Conditional Genetic Deletion of CSF1 Receptor in Microglia Ameliorates the Physiopathology of Alzheimer's Disease

Vincent Pons

CHUQ

Pascal Lévesque

U. Laval

Marie-Michèle Plande

CHUQ

Serge Rivest (✉ Serge.Rivest@crchudequebec.ulaval.ca)

CHU de Québec Research Center, 2705, Laurier boulevard, Québec, QC, G1V 4G2, Canada

<https://orcid.org/0000-0002-6082-770X>

Research article

Keywords: mCSF, TREM2, β -Catenin, IL-34, Microglia, Innate immunity, Amyloid, Phagocytosis, Cognitive decline

Posted Date: October 6th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-80698/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Alzheimer's Research and Therapy on January 5th, 2021. See the published version at <https://doi.org/10.1186/s13195-020-00747-7>.

Abstract

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia in the world. Microglia are the innate immune cells of CNS, their proliferation, activation and survival in pathologic and healthy brain have previously been shown to be highly dependent on CSF1R.

Methods: Here we investigate the impact of such receptor on AD etiology and microglia. We deleted CSF1R using Cre/Lox system, the knock-out (KO) is restricted to microglia in the APP/PS1 mouse model. We induced the knock-out at 3-month-old, before plaque formation and evaluated both 6 and 8-month-old groups of mice.

Results: Our findings demonstrated that CSF1R KO did not impair microglial survival and proliferation at 6 and 8 months of age in APP cKO compared to their littermate controls groups APP_{Swe}/PS1. We have also shown that cognitive decline is delayed in CSF1R-deleted mice. Ameliorations of AD etiology is associated with a decrease in plaque volume in cortex and hippocampus area. A compensating system seems to take place following the knock-out, since TREM2/ β -Catenin and IL-34 expression are significantly increased. Such a compensatory mechanism may promote microglial survival and phagocytosis of A β in the brain.

Conclusions: Our results provide new insights on the role of CSF1R in microglia and how it interacts with the TREM2/ β -Catenin and IL-34 system to clear A β and ameliorates the physiopathology of AD.

Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia in the world. The number of new cases is growing daily due to a lack of reliable biomarkers for diagnosis and efficient treatments¹. Beta amyloid (A β) is the hallmark of AD. A β aggregation leads to the formation of senile plaques. The cleavage of amyloid protein precursor (APP) by BACE1 and the enzymatic complex γ -secretase generates A β resulting in two main isoforms A β_{1-40} (A β_{40}) and A β_{1-42} (A β_{42}), the latter being the most toxic form (2). 4-6% of AD patients have genetic predisposition, early AD is associated with APP, PS1 and PS2 gene mutation, whereas late AD is related to a mutation of APOE 4 (3,4).

A β deposits activate microglia, the innate immune cells of the central nervous system (CNS). Amyloid-exposed microglia synthesize tumor necrosis factor (TNF), which participates to the recruitment microglia and worsen inflammatory response (5). Microglia originate in the embryonic yolk sac and migrate to the brain between embryonic days 8.5 and 10 in mice (6). In adult CNS, microglia serve as brain macrophages, although they are distinct from other resident macrophages. Microglial cells dynamically survey the environment, they are responsible for the elimination of pathogens, cellular debris, dead cells, remodeling synapses, and the clearance of toxic proteins (7). They are partly dependent on colony-

stimulating factor-1 receptor (CSF1R) signaling for their maintenance, activation, proliferation and self-renewal (8,9).

CSF1R belongs to tyrosine kinase receptor family. It is broadly expressed in the organism by monocytes, macrophages, osteoclasts and microglia (10). CSF1R can bind two ligands namely macrophage-colony stimulating factor (mCSF) and Interleukine-34 (IL-34). They have different binding sites, but their roles are quite equivalent (11). In the brain, mCSF is primarily expressed by astrocytes, oligodendrocytes and microglia, whereas IL-34 is mainly expressed by neurons (Nandi et al. 2012). Previous studies have demonstrated that administration of mCSF can generate macrophages from bone-marrow precursor cells and a substantial increase in the number of blood monocytes (13–15). Blood levels of mCSF and other hematopoietic cytokines were found to decrease in AD patients, which was proposed as a good predictor for a rapid evolution from mild cognitive impairment pre-symptomatic to dementia (16). In 2009, Boissoneault and colleagues have performed a study using multiple systemic mCSF injections in APP_{swe/PS1} mice and they reported a powerful effect of such a treatment to prevent the cognitive decline even at a critical age of the disease. MCSF-treated mice presented a lower number of A β plaques, which were associated with an increased number of microglial cells in the brain. The cytokine stimulates the clearance of A β ₄₂ by microglia and infiltrating bone marrow-derived cells (17). In line with these findings, hippocampal injection of mCSF in AD mouse model induced the differentiation of bone marrow cells into bone marrow-derived microglia (BMDM), which resulted in improved cognitive decline (18).

