p38α-MAPK-deficient myeloid cells ameliorate symptoms and pathology of APP-transgenic Alzheimer’s disease mice

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Abstract
Alzheimer’s disease (AD), the most common cause of dementia in the elderly, is pathologically characterized by extracellular deposition of amyloid-β peptides (Aβ) and microglia-dominated inflammatory activation in the brain. p38α-MAPK is activated in both neurons and microglia. How p38α-MAPK in microglia contributes to AD pathogenesis remains unclear. In this study, we conditionally knocked out p38α-MAPK in all myeloid cells or specifically in microglia of APP-transgenic mice, and examined animals for AD-associated pathologies (i.e., cognitive deficits, Aβ pathology, and neuroinflammation) and individual microglia for their inflammatory activation and Aβ internalization at different disease stages (e.g., at 4 and 9 months of age). Our experiments showed that p38α-MAPK-deficient myeloid cells were more effective than p38α-MAPK-deficient microglia in reducing cerebral Aβ and neuronal impairment in APP-transgenic mice. Deficiency of p38α-MAPK in myeloid cells inhibited inflammatory activation of individual microglia at 4 months but enhanced it at 9 months. Inflammatory activation promoted microglial internalization of Aβ. Interestingly, p38α-MAPK-deficient myeloid cells reduced IL-17a-expressing CD4-positive lymphocytes in 9 but not 4-month-old APP-transgenic mice. By cross-breeding APP-transgenic mice with Il-17a-knockout mice, we observed that IL-17a deficiency potentially activated microglia and reduced Aβ deposition in the brain as shown in 9-month-old myeloid p38α-MAPK-deficient AD mice. Thus, p38α-MAPK deficiency in all myeloid cells, but not only in microglia, prevents AD progression. IL-17a-expressing lymphocytes may partially mediate the pathogenic role of p38α-MAPK in peripheral myeloid cells. Our study supports p38α-MAPK as a therapeutic target for AD patients.

Abbreviations: AD, Alzheimer’s disease; Aβ, amyloid-β peptide; APP, amyloid precursor protein; APPΔtg, APP-transgenic; APPΔwt, non-APP-transgenic; BMDMs, bone marrow-derived macrophages; GFP, green fluorescence protein; IL-17a, interleukin-17a; LPS, lipopolysaccharide; NFT, neurofibrillary tangles; p-tau, hyper-phosphorylated tau protein; p38α-MAPK, p38α mitogen-activated protein kinase; RFP, red fluorescent protein; SR-A, scavenger receptor A; Th17, T-helper 17; TREM2, triggering receptor expressed on myeloid cells-2.

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1 | INTRODUCTION

Alzheimer’s disease (AD), the major cause of dementia in the elderly, is pathologically characterized by three components: (i) extracellular deposits of amyloid-β peptide (Aβ), (ii) intracellular neurofibrillary tangles (NFT) that is composed of hyper-phosphorylated tau protein (p-tau), and (iii) microglia-dominated inflammatory activation in the brain parenchyma (Scheltens et al., 2021). Interactions between Aβ, p-tau and inflammatory activation are primarily responsible for the progressive neurodegeneration in AD. However, many clinical trials to reduce Aβ accumulation or p-tau aggregation or inflammatory activation (Pleen & Townley, 2022) have failed to produce AD therapies that modify the disease progression. A simple explanation for these failures is that the study population may have already reached a disease stage too late for effective intervention. However, it is important to recognize that AD is a heterogeneous disease. For example, the pathological and biochemical features of Aβ deposits or molecular structure of Aβ aggregates in the brain (Thal et al., 2015) varies among AD patients. Variations in Aβ structure affect how microglia respond to the Aβ deposits, which, in turn, affects inflammatory activation and Aβ internalization (Parvathy et al., 2009). A growing number of subtypes of activated microglia have recently been identified in AD brains (Chen & Colonna, 2021). Moreover, pathological examination of postmortem brain tissues and imaging studies show different distributions of tau-related pathology and patterns of brain atrophy in AD patients (Ferreira et al., 2020). Therefore, targeting multiple pathogenic pathways might be more effective as a therapeutic intervention than focusing on a single step in AD disease progression.

p38α mitogen-activated protein kinase (p38α-MAPK) is a protein kinase present in a variety of cells that respond to external stress stimuli (Kumar et al., 2003). p38α-MAPK is activated in both neurons and microglia in brains of AD patients (Hensley et al., 1999). Our recent study indicates that p38α-MAPK deficiency in neurons reduces both Aβ and p-tau levels in the brain of AD mice (Schnöder et al., 2016, 2020, 2021). A systemic administration of chemical p38α-MAPK inhibitor has been observed to reduce inflammatory activation in the brain of APP- or tau-transgenic mice (Bachstetter et al., 2012; Maphis et al., 2016). Thus, p38α-MAPK inhibition might simultaneously target Aβ, p-tau and inflammation in AD. A recent phase 2 clinical trial showed that a 24-week treatment with p38α-MAPK inhibitor decreased tau proteins in the cerebral spinal fluid of mild AD patients; although it did not improve the cognitive function (Prins et al., 2021). We believe that the therapeutic protocol can be optimized, if the pathogenic mechanisms of p38α-MAPK are better understood. Pharmacological treatments with p38α-MAPK inhibitors affect both microglial p38α-MAPK and neuronal p38α-MAPK, without the ability to distinguish their effects. The inhibition of inflammatory activation in the brain might come from neuronal p38α-MAPK inhibition-mediated attenuation of Aβ and p-tau generation, or even from neuronal protection (Schnöder et al., 2020). In this study, we investigated specific effects of p38α-MAPK in microglia or myeloid cells on AD pathogenesis.

The pathogenic role of microglia in AD is extremely heterogeneous. For example, the rare variants in the triggering receptor expressed on myeloid cells-2 (TREM2) gene increase the risk of developing AD. One group reported that TREM2 deficiency in APP-transgenic mice increases hippocampal Aβ burden and accelerates neuron loss (Wang et al., 2015); however, another group showed that TREM2 deletion reduces cerebral Aβ accumulation (Jay et al., 2015). Subsequent work suggested that the effect of TREM2 deficiency on cerebral Aβ accumulation depends on the stage of disease (Jay et al., 2017). Consistent with this conclusion is the observation from a longitudinal imaging study of human subjects with mild cognitive impairment that several peaks of microglial activation appear over the disease trajectory (Fan et al., 2017). These studies underscore the effects of the changing cellular environment and reinforce the idea that the pathogenic role of microglial activation should be dynamically investigated during disease progression.

