages, actions inhibited variously by receptor antagonists or genetic ablation [219–222]. Notably, we and others have shown that FPR2 can play a similar role in microglial efferocytosis and the removal of apoptotic neurons [223–225], thereby preventing their progression to secondary necrosis and initiation of further inflammatory activity. Notably, many of the genetic risk variants associated with AD are in genes associated with phagocytosis and efferocytosis [226]; hence, systems involved in promoting efferocytosis, such as FPR2, may be of significant use in disease treatment.

The role of FPR2 in inflammation extends beyond monocyte recruitment and efferocytosis however, as the receptor is central to the phenotypic plasticity that characterizes macrophage responses to inflammatory challenge [227]. The FPR2 ligand annexin A1, derived

from apoptotic neutrophils in an inflammatory locus, has been shown to engage the receptor on resident and recruited macrophages, triggering expression of an anti-inflammatory surface phenotypic marker profile and production of anti-inflammatory cytokines such as interleukin 10 and TGF $\beta$  through activation of AMPK signalling [227]. This effect is particularly intriguing as it links FPR2 signalling with the control of cellular energy homeostasis, a function we are increasingly appreciating as a major regulator of immune cell activity [228,229].

## FPR2 & Neuroinflammation in AD

The involvement of FPR2 in neuroinflammation has been relatively understudied by comparison with its effects in the periphery, but evidence does still implicate it as both a mediatory of neuroinflammatory resolution and as a potential target in AD. Initial studies of FPR2 expression were limited by difficulties in distinguishing it from FPR1, but more recent analyses indicate that the receptor is present at a relatively low level within microglia throughout the brain [184,230,231]. While whole-brain FPR2 content does not appear to change markedly in AD [190], microglial FPR2 expression is markedly upregulated following exposure to inflammatory stimuli, including TNF $\alpha$  and agonists of Toll-like receptors 2, 3, 4 and 7 [232–236], and most notably fibrillar A $\beta$ , such that microglia associated with amyloid plaques express high levels of the receptor [184,237]. This regulation of FPR2 expression by inflammatory stimuli has parallels with the behaviour of the receptor in macrophages where FPR2 expression declines as a pro-resolving phenotype is adopted [214,227]. Given that several studies have now indicated microglia can also be induced to take an anti-inflammatory phenotype by FPR2 activation [193,238,239], this may represent a mechanism to maintain a pro-reparative microglial phenotype.

The increase in FPR2 expression seen in plaque-associated microglia may be associated with the ability of microglia to phagocytose and break down fA $\beta$  [132,190,240], as treatment of microglia with pro-resolving FPR2 ligands such as annexin A1 has been shown to stimulate fA $\beta$  phagocytosis [190], alongside reducing inflammatory cytokine production [224,241]. Intriguingly, the ability of primary microglia to phagocytose fA $\beta$  is inhibited by the actions of oligomeric forms of the peptide [132], providing further support for the idea that oA $\beta$  may have greater toxicity. In this vein, micromolar concentrations of oA $\beta$  are potent activators of microglia *in vitro* through FPR2, provoking release of inflammatory cytokines and ROS and

stimulating chemotaxis [230,235,242], although it should be noted that treatment of microglia with more AD-relevant concentrations of oA $\beta$  had a more selective effect, triggering oxidative stress without notable inflammatory change [10]. Significantly, the effects of oA $\beta$  on microglia ROS production and metabolic phenotype could be reversed by subsequent treatment with small molecule agonists of FPR2 [10], suggesting a potential avenue for further therapeutic investigation.

Importantly, these interactions between FPR2 and microglial behaviour feeds forward to improvements in cognition in several different animal models of AD. For example, using a rat model of AD in which A $\beta$ <sub>1-42</sub> was infused intracerebroventricularly, upregulation of Fpr2 expression was shown to improve working and spatial memory deficits, suppress hippocampal inflammation and A $\beta$  deposition and induce an anti-inflammatory microglial profile [235]. Similarly suggestive of a beneficial role for FPR2, administration of lipoxin A4 improves recognition memory in the 3xTg-AD mouse [180], and the stable aspirin-triggered 15-epi-lipoxin A4 enhanced performance on the Morris water maze task in the Tg2576 tau transgenic mouse [181]. In contrast, long-term administration of the mixed FPR1/FPR2 antagonist Boc2 improved performance on the Morris water maze task, prevented hippocampal neuron loss and limited microglial activity in APP/PS1 mice [243]. These data further support a role for FPRs in amyloid pathology, although it should be noted that Boc2 has also been shown to possess FPR-independent activity [244]. Thus, while further work is needed to fully define the role of FPR2 in AD and its models, these studies highlight the potential of the receptor as a target to not only slow neuroinflammation, but to actually improve ultimate cognitive outcomes.

## Could FPR2 have therapeutic utility for AD?

Dementia and AD are major and growing healthcare challenges, particularly as despite the significant work and resources that have gone into therapeutic development, there remain no clinically effective treatments. Primarily, efforts have been guided by the amyloid hypothesis, that is, that neurodegeneration is ultimately driven by the toxic actions of A $\beta$ , and that its suppressed production or enhanced removal would be beneficial [245]. Given that over 300 clinical trials based on this hypothesis have now been performed and have yet to result in a clinically usable therapy, it may be time to expand our horizons beyond the direct role of A $\beta$  and investigate other aspects of AD

[246,247]. Neuroinflammation is central to the progression of AD and is thus a key candidate process for investigation, although as directly acting anti-inflammatory agents such as the NSAIDs have also shown little effect in prospective trials, a more considered approach may be needed.

The endogenous processes governing neuroinflammatory resolution have significant potential to control microglial activity in AD, with FPR2 in particular offering an attractive target for pharmacological treatment. Moreover, the evidence describing the intricate allosteric interactions between FPR2 agonists and the complex signalling patterns that result raises the possibility of selective targeting of pro-resolving functions for therapeutic development. Such pro-resolving FPR2 agonists will be able to exert a number of beneficial effects in the brain, including suppression of pro-inflammatory cytokine and ROS production [10,190,235], improving clearance of  $\text{fA}\beta$  [180,190] and apoptotic cells and debris [224] and promoting an anti-inflammatory microglial phenotype [193,238]. More hypothetically, if microglia do behave in a similar manner to their peripheral macrophage cousins [214,227], induction of a pro-resolving phenotype by FPR2 activation may also downregulate expression of the receptor itself, helping to shield microglia from further pro-inflammatory activation by  $\text{oA}\beta$ . The ability of FPR2 to regulate such a diverse array of microglial processes places the receptor in a key position to govern how microglia behave under neuroinflammatory conditions, and we would argue, positions it as a critical target for pharmacological exploitation in dementia. Given the lack of therapies available to even slow down AD progression, targeting a system, such as FPR2, that activates the endogenous control pathways that reduce inflammatory activity and promote healing hold significant promise for an intervention that is sorely needed in dementia treatment.

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## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

ESW and SM prepared the majority of the text, with contributions from MAI. SJG provided significant comments and suggestions for improvement.

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