IL-34, triggering receptor expressed on myeloid cells 2 (TREM2) and its adaptor DNAX-activating protein of 12 KDa (DAP12) have also an important role in the pathology. IL34 stimulates proliferation of monocytes and macrophages by binding CSF1R. Mizuno et al. have demonstrated that IL-34 injections ameliorated cognitive decline and reduced A β burden by up-regulating insulin-degrading enzyme (IDE) in a mouse model of AD (19). TREM2/DAP12 has a critical role in microglia survival and proliferation and their ability to phagocytose A β (20). A significant increase in TREM2 expression was found in plaque-associated microglia in AD patients (21–23). A mutation leading to a loss-of-function in TREM2 gene could decrease microglia survival and proliferation and a greater A β burden (24). Indeed, experiments have shown that TREM2 deficiency led to an impaired phagocytosis of A β by microglia in a mouse model of AD (25). β -Catenin has emerged as a crucial molecule for several pathologies (26–29).

CSF1R/TREM2/ β -Catenin are linked by Src tyrosine kinase, the principal effector of CSF1R signaling that can phosphorylate DAP12. DAP12 downstream intermediate molecule Pyk2 promotes stabilization of β -Catenin (23,30). In AD, Wnt/ β -Catenin is downregulated while Wnt antagonist Dkk1 is upregulated (26). Some research groups have reported that restoring β -Catenin in adult hippocampus was able to reverse synaptic loss, whereas administration of Dkk1 provokes a decrease in β -Catenin, TCF, LEF, and PSD-95 protein levels (31,32).

Altogether these data suggest that CSF1R/TREM2/ β -Catenin and IL-34 pathways are important to prevent cognitive impairment in AD. In this study, we investigated the role of CSF1R in a mouse model of AD using a conditional and specific microglial knock-out (KO). We have found that CSF1R gene deletion failed to affect microglia survival and activation probably due to several compensatory mechanisms. The

KO caused a significant increase in IL-34, TREM2 and β -Catenin gene expression together with improvement of cognitive decline and a decrease in A β burden. These molecules together with their signaling pathways may compensate for the suppression of CSF1R in microglia.

Methods

The aim of this study is to compare APP_{PS1/swe} to APP CSF1R^{-/-} to understand the role of CSF1R in Alzheimer's disease.

Animals:

We used only male for this study.

Conditional CSF1R knock out mice CSF1R-lox/CX3CR1-Cre/ER (called, cKO) B6.Cg-CSF1R *tm1jwp*/J mice (JaxMice; stock number 02212) were crossed with the B6.129-Cx3cr1tm2.1 (CreER)Jung/Orl mice (EMMA mouse respiratory; EM:06350). The resulting mouse has a tamoxifen-inducible CRE activity specifically in microglial cells, leading to a non-functional CSF1R protein. Mice were injected with tamoxifen at 10-week-old.

Rosa^{redTm}-CSF1R-lox/CX3CR1-Cre/ER mice: We crossed B6.Cg-Gt(ROSA)26Sor^{tm14}(CAG-tdTomato)Hze (JaxMice cat# 007914) mice with CSF1R-lox/CX3CR1-Cre/ER to confirm the deletion of CSF1R. Mice were injected with tamoxifen at 10 weeks-old for 4 consecutive days.

APP_{Swe/PS1} male transgenic mice bearing a chimeric human/mouse β -amyloid precursor protein (APP_{Swe}) gene and the human presenelin 1 gene (A246E variant). These mice were purchased from The Jackson Laboratory [Strain: B6C3-Tg (APP695)3Dbo Tg(PSEN1)5Dbo/J] and maintained on a C57BL/6J background.

cKO mice and APP_{Swe/PS1} mice were crossed to obtain an APP_{Swe/PS1}-CSF1R-lox; CX3CR1-Cre/ER (called, APP cKO). The resulting mouse has a tamoxifen-inducible CRE activity specifically in microglial cells, leading to a non-functional CSF1R protein. Mice were injected with tamoxifen at 3-month-old.

All animals were acclimated to standard laboratory conditions (14 h light, 10 h dark cycle; lights on at 06:00 and off at 20:00 h) with free access to rodent chow and water. Protocols were conducted according to the Canadian Council on Animal Care guidelines, administered by the Laval University Animal Welfare Committee.