In this study, we conditionally knocked out Mapk14 gene (encoding p38α-MAPK) in the myeloid cell lineage or specifically in microglia in amyloid precursor protein (APP)-transgenic mice and investigated the AD pathology and microglial activation in early and late disease stages. We observed that deletion of p38α-MAPK attenuated Aβ load and neuronal deficits of AD mice; however, the pathogenic mechanism of p38α-MAPK is evolving during the disease progresses, which potentially involves peripheral interleukin (IL)-17α-expressing T lymphocytes.

2 | RESULTS

2.1 | Establishment of APP-transgenic mice deficient of p38α-MAPK in myeloid cells

To investigate the pathogenic role of p38α-MAPK in microglia and peripheral myeloid cells in AD, we cross-bred APP-transgenic (APPtg) mice with p38α-MAPK-encoding gene Mapk14 floxed mice, and LysM-Cre+/− mice expressing Cre specifically in the myeloid cell lineage, to obtain APPtgp38fl/fLysM-Cre+/− (p38α deficient) and APPtgp38fl/flLysM-Cre−/− (p38α wild type) of genotypes. By measuring Mapk14 gene transcripts and p38-MAPK proteins in CD11b+ brain cells from APPtgp38fl/fLysM-Cre+/− and APPtgp38fl/flLysM-Cre−/− mice, we found that the rate of LysM-Cre-mediated Mapk14 gene recombination in microglia of 9-month-old AD mice was ~45% (Figure S1a,d,e). In 4-month-old AD mice, LysM-Cre altered neither
Mapk14 transcription nor p38-MAPK protein in CD11b+ brain cells, but decreased Mapk14 transcription by 88% in CD11b+ blood cells (Figure S1b–e).

We further constructed APP-transgenic green fluorescence protein (GFP)-expressing LysM-Cre reporter mice (APP <sup>tg</sup>Rosa<sup>tm1Eco</sup>LysM-Cre<sup>+</sup>; Muzumdar et al., 2007). GFP was mainly expressed in microglia associated with Aβ deposits (Figure S1f); Aβ deposits were also surrounded by microglia without expression of GFP, indicating heterogeneity of Aβ plaques. GFP was rarely expressed in neurons (Figure S1g). APP<sup>tg</sup>p38<sup>fl/fl</sup>LysM-Cre<sup>+/−</sup> mice were also mated to CCR2-RFP reporter mice expressing red fluorescent protein (RFP) under the control of Ccr2 gene promoter (Saederup et al., 2010). Both histological and flow cytometric analysis showed that p38α-MAPK deficiency does not affect the recruitment of peripheral myeloid cells into the brain of 9-month-old APP-transgenic mice (Figure S2).

2.2 | Deficiency of p38α-MAPK in myeloid cells improved the cognitive function of APP-transgenic mice

We used the Morris water maze test to examine cognitive function of 9-month-old APP<sup>wt</sup> and their non-APP-transgenic (APP<sup>wt</sup>) littermate mice. During the acquisition phase, APP<sup>wt</sup> mice with or without deletion of p38α-MAPK in myeloid cells (APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> and APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>+/−</sup>) showed no significant differences in either swimming time or swimming distance before climbing onto the escape platform (Figure 1a,b). Compared to APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermates, 9-month-old APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>+/−</sup> mice with normal p38α-MAPK expression travelled significantly longer distances (Figure 1a) and spent significantly more time (Figure 1b) to reach the escape platform. Interestingly, APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice with the deletion of myeloid p38α-MAPK performed significantly better than their APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermates in searching and finding the platform after 3 days of training (Figure 1a,b). The swimming velocity did not differ between p38α-MAPK-deficient and wildtype APP-transgenic mice or for the same mice on different training dates (Figure 1c).

Twenty-four hours after the end of training phase, the escape platform was removed and a 5-min probe trial was performed to test the memory of mice. Compared to APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermates, APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice remained for a significantly longer time in their first visit to the region where the platform had been located, and crossed the original platform region with significantly less frequency during the total 5-min probe trial (Figure 1d,e). Interestingly, when compared to APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice, APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>+/−</sup> mice were able to reach the original platform region in significantly less time and crossed the region more frequently (Figure 1d,e). We observed differences in neither parameter analyzed in the probe trial between APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> and APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermate mice (Figure 1d,e).

We further used Western blot analysis to quantify the levels of four synaptic proteins: Munc18-1, synaptophysin, SNAP-25, and PSD-95 in the brain homogenate of 9-month-old APP<sup>wt</sup> and APP<sup>wt</sup> littermate mice. As shown in Figure 1f–k, protein levels of Munc18-1, synaptophysin and SNAP-25 in APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice were significantly lower than levels of these proteins derived from APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermate mice. The reduction in Munc18-1, synaptophysin and SNAP-25 proteins due to APP-transgenic expression was rescued by the deletion of p38α-MAPK in myeloid cells (Figure 1g,h,k). PSD-95 protein levels were significantly higher in brains from APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice than that from APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> control mice (Figure 1j). Comparison of APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> and APP<sup>wt</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermate mice showed no significant differences in protein levels of these four tested synaptic proteins (Figure 1f–k).

2.3 | Deficiency of p38α-MAPK in myeloid cells reduces Aβ load in the brain of APP-transgenic mice

As Aβ is the key molecule leading to neurodegeneration in AD (Scheltens et al., 2021), we analyzed the effects of myeloid p38α-MAPK on Aβ pathology in the APP-transgenic mice. Using immunohistological and stereological Cavalierei methods, we observed that the volume of immunoreactive Aβ load in 9-month-old APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice was significantly lower than that in APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermate mice (Figure 2a,b). The brain tissue was also stained with Congo red that typically binds to the β sheet structure of Aβ plaques, which showed that deficiency of p38α-MAPK decreased the cerebral level of Aβ aggregates (Figure 2c,d), corroborating the results from immunohistochemistry.