Tamoxifen preparation and administration:

Tamoxifen was dissolved in corn oil (Sigma-Aldrich cat#C8267) and Ethanol 100% for 1 h at 37 degrees Celsius, vortexed every 15 min. We used ~75 mg tamoxifen/kg body weight and 100 μ l tamoxifen/corn oil solution was administered via intraperitoneal injection for 4 consecutive days.

Sacrifices:

All mice were deeply anesthetized with ketamine/xylazine (90:10) and sacrificed via intracardiac perfusion with 0.9% saline. Brains were retrieved and post-fixed 24 h in 4% PFA pH 7.4 and transferred in 4% PFA pH 7.4 + 20% sucrose for a minimum of 15 h. Brains were sliced in coronal sections of 20- μ m thickness with a freezing microtome (Leica Microsystems), serially collected in anti-freeze solution and kept at -20 C until usage.

Western blot:

Brain protein lysate was extracted and quantified as previously described (63). Proteins were loaded in 8-16% agarose precast gels (BioRad) and electroblotted onto 0.45 μ m Immibilon PVDF membranes. Membranes were incubated with primary antibodies anti-b Catenin (Cell signaling rabbit 1:1000 cat#9562), TREM2 (R&D sheep 1:1000 cat#AF-1729), BDNF (Millipore rabbit 1:1000 cat#AB-1534), Syndecan-1 (Abcam mouse 1:1000 cat#AB-34164), IL-34 (R&D sheep 1:1000 cat#AF-5195), PSD95 (Millipore mouse 1:1000 cat#MAB-1596), BACE1 (Cederlane rabbit 1:1000 AB-108394), APC (Novus biological rabbit 1:1000 cat#NBP2-15422), ABCB1 (Novus biological rabbit 1:1000 cat#NB-100-80870), followed by the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies and revealed by clarity (ECL) substrate (BioRad). Quantification was done by determining integrative density of bands using Thermo Scientific Pierce by Image Analysis software v2.0. Optical values were normalized over actin.

Immunofluorescent staining:

Brain sections were washed (4 x 5 minutes) in KPBS then blocked in KPBS containing 1% BSA and 1% Triton X-100 (Sigma-Aldrich cat#T8787). Tissues were incubated overnight 4 degrees Celsius with primary antibodies Iba1 (Wako rabbit 1:1000 cat#09-19741), 6E10 (Biolegend mouse 1:1000 cat#SIG-39320), CD31 (Ebioscience rat 1:1000 cat#11-0311-81). After washing sections in KPBS (4 x 5 minutes), tissues were incubated with the appropriate secondary antibodies for 2 hours at room temperature. Followed by washes in KPBS (4 x 5 minutes), tissues were incubated 10 minutes with DAPI (Molecular Probes 1:10000 cat#D3571). After washes in KPBS (4 x 5 minutes) sections were mounted onto MicroSlides Superforst® (Fisherbrand cat#22-037-246) and cover slipped (Globe scientific cat#1419-10) with Fluoromount-G (Electron microscopy sciences cat#17984-25).

Immunohistochemistry:

Brain sections were washed (4 x 5 minutes) in KPBS then blocked in KPBS containing 1% BSA and 1% Triton X-100 (Sigma-Aldrich cat#T8787). Tissues were incubated overnight 4 degrees Celsius with primary antibodies CSF1R (R&D sheep 1:750 cat#AF-3818). After washing sections in KPBS (4 x 5 minutes), tissues were incubated with the appropriate secondary antibodies for 2 hours at room temperature. Followed by washes in KPBS (4 x 5 minutes), tissues were incubated with ABC mix (Vector Laboratories Vectasin® Elite ABC-HRP Kit, Peroxidase (Standard) 2,25 μ l/ml sol A and 2,5 μ l/ml sol B

cat#PK-6100) for 1 hour at room temperature. After incubation sections were washed in KPBS (4 x 5 minutes) and incubated up to 15 minutes with a DAB solution (Sigma-Aldrich H₂O₂ 0.003% cat#H1009 and Sigma-Aldrich DAB 0.05% cat#D7304-1SET), followed by washed in KPBS (4 x 5 minutes). Sections were mounted onto MicroSlides Superforst® (Fisherbrand cat#22-037-246) and cover slipped (Globe scientific cat#1419-10) after dehydration with DPX (Electron microscopy sciences cat#13510).

Behavioral analyses:

Novel object recognition task protocol (34) consists of 3 phases: habituation, familiarization and test phase. The experimenter who observed and recorded the behavior was not aware of treatment and genotype of the tested animals (WT+vehicle, n=10, APP_{Swe}/PS1 + vehicle, n=10 and APP + tamoxifen, n = 10). Baseline data were obtained at 3 months of age, whereas the effects of the treatment were determined at 6 or 8 months. Tests were made at 15 lux.

Nesting protocol (64), mice were placed 1 per cage at 3 month-old. The experimenter who observed and recorded the behavior was not aware of treatment and genotype of the tested animals (WT+vehicle, n=10, APP_{Swe}/PS1 + vehicle, n=10 and APP + tamoxifen, n = 10). After each NOR task, cages were cleaned a new pad was placed inside. After 24h, the nests were analysed and scored. The scores vary from 1 (untouched pad) to 5 (perfect nest).

ELISA:

Total brain homogenates were used for all the ELISA. For the A β ₁₋₄₀ (Millipore cat#EZHS40) we analyzed 50 μ g per well following the protocol provided.

Unbiased Stereological count:

Brains were serially sectioned as previously described and were stained with Iba1, 6E10 and DAPI as previously described. We counted microglia and plaques at 20x magnification using an Axio Observer microscope equipped with AxioCam 503 monochrome camera and processed using with ZEN Imaging Software (Carl Zeiss Canada, Toronto, ON, Canada). Sampling characteristics for cortex sections: Sampling interval 12, total number of sections 7, section sampling interval 3 and starting selection 1. For hippocampal sections: Sampling interval 12, total number of sections 7, section sampling interval 2 and starting selection 1. After counting plaques using unbiased disector, the mean plaque volume (MPV) was estimated using the rotator method. The estimated MPV was based on the length of line crossing each plaque using randomly oriented line. 4 locations for each hippocampus and 3 for each cortex were sampled in 7 sections. Plaques and microglia were counted according to Gundersen unbiased counting rules, with optical fractionator method and sampling continued to a coefficient of error of 10% or less (65).

Image Acquisition:

Image acquisition of Fluorescent staining images was performed using a Zeiss LSM800 confocal microscope supported by the Zen software (2.3 system) using the 10×, 20×, and 40× lenses. Confocal images were then processed using Fiji (ImageJ Version 2.0.0-rc-43/1.51n). For analyses and bright field image acquisition of staining, Iba-1 and CSF1R, 8-bit grayscale TIFF images of the regions of interest were taken in a single sitting for whole protocols with a Qimaging camera (Qcapture program, version 2.9.10) attached to Nikon microscope (C-80) with the same gain/exposure settings for every image. To evaluate the level of Iba-1+ immune response in the regions of interest, the images were imported into ImageJ (1.37) and the percentage of area occupied by the staining was measured using the threshold parameter. Cell count was assessed manually using ImageJ (1.37). Analysis was performed in double blinded to avoid bias of analysis. Fluorescent staining of images was performed using a Zeiss LSM800 confocal microscope supported by the Zen software (2.6 system). Confocal images were then processed using Fiji (ImageJ Version 2.0.0-rc-43/1.51n).

Statistical analyses and Figure preparation:

Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical analyses were carried with the Prism software (version 8.0, GraphPad Software Inc.), comparing control groups vs. tested groups. Values were considered statistically significant if $p < 0.05$. All panels were assembled using Adobe Photoshop CC 2018 (version 19.1.0) and Adobe Illustrator CC 2018 (version 23.0.1).

Results

CSF1R is solely deleted in microglia.

CSF1R conditional knock-out (cKO) was made by a Cre/Lox system (Figure 1B). Cre recombinase is under the CX3CR1 promoter. Lox sites are on each side of the CSF1R Exon 5. When tamoxifen is injected, the Cre recombinase complex translocates to the nucleus to interact with lox sites thereby CSF1R gene is non-functional. Mice were injected with tamoxifen at 3-month-old (Figure 1 A). To determine whether the KO was efficient, we used Rosa^{redTm}-CSF1R-lox/CX3CR1-Cre/ER mice. Mice express robust redtm fluorescence following Cre-mediated recombination in CX3CR1 cells in the brain, meaning the knock-out is effective (Figure 1C, D). Quantification shows an endogenous activity of Cre-recombinase in mice without tamoxifen is around 20%, however, after induction the knock-out cells reach 89%. To confirm these results, we quantified by immunohistochemistry the percentage of CSF1R⁺ cells in parenchyma of cKO mice. Results shown a robust decrease of CSF1R expression in cKO mice. These data indicate that our model is reliable, and strongly efficient to induce the knock-out selectively in microglia.

The deletion of CSF1R does not affect microglia survival and delay cognitive decline in APP cKO mice.