The amount of differently aggregated Aβ in brain tissue homogenates was measured with Western blot (The establishment of method was shown in Figure S3) and ELISA. Protein levels of monomeric and dimeric Aβ in 9-month-old APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice were significantly lower than that in APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermates (Figure 2e,f). Similarly, p38α-MAPK deficiency significantly decreased concentrations of both Aβ40 and Aβ42 in TBS plus 1% Triton X-100 (TBS-T)-soluble, and Aβ42 in guanidine hydrochloride-soluble brain homogenates of APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice compared with APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> littermate mice (Figure 2g).

In order to learn the effects of myeloid p38α-MAPK on Aβ pathology during the disease progression, Aβ deposits in 4-month-old APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> and APP<sup>tg</sup>p38α<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice were also analyzed. Deletion of p38α-MAPK in myeloid cells already reduced Aβ deposits in APP-transgenic mouse brain at this early disease stage compared with p38α-MAPK-wildtype APP-transgenic mice (Figure 2h,i); however, Western blot did not show significant effects of p38α-MAPK deficiency on cerebral oligomeric Aβ levels (Figure S4).
FIGURE 1 Deficiency of p38α-MAPK in myeloid cells improves cognitive function and attenuates AD-associated loss of synaptic proteins in APP-transgenic mice. (a–c) Nine-month-old APP-transgenic (APPtg) and non-APP-transgenic (APPwt) littermate mice with (p38α ko) and without (p38α wt) deletion of p38α-MAPK in myeloid cells were assessed for cognitive function using the water maze test. In the training phase, deficiency of p38α-MAPK decreases swimming distance (a) and latency (b) to reach the escape platform in APPtg but not in APPwt mice. Deficiency of p38α-MAPK does not affect the traveling velocity in APPtg mice (c). Two-way ANOVA from day 3 to day 6 followed by Bonferroni’s post hoc test, n is shown in the figure. The latency of first visit to the region where the escape platform was previously located (d) and the frequency, with which mice crossed the platform region (e), were recorded in the 5-min probe trial. One-way ANOVA followed by Bonferroni’s post hoc test. (f–k) The amount of synaptic proteins, Munc18-1, SNAP25, synaptophysin, and PSD-95 in the brain homogenate of 9-month-old APPtg and APPwt mice was determined using Western blotting. One-way ANOVA followed by Bonferroni’s post hoc test, n ≥ 11 per group for APPtg mice and n ≥ 6 per group for APPwt mice. Here, representative Western blot images from five independent experiments are shown. Munc18-1 and SNAP15, PSD-95 and synaptophysin, and their corresponding α-tubulin immunoblots were performed on the same membrane. Data was represented as mean ± SEM.
2.4 | Deficiency of myeloid p38α-MAPK differently regulates microglial inflammatory activation in early and late disease stages of APP-transgenic mice

Inflammatory activation of microglia is another pathogenic factor in AD (Scheltens et al., 2021). After immunofluorescent staining of Iba-1, we used the stereological method, Optical Fractionator probe, to count microglia in the hippocampus and cortex. Deficiency of p38α-MAPK in myeloid cells significantly decreased Iba-1-immunoreactive microglia in both 4 and 9-month-old APP-transgenic, but not in 9-month-old non-APP-transgenic mice (Figure 3a,b,x). We also observed that deficiency of p38α-MAPK decreased the number of P2RY12-immunoreactive microglia in the hippocampus (Figure S5). It has been reported that P2RY12 is a more specific protein marker for endogenous microglia (McKinsey et al., 2020).

Deficiency of p38α-MAPK in myeloid cells reduced II-1β, Ccl-2 and II-10, but not Tnf-α, Inos, Arg1, Chi3l3 and Mcr1 gene transcripts in the brain of 9-month-old APPtg/p38αfl/flCre−/− mice compared with APPtg/p38αfl/flCre−/− littermates (Figure 3c–i). Notably, p38α-MAPK decreased the transcriptional level of Ccl-2, but not Tnf-α, II-1β, and II-10 in the brain of 9-month-old non-APP-transgenic mice (Figure 5a–d). In the brain of 4-month-old APP-transgenic mice, p38α-MAPK deficiency changed the transcription of neither pro- (Tnf-α, II-1β, Inos, and Ccl-2) nor anti-inflammatory genes (II-10, Chi3l3, and Mcr1; Figure S7a–g).

II-10 activates Stat3. The levels of phosphorylated Stat3 in both 4- and 9-month-old APPtg/p38αfl/flLysM-Cre−/− mice were significantly lower than that in APPtg/p38αfl/flLysM-Cre−/− littermate controls (Figure 3k,l,y,z). Phosphorylated Stat3 was undetectable in the brain of 9-month-old APPwt mice (Figure 3k), suggesting that II-10/Stat3-mediated inflammatory signaling was activated in the brain of APP-transgenic mice and inhibited by p38α-MAPK deficiency in myeloid cells in both early and late disease stages.

To further analyze the inflammatory activity of microglia in p38α-MAPK-deficient AD mice, we isolated CD11b+ microglia from both 4- and 9-month-old APP-transgenic mouse brains and detected inflammatory gene transcripts. Surprisingly, deficiency of p38α-MAPK in myeloid cells significantly reduced the transcription of II-1β, Ccl-2 and II-10 genes in cells from 4-month-old APP-transgenic mice (Figure 3a–ad), but increased the transcription of II-1β and Ccl-2 genes in cells from 9-month-old APP-transgenic mice (Figure 3n,o), compared with p38α-MAPK-wildtype APP mice. Transcription of other tested inflammatory genes, for example, Tnf-α in both 4 and 9-month-old APP-transgenic mice (Figure 3m,aa) and II-10 in 9-month-old APP-transgenic mice (Figure 3p), was not altered by p38α-MAPK deficiency. In microglia isolated from 9-month-old APPwt mice, p38α-MAPK deficiency did not alter transcripts of all tested genes, Tnf-α, II-1β, Ccl-2 and II-10 genes (Figure S6e–h).