As previously described, CSF1R is largely depleted in microglia. Here we show using an unbiased stereological analysis of Iba1-positive cells, no significative difference in microglia number into hippocampus and cortex at 6 and 8-month-old in APP cKO compared to APP_{Swe/PS1} (Figure 2A-C). These data are corroborating our previous findings, suggesting that KO has no impact on microglia survival in a

specific model of neuronal injury (33). To investigate the impact of the KO on cognition, the memory of APP cKO and their littermate control mice were tested at 3, 6 and 8-month-old using novel object recognition task (NOR). This test is a standard to evaluate the cognitive decline in mouse models of AD (34). The test measures the time spent on exploring the novel object compared to the common object. During the acquisition phase, every animal explored more than 10 seconds each object (Figure 2E). The test phase analysis shown no differences between groups at 3-month-old. However, at 6-month-old APP_{Swe/PS1} mice present an expected sign of cognitive decline (**p=0.0002), which was also confirmed at 8-month-old (**p=0.0007). APP_{Swe/PS1} mice spent equal time at exploring both objects rather than exploring the novel one. Interestingly, APP cKO mice did not have the same cognitive decline at 6 or 8-month-old, they spent an equivalent time to explore the novel object as the wild-type group did (Figure 2D). Our data suggest a protective effect of CSF1R gene deletion to prevent cognitive decline in APP cKO mice.

The nesting behavior was conducted to evaluate the effect of CSF1R knock-out on social withdrawal and apathy linked to AD. As depicted by the Figure 2F, the nest scores were equivalent between groups at 3 and 6-month-old. However, 8-month-old APP_{Swe/PS1} mice had a lower score compared with both WT and APP cKO groups (Figure 2E, 2.5, APP_{Swe/PS1} ***p=0.0009, 4.5, WT and 4.3 APP cKO). These data suggest that APP cKO do not exhibit a significant decrease in apathy and social withdrawal.

Altogether these results indicated that APP cKO mice do not present memory and social behavior impairment associated with AD. NOR and nesting tests did not show a significative difference between APP cKO mice and WT, unlike APP_{Swe/PS1}. as expected arbor a robust cognitive decline. Moreover, the number of microglia was equal between APP cKO and their control APP_{Swe/PS1}, suggesting CSF1R is not the only receptor involved in microglia proliferation and survival in this model.

Long-term knock-out reduces volume and plaque number, with the onset of cerebral amyloid angiopathy (CAA).

Cognitive impairment is correlated with the onset of A β plaque formation in the cortex and hippocampus in APP_{Swe/PS1}. In this animal model, plaques are established and observable at 6-month-old. Since CSF1R-deleted mice performed as well as WT mice, they should have fewer amyloid deposits in hippocampus and cortex compared to APP mice. Sections were stained with Iba1 and anti-A β (6E10) at 6 and 8-month-old (Figure 3A). Here we observed a difference in plaque number and volume in the cortex area. Using unbiased stereology, we quantified the number of plaques per region and volume of A β deposit in APP cKO at 6 and 8-month-old and their littermate controls (Figure 3B-E). We observed a diminution by 2.1-fold of plaque volume in APP cKO group at 6-month-old (*p=0.0354 hippocampus, cortex *p=0.0479). We also wanted to see if the number of plaques in each structure was changed. For this matter we counted every plaque in both hippocampus and cortex and then normalized the data with the volume of these structures. Results are expressed in number of plaques per mm³, the software gave an unbiased count on the whole brain. The relative number of plaques is significantly decreased by 1.8-fold in the cortex of the APP cKO 6-month-old group (*p=0.0270). At 8-month-old, the volume of plaques

is also reduced in hippocampus (*p=0.0320) and cortex (*p=0.0227) compared to their control littermates, respectively by 6.6-fold and 10-fold. Actually, the volume of plaque does not differ between 6 and 8 months in APP cKO. However, regarding the number of plaques in hippocampus or cortex, it remains similar in both groups at 8-month-old (Figure 3E).

These data provide clear evidence that deletion of CSF1R prevents the accumulation and/or induces a better clearance of A β in the brain.

The equilibrium between A β burden in the parenchymal and blood vessels is well described (35). AD patients present a diminution of the transporter ABCB1 that impairs the efflux of A β in blood vessels. According to previous data, we studied the vascular amyloid to see if this transport was maintained. We used Elisa kits to quantify A β_{40} levels in blood vessels. We did not observe any difference at 6 months in APP_{Swe/PS1} or APP cKO groups. However, at 8-month-old, we detected a significant augmentation of A β_{40} in APP cKO group (**p=0.0098) (Figure 3F) associated with a stable expression of ABCB1 at 8 months for APP cKO (*p=0.023) (Figure 3G). These data indicate that CSF1R could play a role in CAA onset or at least it may contribute to down-regulate ABCB1. These results also show that peripheral immune system is not adequately activated to clear vascular amyloid.