Regarding other molecular signatures of disease-associated microglia (DAM; Keren-Shaul et al., 2017), we observed a significant increase of Cx3cr1 transcription (Figure 3s), and a decrease of Trem2 transcription (Figure 3q) in CD11b+ microglia from 9-month-old APPtg/p38αfl/flLysM-Cre−/− mice compared with APPtg/p38αfl/flLysM-Cre−/− littermates. Deficiency of p38α-MAPK did not affect the transcription of Apoe, P2ry12, Lpl, Clec7a and Itgax genes in microglia of 9-month-old APP-transgenic mice (Figure 3r,t–w). In 4-month-old APP-transgenic mice, transcription of none of the DAM-associated genes tested in 9-month-old mice was altered by the deficiency of p38α-MAPK in microglial cells (Figure S7h–n).

2.5 | Deficiency of myeloid p38α-MAPK increases microglial clearance of Aβ in APP-transgenic mice at the late disease stage

Microglia play like a double-edged sword. Their uptake of Aβ is an important mechanism of Aβ clearance in AD brain (Scheltens et al., 2021). We asked whether deficiency of p38α-MAPK facilitates microglial internalization of Aβ in AD mice. After observing that there were more microglia surrounding Aβ deposits in 9-month-old APPtg/p38αfl/flLysM-Cre−/− mice than in APPtg/p38αfl/flLysM-Cre−/− littermate mice (Figure 4a,b), we isolated microglia from these two groups of AD mice and quantified intracellular Aβ with Western blot (Figure S3b,c). As shown in Figure 4c,d, the protein level of intracellular Aβ in p38α-MAPK-deficient cells was higher than that in p38α-MAPK-wildtype microglia. Furthermore, we evaluated expression levels of Aβ internalization-associated receptors in microglia with quantitative RT-PCR and flow cytometry. Deficiency of p38α-MAPK increased expression of scavenger receptor A (SR-A) in microglia at both transcriptional and protein levels compared with microglia from p38α-MAPK-wildtype APP-transgenic mice (Figure 4e,h,i). The transcription of other Aβ internalization-associated receptors, such as CD36 and RAGE, was not changed by deficiency of p38α-MAPK (Figure 4f).

In 4-month-old APP-transgenic mice, we repeated all experiments for 9-month-old mice. We observed that p38α-MAPK deficiency neither altered the intracellular Aβ in microglia, nor affected the transcription of Aβ internalization-associated receptors, including SR-A, CD36, and RAGE (Figure S1d and Figure S8).

In order to verify our in vivo observation that p38α-MAPK deficiency enhances Aβ internalization in microglia, we cultured p38α-MAPK-deficient and wildtype bone marrow-derived macrophages (BMDMs) and primed them with and without 100 ng/ml lipopolysaccharide (LPS) for 48 h. Deficiency of p38α-MAPK did increase Aβ internalization in inflammasorily activated macrophages in association with an up-regulation of SR-A, but not in resting cells (Figure S9a–f). Co-treatment with fucoidan, an antagonist of SR-A, abolished p38α-MAPK deficiency-enhanced Aβ internalization (Figure S9g,h).

2.6 | Deficiency of p38α-MAPK specifically in microglia reduces AD-associated pathologies in the brain of APP-transgenic mice, but with low efficiency

After observing that deficiency of p38α-MAPK in whole myeloid cells prevented AD progression, we asked whether p38α-MAPK deficiency...
FIGURE 2 Deficiency of p38α-MAPK in myeloid cells reduces Aβ load in the brain of APP-transgenic mice. Four and 9-month-old APP-transgenic mice with p38α ko and without (p38α wt) deletion of p38α-MAPK in myeloid cells were analyzed with stereological Cavalieri methods for cerebral Aβ volumes (adjusted by the volume of analyzed tissues) after immunohistochemical (a, b), Congo red (c, d) and immunofluorescent (h, i) staining. T test, n ≥ 7 per each group. (e, f) Aβ was also detected in brain homogenates of 9-month-old p38α-MAPK-ko and -wt APP-transgenic mice with Western blot. Here, representative Western blot images from two independent experiments are shown. Aβ and β-Actin immunoblots were performed on the same membrane. T test, n ≥ 4 per each group. (g) APP-transgenic mouse brains were further serially homogenized in TBS-, TBS-T-, and guanidine-soluble fractions, in which monomeric, oligomeric and high-molecular-weight Aβ aggregates were enriched, respectively. Aβ40 and Aβ42 were measured by ELISA and normalized to the amount of homogenate protein. T test, n = 10 per group. Data was represented as mean ± SEM.

2.7 | IL17a-expressing lymphocytes might be involved in attenuating Aβ pathology in myeloid p38α-MAPK-deficient APP-transgenic mice

The comparison of APPp38fl/fl/LysM-Cre+/− and APPp38fl/fl/Cx3Cr1-Cre−/− mouse models strongly suggested that peripheral p38α-MAPK-deficient myeloid cells were more efficient than p38α-MAPK-deficient microglia in reducing cerebral Aβ in AD mice. We hypothesized that deficiency of myeloid p38α-MAPK cells regulates peripheral immune cells and indirectly affects brain pathology. Interestingly, 6-month-old APP-transgenic mice showed that transcription of Il-17a, but not Il-13, Il-4 and Il-10 genes, was significantly up-regulated in CD4+ spleen cells compared with APP-wildtype littermate mice (Figure S12a–d). By cross-breeding APPtg mice with IL-17a-eGFP reporter mice, in which eGFP is expressed under the control of endogenous Il-17a gene promoter (Esplugues et al., 2011), and detecting GFP-expressing lymphocytes in the intestine, we observed that there were significantly more GFP-expressing CD4+ lymphocytes in both lamina propria and Peyers patches of 6-month-old APP-transgenic mice than in APP-wildtype littermates (Figure S12e–i).
FIGURE 3  Deficiency of p38α-MAPK in myeloid cells differently regulates microglial inflammatory activation in the brain of APP-transgenic mice at early and late disease stages. (a, b, x) microglia stained with red fluorescent Iba-1 antibody were counted with the optical fractionator stereological probe in brains of 4 and 9-month-old APP-transgenic (APPtg) and non-transgenic (APPwt) mice with (p38α-ko) and without (p38α-wt) deletion of p38α-MAPK in myeloid cells. The cell number was adjusted by the volume of analyzed tissues. One-way ANOVA followed by Bonferroni’s post hoc test for 9-month-old mice, n ≥ 7 per group for APPtg mice and ≥3 per group for APPwt mice; t test for 4-month-old mice, n ≥ 7 per group. (a) Images show the immunofluorescent staining of 9-month-old mouse brains. (c–j) The inflammatory gene transcripts in brains of 9-month-old APPtg mice were measured with real-time PCR. T test, n ≥ 8 per group. (k, l, y, z) four and nine-month-old APP<sup>fl/fl</sup>LysCre<sup>+</sup>−/− and APP<sup>fl/fl</sup>LysM-Cre<sup>−/−</sup> mice were further analyzed with Western blot for the levels of phosphorylated Tyr705; p-) and total (t-) Stat3 in the brain. The same membrane was serially blotted with antibodies against p-Stat3, t-Stat3 and β-Actin. The activity of Stat3 is shown in the ratio of p-/t-Stat3. T test, n ≥ 7 per group for 9-month-old mice and n = 5 per group for 4-month-old mice. (y) To avoid overexposure of the film, the membrane for t-Stat3 blotting was additionally washed for 17 h after the first exposure to the film after 1 h of washing. Here, representative Western blot images from two independent experiments are shown. (m–w, aa–ad) in following experiments, microglia were selected from brains of 4- and 9-month-old p38α-wt and ko APPtg mice. The transcriptional level of inflammatory genes and other DAM-associated genes was determined by real-time PCR. T test, n ≥ 6 and 4 per group for 9 and 4-month-old mice, respectively. Data was represented as mean ± SEM.
We then isolated CD4+ spleen cells from 4- and 9-month-old APP\textsuperscript{tg}p38\textsuperscript{fl/fl}LysM\textsuperscript{-}Cre\textsuperscript{+}/− and APP\textsuperscript{tg}p38\textsuperscript{fl/fl}LysM\textsuperscript{-}Cre\textsuperscript{−}/− littermate mice, and observed that p38\textalpha-MAPK deficiency significantly reduced the transcription of Il-17a, but not Ifn-γ, Il-4 and Il-10 in AD mice at the age of 9, but not 4 months (Figure 6a–d). Thus, we hypothesized that IL-17a-expressing cells might be involved in cerebral...
Aβ reduction induced by peripheral p38α-MAPK-deficient myeloid cells.

We cross-bred APPtg mice with IL-17a−/− mice (Nakae et al., 2002) and observed that the extent of immunoreactive Aβ deposits in both the cortex and hippocampus of 6-month-old APPfl/flLysM-Cre−/− (IL-17a knockout) mice was significantly less than that in APPfl/flLysM-Cre+/+ (IL-17a wildtype) littermates (Figure 6e–g).

To investigate whether IL-17A deficiency models p38α-MAPK-deficient myeloid cells in regulating microglial activation, we analyzed and compared the morphology of microglia surrounding Aβ deposits. Deletion of p38α-MAPK in myeloid cells decreased the total number and end points of branches of microglial processes in 9- but not 4-month-old APP-transgenic mice (Figure 6h–p). In Sholl analysis, microglial branches crossed concentric circles significantly less in 9-month-old APPfl/flLysM-Cre−/− compared with APPfl/flLysM-Cre+/− littermates, especially at 40–70 μm from the soma (Figure 6m). Interestingly, all changes of microglial morphology in 9-month-old APPfl/flLysM-Cre−/− mice relative to APPfl/flLysM-Cre+/− littermates could be produced in 6-month-old APPfl/flLysM-Cre−/− mice compared with APPfl/flLysM-Cre+/+ mice (Figure 6q–v).

3 DISCUSSION

We constructed two AD mouse models with deletion of p38α-MAPK in all myeloid cells (APPfl/flLysM-Cre−/−) and specifically in microglia (APPfl/flLysM-Cre+/−). In APPfl/flLysM-Cre−/− mice, LysM-Cre reduced Mapk14 transcription in microglia by 45% by 9 months of age and in CD11b+ blood cells by 88% as early as 4 months of age. Twelve-month-old APPfl/flLysM-Cre−/− mice showed a 97% decrease in Mapk14 transcription in microglia, whereas there was no change in peripheral myeloid cells. Of note, deletion of p38α-MAPK attenuated cerebral Aβ and neuronal damage in APPfl/flLysM-Cre+/− mice but had little effect on these two pathologies in APPfl/flLysM-Cre−/− mice. Thus, it may be peripheral p38α-MAPK-deficient myeloid cells rather than p38α-MAPK-deficient microglia that can effectively prevent disease progression in APP-transgenic mice.

Deficiency of p38α-MAPK in myeloid cells inhibited inflammatory activation in individual microglia early in disease (by 4 months), but enhanced it after disease progression (by 9 months). The decrease of inflammatory gene transcripts (e.g., IL-1β and Ccl2) in the whole brain of 9-month-old APPfl/flLysM-Cre−/− mice was likely due to the decreased number of microglia beginning earlier in the disease (e.g., at 4 months of age). Interestingly, microglia internalized more Aβ in APPfl/flLysM-Cre−/− than in APPfl/flLysM-Cre+/− littermates at 9 but not 4 months of age. In cell cultures, we observed that p38α-MAPK deficiency increased Aβ uptake by LPS-primed cultured macrophages. Thus, p38α-MAPK deficiency could promote microglial Aβ clearance in the context of inflammatory activation. Part of the possible mechanisms is that deficiency of p38α-MAPK upregulates the expression of SR-A, a typical Aβ-phagocytic receptor (Paresce et al., 1996), in microglia. We also observed that p38α-MAPK deficiency in myeloid cells inhibited IL-10-Stat3 signaling in the brain of APP-transgenic mice. It has been reported that deficiency of IL-10 or Stat3 facilitates microglial clearance of Aβ in AD mice (Guillot-Sestier et al., 2015; Reichenbach et al., 2019). In APPfl/flLysM-Cre−/− mice, p38α-MAPK deficiency inhibited inflammatory activation in microglia, which may prevent p38α-MAPK deficiency from enhancing Aβ internalization. Indeed, mild inflammatory activation has the potential to increase Aβ clearance in the brain. Systemic injection of TLR4 or TLR9 agonists induces both pro- and anti-inflammatory activation and decreases Aβ in the brain of APP-transgenic mice (Michaud, Halle, et al., 2013; Scholtzova et al., 2014). TREM2 antibody administration also decreases Aβ load in the presence of increased expression of inflammatory cytokines and chemokines in the brain of APP-transgenic mice (Price et al., 2020). However, the mechanisms of inflammatory regulation of microglial Aβ clearance remain unclear. The gene transcription in microglia from our APPfl/flLysM-Cre−/− mice showed partial DAM signatures (e.g., induction of proinflammatory genes); however, transcription of homeostatic genes (e.g., Cx3cr1) was also up-regulated and transcription of Trem2 gene was reduced.