Altogether these data indicate that the KO has a beneficial effect in this model on cerebral amyloid load by decreasing the volume of senile plaques (Figure 3B, E) and may accelerate vascular A β deposits and CAA (Figure 3F, G, H).

TREM2/ β -Catenin and IL-34 brain protein levels following CSF1R gene deletion.

We have previously demonstrated that a genetic ablation of CSF1R in a non-inflammatory model did not affect microglia proliferation and activation. Moreover, CSF1R knockout microglia overexpress TREM2 following nerve section. However, in acute inflammatory model such as cuprizone, microglia are unable to proliferate and activate properly (33), indicating that the role of CSF1R is dependent on the microglial environment. Here, we detected the same amount of microglia in APP_{Swe/PS1} and APP cKO and microgliosis around plaques was detected in both groups (Figure 2A, 3A). This suggests that the genetic deletion in this model did not impair microglial proliferation and survival that may depend on other factors or compensatory mechanisms due to the CSF1R gene deletion.

We then studied the expression of TREM2, β -Catenin and IL-34 in young WT and cKO 10-week-old mice, because we postulated that these molecules can play an important role in AD by compensating for the KO. TREM2 protein levels increased by 2-fold in cKO mice compared to littermate controls (*p=0.0384), which was associated with the stabilization of β -Catenin (*p=0.0360) and the diminution of adenomatous polyposis coli (APC), a member of β -Catenin destruction complex (**p=0.0023) (Figure 4A).

We then looked at our both groups of 6 and 8-month-old mice and found a robust expression of TREM2, especially at 6-month-old APP cKO mice compared to APP_{Swe/PS1} age matched animals (Figure 4B) (*p=0.036). This was again associated with an augmentation of β -Catenin protein levels (*p=0.0027).

Such up-regulation of TREM2/ β -Catenin may suggest that these pathways could compensate for the loss of CSF1R in order to keep microglia alive and functional. Interestingly, other very critical molecules increased in the 6 month-old cKO group, namely BDNF (**p=0.0071), Syndecan-1 (*p=0.0498), IL-34 (*p=0.0274) and PSD-95 (*p=0.0120) (Figure 4B). These increases suggest a beneficial effect of CSF1R deletion on neurons, synapses and microglia in the onset of the disease. Similar profile was found at 8-month-old (Figure 4C), at least for BDNF (*p=0.0494) and syndecan-1 (*p=0.0378), but not for PSD-95 and IL-34. It is noteworthy that BACE-1 significantly decreased (**p=0.001) in the brain of 8-month-old cKO mice (Figure 4C-D).

The number of microglia following knock-out induction remains unchanged.

Several studies have shown a robust microglia lethality following pharmacological inhibition of CSF1R (36,37). We have previously demonstrated that number of microglia in hippocampus and cortex between APP_{Swe/PS1} and APP cKO is not statistically different (Figure 2A). We aimed to show whether microglia following the KO died and repopulated the brain or signalling pathways previously described are sufficient to keep microglia alive. We have injected tamoxifen in WT and cKO mice at 3-month-old every day for 4 days. We then have sacrificed mice every 2 days after the last injection, until 20th days post-injection. The count of microglia over the time indicates that microglia survived even depleted from CSF1R (Figure 5A). Interestingly, at D4 we can observe that almost all microglia are KO, unlike at D2 suggesting that tamoxifen induces KO within 4 days after the last injection (Figure 5B). These data strongly suggest that compensatory mechanisms must take place immediately following the conditional gene deletion to allow such microglial survival and activation in the brain of APP mice or CSF1R is far from being the only receptor involved in such process, at least in this mouse model of AD.

Discussion

In this article, we aimed to study the role of CSF1R in AD. For this purpose, we have bred APP_{Swe/PS1} with CSF1R-lox/CX3CR1-Cre/ER mice. We induced the KO at 3-month-old by injecting tamoxifen, before plaque formation and we sacrificed animals at 6 or 8-month-old. AD is a multifactorial disease in which CSF1R could play a major role. MCSF/CSF1R axis promotes microglial proliferation, survival and activation (8). In 2003, Mitrasinovic and colleagues have stimulated microglia with mCSF and they found that the cytokine promoted phagocytosis of amyloid. In the same line, Boissonneault and colleagues found similar results by injecting mCSF in APP_{Swe/PS1} mice. CSF1R stimulation has beneficial effects on A β clearance by microglia and bone marrow-derived microglia (BMDM) (17). However, growing evidence has shown recently that CSF1R inhibition could also prevent cognitive decline and amyloid deposition. Most of research groups used a molecule which inhibits CSF1R signalization, namely PLX5622. Interestingly, by impeding mCSF signal they observed a massive microglial depletion, suggesting a central role of mCSF/CSF1R axis in microglia survival (36,38–42). Lately, we have demonstrated the effect of CSF1R ablation on microglia is depending on the context. Actually, CSF1R-deleted microglia still survive and proliferate in a non-inflammatory environment, whereas they are unable to proliferate in a model of cuprizone-induced demyelination and inflammation (33).