Our study was not yet able to answer the question of how peripheral p38α-MAPK-deficient myeloid cells reduced cerebral Aβ in 4-month-old APPfl/flLysM-Cre−/− mice. The decrease in...
Microglia at 4 months of age may be secondary to the Aβ reduction. It is generally accepted that microglia play a primary role in AD pathogenesis. However, AD is a systemic disease associated with dysregulation of the peripheral immune system. Peripheral myeloid cells have been reported to directly clear Aβ in blood vessels (Michaud, Bellavance, et al., 2013). Our cell culture experiments showed that p38α-MAPK-deficient macrophages took up more Aβ42 oligomers than p38α-MAPK-wildtype cells. In the following study, we generate bone marrow–chimeric AD mice as we have done previously (Hao et al., 2011) by transplanting p38α-MAPK-deficient and wildtype hematopoietic stem cells into APP-transgenic mice that have received selective-body irradiation (omitting the brain). This experiment may answer the question whether deficiency of p38α-MAPK in peripheral myeloid cells is sufficient to reduce the cerebral Aβ load.

It remains unclear how peripheral p38α-MAPK-deficient myeloid cells regulate the inflammatory activation of microglia. Since the number of cells expressing CD11b and CCR2-RFP reporter in the brain did not differ between APP^{p38^+/+LysM-Cre^+/--} and APP^{p38^+/+LysM-Cre^+/+} littermate mice, it is unlikely that peripheral p38α-MAPK-deficient myeloid cells migrate into the brain parenchyma and interact directly with microglia. We observed that p38α-MAPK-deficient myeloid cells decreased IL-17a gene transcription in CD4-positive splenocytes at nine but not 4 months of age, which was correlated with changes in microglial inflammatory activation and morphology and Aβ internalization. The morphology of microglia surrounding Aβ deposits in nine (but not 4)-month-old myeloid p38α-MAPK-deficient APP-transgenic mice was characterized by a reduction in the overall length, branches and end points of the processes, which are markers of microglial maturation and activation (Enry et al., 2021), and may also indicate active Aβ internalization (Huang et al., 2021). Very interestingly, the morphological pattern of microglia in p38α-MAPK-deficient mouse can be generated in IL-17a-deficient APP-transgenic mice. It is known that p38α-MAPK signaling in dendritic cells drives differentiation of T helper 17 (Th17) cells and sustains autoimmune inflammation (Huang et al., 2012). We have observed that APP is expressed in myenteric neurons of the gut (Seman et al., 2013) and is able to increase IL-17a expression in CD4-positive gut lymphocytes (Figure S12). During disease progression, AD pathology in the gut is sufficient to induce differentiation and activation of T lymphocytes and myeloid p38α-MAPK has the opportunity to alter the immune response. Our study suggests that IL-17a may at least partially mediate the pathogenic role of myeloid p38α-MAPK in AD pathogenesis. It has been reported that the number of Th17 cells increases in the blood of AD patients (Oberstein et al., 2018). IL-17a-expressing T lymphocytes accumulate in the means and brain of triple-transgenic AD mice (3x Tg-AD; Brigas et al., 2021). It is worthwhile to reanalyze the pathogenic effects of myeloid p38α-MAPK in AD mice on the basis of IL-17a deficiency in our future studies.

It should be noted that deficiency of p38α-MAPK promotes the recruitment of microglia around Aβ deposits in both APP^{p38^+/+LysM-Cre^+/--} and APP^{p38^+/+Cx3Cr1-Cre^+/+} mice, possibly favoring Aβ clearance (Hao et al., 2011; Quan et al., 2021). It has also been suggested that microglia clustered around Aβ deposits protect local neurites from damage by forming a physical barrier and condensing Aβ into dense plaques (Condello et al., 2015). Indeed, we observed p38α-MAPK deficiency in all myeloid cells as well as specifically in microglia protecting neurons in APP-transgenic mice, albeit with varying efficiency. The mechanism that drives microglia to migrate to Aβ deposits needs to be further identified.

In summary, deficiency of p38α-MAPK in all myeloid cells, not just microglia, triggers efficient Aβ clearance in the brain and improves cognitive function of APP-transgenic mice. Together with our previous observations that neuronal deficiency of p38α-MAPK reduces Aβ and phosphorylated tau proteins in the brains of AD mice (Schnöder et al., 2016, 2020, 2021), our serial studies support that inhibition of p38α-MAPK is a novel therapeutic option targeting multiple pathogenic processes in AD. As a potential anti-AD mechanism,
deficiency of p38α-MAPK in peripheral myeloid cells decreases the generation of IL17a-expressing T lymphocytes, which subsequently activates microglia to internalize Aβ. Further studies on pathophysiological mechanisms associated with IL-17a-expressing T lymphocytes may be helpful in optimizing AD therapy with p38α-MAPK inhibitors.