In the present study, we used a Cre/Lox system to suppress CSF1R gene expression. The knock-out affects 89% of microglia in the brain (Figure 1D-F). Importantly, we observed a similar number of microglia following the KO (Figure 5A) indicating that CSF1R is not essential for microglia to survive and proliferate in APP/PS1 mice (Figure 2 A-C). These findings are contradictory with studies using PLX5622, raising the question about the specificity of CSF1R inhibitor molecules or that the proposed compensatory mechanisms may not take place following the chemical inhibition of the receptor. TREM2 and β -Catenin may have a critical role to allow the survival and proliferation of microglia in the CSF1R cKO APP mice. TREM2 is an immunoreceptor expressed in the brain by microglia (23) and activates survival pathway via β -Catenin (23,25,29,43). Moreover, TREM2/ β -Catenin pathway is important for microglial survival and proliferation (43). Indeed, β -Catenin initiates the transcription of cyclin D1 and c-Myc. Interestingly, TREM2-deficient microglia have a reduced level of these controlling cell cycle molecules. In this model this signaling pathway may compensate CSF1R deletion. We observed that TREM2 increased by 2-fold in cKO mice compared to their littermate controls and this is accompanied with a stabilization of β -Catenin since there is a diminution by 2.2-fold of APC (Figure 4A). Adenomatous polyposis coli activation leads to β -Catenin degradation (44).

These data strongly suggest that the TREM2/ β -Catenin signaling pathway compensates for the CSF1R KO in this mouse model of AD. Such augmentation of TREM2 and stabilization of β -Catenin at 6 and 8 months of age suggest a positive effect on microglia proliferation and survival, which has been found to be beneficial in a mouse model of AD (45). IL-34 and Syndecan-1 also increased in APP cKO, especially at 6 months of age and IL34 was recently proposed to be the main brain factor to stimulate proliferation of microglia in the ME7 model of prion disease (46). On interest, elevation of IL-34 and Sydecan-1 was also found in CSF1R^{op/op} mice. Indeed, osteoclasts compensate by overexpressing IL-34 indicating a crosstalk or a similar role of both cytokines (47). Furthermore, Mizuno and colleagues demonstrated that injections of IL-34 ameliorate cognitive decline and reduce A β burden in APP/PS1 mice (19). Importantly, CSF1R-deficient mice present different phenotypes whether CSF1R is constitutively deleted or not (48). Growing evidence show that IL-34 signaling could be more important in adulthood than mCSF (49). CSF1R is the main receptor of IL-34, but recently alternative receptors have been proposed. Syndecan-1 is one of them. *In vitro* syndecan-1 is positively expressed when IL-34 binds CSF1R in macrophages (50). In APP cKO model, IL-34/syndecan-1 could be an alternative pathway for survival and proliferation of microglia. Overexpression of both TREM2/ β -Catenin and IL-34/syndecan-1 are in line with other findings, corroborated by NOR and nesting test (Figure 2 D, E). NOR shows a delayed cognitive decline in APP cKO 6 and 8-months-old associated with a decreased volume of plaques in hippocampus and cortex (Figure 3 B, C). Microglia may be more efficient to phagocyte amyloid due to the overexpression of TREM2. These data together with the fact that BDNF increased by 3.3-fold in APP cKO in 6-month-old and 1.8-fold in 8-month-old mice clearly underline the beneficial outcome of the compensatory mechanisms (Figure 4B, C). Of interest is the recent papers showing that TREM2 and β -Catenin up-regulation is linked to microglia expressing BDNF (51,52). Finally, the inverse correlation of BACE-1 and TREM2 in AD cKO mice is in line with the overall beneficial property of the conditional CSF1R gene deletion. In this regard is the ability of A β ₄₂ to stimulate NF- κ B pathway and BACE-1 gene transcription together with TREM2 inhibition (53).

To summarize molecular expression changes, we proposed a representative diagram in Figure 5C.