4 | EXPERIMENTAL PROCEDURES

4.1 | Animal models and cross-breeding

APP/PS1-double transgenic mice (APP\(^{Fl}\)) over-expressing human mutated APP (KM670/671NL) and PS1 (L166P) under Thy-1 promoters (Radde et al., 2006) were kindly provided by M. Jucker, Hertie Institute for Clinical Brain Research, Tübingen, Germany; p38\(^{α/−}\) mice with loxP site-flanked Mapk14 gene (Nishida et al., 2004) were kindly provided by K. Otsu (Osaka University) though the RIKEN Bioresource Center, RIKEN Tsukuba Institute, Japan; Cx3Cr1-CreERT2 mice that express a fusion protein of Cre recombinase and an estrogen receptor ligand binding domain under the control of endogenous Cx3cr1 promoter/enhancer elements (Goldmann et al., 2013) were kindly provided by M. Prinz, University of Freiburg, Germany; and LysM-Cre knock-in mice expressing Cre from the endogenous Lysozyme 2 gene locus (Clausesen et al., 1999) were bought from The Jackson Laboratory, Bar Harbor, ME (stock number 004781) and were back-crossed to C57BL/6J mice for >6 generations. APP-transgenic mice deficient of p38α-MAPK specifically in myeloid cells (e.g., microglia, macrophages and neutrophils; APP\(^{Fp38^{α/−}/α}\)LysM-Cre\(^{−/−}\)) were established by cross-breeding APP-transgenic mice with p38\(^{α/−}\) and LysM-Cre mice. To generate AD mice with deficiency of p38α-MAPK specifically in microglia (APP\(^{Fp38^{α/−}/α}\)Cx3Cr1-Cre\(^{−/−}\)), APP-transgenic mice were cross-bred with p38\(^{α/−}\) and Cx3Cr1-Cre mice and induced for the recombination of Mapk14 gene by intraperitoneal injection of tamoxifen (100 mg/kg; Sigma-Aldrich Chemie) in corn oil once a day over 5 days. Our study only used mouse litters containing both APP\(^{Fp38^{α/−}/α}\)LysM (or Cx3Cr1)-Cre\(^{−/−}\) and APP\(^{Fp38^{α/−}/α}\)LysM (or Cx3Cr1)-Cre\(^{−/−}\) of genotypes, so that p38α-MAPK-deficient and wildtype APP-transgenic mice were compared between siblings.

To delete IL-17a in AD mice, APP-transgenic mice were cross-bred with Il-17a knockout mice (Nakae et al., 2002), which were kindly provided by Y. Iwakura, Tokyo University of Science, Japan. Moreover, to investigate the location of LysM-Cre-expressing cells in the brain, APP-transgenic mice were cross-bred with ROSA\(^{mT/mG}\) Cre reporter mice (Muzumdar et al., 2007) and LysM-Cre\(^{−/−}\) mice to obtain APP\(^{Fp38^{α/−}/α}\)ROSAM\(^{mT/mG}\) LysM-Cre\(^{−/−}\) of genotype, which express enhanced green fluorescence protein (eGFP) in LysM-Cre-expressing cells. To examine whether peripheral myeloid cells migrate into the brain parenchyma, APP\(^{Fp38^{α/−}/α}\)LysM-Cre\(^{−/−}\) were mated to CCR2-RFP reporter mice (The Jackson Laboratory; stock number 017586), in which the chemokine (C-C motif) receptor 2 (CCR2) -coding sequence has been replaced with monomeric RFP-encoding sequence (Saelerup et al., 2010). To track IL-17a-expressing cells in APP-transgenic mice, APP\(^{Fl}\) mice were cross-bred with IL-17a-eGFP reporter mice (kindly provided by R. Flavell, Yale University, USA), which express eGFP under the control of mouse Il-17a gene promoter (Esplugues et al., 2011).

Animal breeding, experimental procedure and methods of killing were conducted in accordance with national rules and ARRIVE guidelines, and were authorized by Landesamt für Verbraucherschutz, Saarland, Germany (registration numbers: 40/2014, 12/2018 and 34/2019).

4.2 | Other experimental methods

Detailed descriptions of: (1) behavior tests, (2) analysis of brain pathology using histological, biochemical, and molecular biological approaches, (3) examination of microglia for their inflammatory activation, morphology, and Aβ internalization, and (4) statistical analysis were provided in the supplemental material.

AUTHOR CONTRIBUTIONS

Y.L. conceptualized and designed the study, acquired funding, conducted experiments, acquired and analyzed data, and wrote the manuscript. Q.L., L.S., W.H., K.L., Y.D., and I.T. conducted experiments, acquired data and analyzed data. M.M. offered an animal facility and supervised animal experiments. K.F. offered a research laboratory and supervised the laboratory work. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. Raw data are available upon reasonable request.
REFERENCES

Bachstetter, A. D., Norris, C. M., Sompol, P., Wilcock, D. M., Goulding, D., Neltner, J. H., St Clair, D., Watterson, D. M., & van Eldik, L. (2012). Early stage drug treatment that normalizes proinflammatory cytokine production attenuates synaptic dysfunction in a mouse model that exhibits age-dependent progression of Alzheimer’s disease-related pathology. The Journal of Neuroscience, 32(30), 10201-10210. https://doi.org/10.1523/JNEUROSCI.1496-12.2012

Brigas, H. C., Ribeiro, M., Coelho, J. E., Gomes, R., Gomez-Murcia, V., Carvalho, K., Faiivre, E., Costa-Pereira, S., Darrigues, J., de Almeida, A. A., Buée, L., Dunot, J., Marie, H., Pousinha, P. A., Blum, D., Silva-Santos, B., Lopes, L. V., & Ribot, J. C. (2021). IL-17 triggers the onset of cognitive and synaptic deficits in early stages of Alzheimer’s disease. Cell Reports, 36(9), 109574. https://doi.org/10.1016/j.celrep.2021.109574

Chen, Y., & Colonna, M. (2021). Microglia in Alzheimer’s disease at single-cell level. Are there common patterns in humans and mice? The Journal of Experimental Medicine, 218(9), e20202717. https://doi.org/10.1084/jem.20202717

Clausen, B. E., Burkhartt, C., Reith, W., Renkawitz, R., & Forster, I. (1999). Conditional gene targeting in macrophages and granulocytes using LysMCre mice. Transgenic Research, 8(4), 265–277.

Condello, C., Yuan, P., Schain, A., & Grutzendler, J. (2015). Microglia constitute a barrier that prevents neurotoxic protobacterial Abeta42 spots around plaques. Nature Communications, 6, 6176. https://doi.org/10.1038/ncomms7176

Erny, D., Dokalis, N., Mezö, C., Castoldi, A., Mossaad, O., Staszewski, O., Frosh, M., Villa, M., Fuchs, V., Mayer, A., Neuber, J., Sosat, J., Tholen, S., Schilling, O., Vlachos, A., Blank, T., Gomez de Agüero, M., Macpherson, A. J., Pearce, E. J., & Prinz, M. (2021). Microbiota-derived acetate enables the metabolic fitness of the brain innate immune system during health and disease. Cell Metabolism, 33(11), 2260–2276.e7. https://doi.org/10.1016/j.cmet.2021.10.010

Fan, Z., Brooks, D. J., Okello, A., & Edison, P. (2017). An early and late peak in microglial activation in Alzheimer’s disease trajectory. Brain, 140(3), 792–803. https://doi.org/10.1093/brain/aww349

Ferreira, D., Nordberg, A., & Westman, E. (2020). Biological subtypes of Alzheimer disease: A systematic review and meta-analysis. Neurology, 94, 436–448. https://doi.org/10.1212/WNL.0000000000009058

Goldmann, T., Wieghofer, P., Müller, P. F., Wolf, Y., Varol, D., Yona, S., Brendecke, S. M., Kierdorf, K., Staszewski, O., Datta, M., Luedde, T., Heikenwalder, M., Jung, S., & Prinz, M. (2013). A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. Nature Neuroscience, 16(11), 1618-1626. https://doi.org/10.1038/nn.3531

Guillot-Sestier, M. V., Doty, K. R., Gate, D., Rodriguez, J., Jr., Leung, B. P., Rezaï-Zadeh, K., & Town, T. (2015). IL10 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. Neuron, 85(3), 534–548. https://doi.org/10.1016/j.neuron.2014.12.068

Hao, W., Liu, Y., Liu, S., Walter, S., Grimm, M. O., Kiliaan, A. J., Penke, B., Hartmann, T., Rübe, C. E., Menger, M. D., & Fassbender, K. (2011). Myeloid differentiation factor 88-deficient bone marrow cells improve Alzheimer’s disease-related symptoms and pathology. Brain, 134(Pt 1), 278–292. https://doi.org/10.1093/brain/awq325

Hensley, K., Floyd, R. A., Zheng, N. Y., Nael, R., Robinson, K. A., Nguyen, X., Pye, Q. N., Stewart, C. A., Geddes, J., Markesbery, W. R., Patel, E., Johnson, G. V., & Bing, G. (1999). p38 kinase is activated in the Alzheimer’s disease brain. Journal of Neurochemistry, 72(5), 2053–2058.

Huang, G., Wang, Y., Vogel, P., Kang, P., Takanashi, T. D., Otsu, K., & Chi, H. (2012). Signaling via the kinase p38alpha programs dendritic cells to drive TH17 differentiation and autoimmune inflammation. Nature Immunology, 13(2), 152-161. https://doi.org/10.1038/ni.2207

Huang, Y., Hanponen, K. E., Burrola, P. G., O’Connor, C., Hah, N., Huang, L., Nimmerjahn, A., & Lemke, G. (2021). Microglia use TAM receptors to detect and engulf amyloid beta plaques. Nature Immunology, 22(5), 586–594. https://doi.org/10.1038/s41590-021-00913-5

Jay, T. R., Hirsch, A. M., Broihier, M. L., Miller, C. M., Neilson, E. L., Ransohoff, R. M., Lamb, B. T., & Landreth, G. E. (2017). Disease progression-dependent effects of TREM2 deficiency in a mouse model of Alzheimer’s disease. The Journal of Neuroscience, 37(3), 637–647. https://doi.org/10.1523/JNEUROSCI.2110-16.2016

Jay, T. R., Miller, C. M., Cheng, P. J., Graham, L. C., Bemiller, S., Broihier, M. L., Xu, G., Margevicius, D., Karlo, J. C., Sousa, G. L., Cotelle, A. C., Butovsky, O., Bekris, L., Staugaitis, S. M., Leverenz, J. B., Pimplikar, S. W., Landreth, G. E., Howell, G. R., Ransohoff, R. M., & Lamb, B. T. (2015). TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer’s disease mouse models. The Journal of Experimental Medicine, 212(3), 287–295. https://doi.org/10.1084/jem.20142322

Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir, Szternfeld, R., Ulland, T. K., David, E., Baruch, K., Lara-Astaiso, D., Toth, B., Itzkovitz, S., Colonna, M., Schwartz, M., & Amit, I. (2017). A unique microglia type associated with restricting development of Alzheimer’s disease. Cell, 169(7), 1276–1290.e17. https://doi.org/10.1016/j.cell.2017.05.018

Kumar, S., Boehm, J., & Lee, J. C. (2003). p38 MAP kinases: Key signaling molecules as therapeutic targets for inflammatory diseases. Nature Reviews, Drug Discovery, 2(9), 717–726. https://doi.org/10.1038/ nrd1177

Mabhis, N., Jiang, S., Xu, G., Kokiko-Cochran, O. N., Roy, S. M., van Eldik, L., Watterson, D. M., Lamb, B. T., & Bhaskar, K. (2016). Selective suppression of the alpha isof orm of p38 MAPK rescues late-stage tau pathology. Alzheimer’s Research & Therapy, 8(1), 54. https://doi.org/10.1186/s13195-016-0221-y

Mckinsey, G. L., Lázama, C. O., Keown-Lang, A. E., Niu, A., Santander, N., Larpthewasarp, A., Chee, E., Gonzalez, F. F., & Arnold, T. D. (2020). A new genetic strategy for targeting microglia in development and disease. eLife, 9, e54590. https://doi.org/10.7554/ eLife.54590

Michaud, J. P., Bellavance, M. A., Prefontaine, P., & Rivest, S. (2013). Real-time in vivo imaging reveals the ability of monocytes to clear vascular amyloid beta. Cell Reports, 5(3), 646–653. https://doi.org/10.1016/j.celrep.2013.10.010

Michaud, J. P., Hallé, M., Lampron, A., Thériault, P., Préfontaine, P., Filali, M., Tribout-Jover, P., Lanteigne, A. M., Jodoin, R., Cluff, C., Brichard, V., Palmantier, R., Pilorget, A., Larocque, D., & Rivest, S. (2013). Toll-like receptor 4 stimulation with the detoxified ligand monophosphoryl lipid a improves Alzheimer’s disease-related pathology. Proceedings of the National Academy of Sciences of the United States of America, 110(5), 1941–1946. https://doi.org/10.1073/pnas.1215165110

Muzumdar, M. D., Tasic, B., Miyamichi, K., Li, L., & Luo, L. (2007). A global double-fluorescent Cre reporter mouse. Genesis, 45(9), 593–605. https://doi.org/10.1002/dvg.20335