Besides positive effects of CSF1R deletion on AD, we have observed CAA onset in APP cKO mice at 8-month-old. CAA is associated with AD in a large majority of cases (54). ABCB1 transports A β ₄₀ from parenchyma to blood vessels and AD patients are known to present a lower expression level of ABCB1. This may allow the accumulation of A β in the brain (55–57). Our data indicate that APP CSF1R KO mice at 8 months of age tend to have a greater accumulation of A β ₄₀ in blood vessels, due to a stable expression of ABCB1 compared to APP_{Swe/PS1} at the same age (Figure 3F-H). β -catenin has a critical role in blood-brain barrier (BBB) regulation, its over-expression may limit BBB damages and prevent CNS immune cell infiltration (58). Moreover, β -catenin is downregulated in AD, but when this pathway is reactivated it restores ABCB1 (59,60). According to these data, the over-expression of β -catenin at 6 and 8-month-old in APP cKO groups can maintain ABCB1 expression and therefore relieve the brain from A β burden. Blood monocytes are more efficient to clear amyloid in blood vessels during AD course than microglia, however, phagocytic capacity of monocytes in AD patients is greatly reduced (61,62).

Conclusions

Our study aimed to understand the role of CSF1R in AD. With a conditional KO mouse model, we have induced CSF1R deletion at 3-month-old, before plaque formation. Here we demonstrated that strong compensatory pathways settled following the KO. Indeed, TREM2/ β -Catenin and IL-34 expression increase leading to a reduction of plaque volume and a delayed cognitive decline. These ameliorations of mouse conditions are associated with the overexpression of molecules acting on maintenance and protection of neurons and synapses. We have also demonstrated that CSF1R deletion did not impair microglia proliferation and survival probably due to the compensating TREM2/ β -Catenin and IL-34 pathways. These signaling pathways seem primordial for microglia and AD etiology when CSF1R gene is deleted in a conditional manner.

List Of Abbreviations

A β Amyloid-Beta

AD Alzheimer's Disease

APC Adenomatous polyposis coli

APP Amyloid precursor protein

cKO Conditional knock-out

CSF1R Colony-stimulating factor-1 receptor

DAP12 DNAX-activating protein of 12 KDa

IL-34 Interleukin 34

KO Knock-out

mCSF Macrophage colony-stimulating factor-1 receptor

NOR Novel object recognition task

TNF Tumor necrosis factor

TREM2 Triggering receptor expressed on myeloid cells 2

WT Wild type

Declarations

Ethics approval and consent to participate:

The animal study was reviewed and approved by CPAUL3, Laval University

Consent for publication:

Not applicable

Availability of data and materials:

All data generated or analysed during this study are included in this published article

Competing interests:

The authors declare that they have no competing interest.

Funding:

This work was supported by the Canadian Institutes in Health Research (CIHR grant no 331652) via the foundation scheme program and les fonds de recherche du Québec-Santé (FRQS) via the research center funding grant. SR is supported by Canadian Research Chair in Neuroimmunology.

Authors' contributions:

VP participated in the design of experiments, analyzed and interpreted the data, wrote the manuscript, and assembled the Figures. PL and MMP participated in experiments. SR formulated the study concept, supervised the project, and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Not applicable

References

1. Perl DP. Neuropathology of Alzheimer's Disease. *Mt Sinai J Med J Transl Pers Med*. 2010 Jan;77(1):32–42.
2. Jucker M, Walker LC. Amyloid- β pathology induced in humans. *Nature*. 2015 Sep;525(7568):193–4.
3. Silva MVF, Loures C de MG, Alves LCV, de Souza LC, Borges KBG, Carvalho M das G. Alzheimer's disease: risk factors and potentially protective measures. *J Biomed Sci*. 2019 Dec;26(1):33.
4. Giri M, Lü Y, Zhang M. Genes associated with Alzheimer's disease: an overview and current status. *Clin Interv Aging*. 2016 May;665.
5. Jana M, Palencia CA, Pahan K. Fibrillar Amyloid- β Peptides Activate Microglia via TLR2: Implications for Alzheimer's Disease. *J Immunol*. 2008 Nov 15;181(10):7254–62.
6. Ginhoux F, Prinz M. Origin of Microglia: Current Concepts and Past Controversies. *Cold Spring Harb Perspect Biol*. 2015 Aug;7(8):a020537.
7. Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. *Nat Rev Neurosci*. 2018 Oct 1;19(10):622–35.
8. Chitu V, Gokhan Ş, Nandi S, Mehler MF, Stanley ER. Emerging Roles for CSF-1 Receptor and its Ligands in the Nervous System. *Trends Neurosci*. 2016 Jun;39(6):378–93.
9. Elmore MRP, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA, et al. Colony-Stimulating Factor 1 Receptor Signaling Is Necessary for Microglia Viability, Unmasking a Microglia Progenitor Cell in the Adult Brain. *Neuron*. 2014 Apr;82(2):380–97.
10. Stanley ER, Chitu V. CSF-1 Receptor Signaling in Myeloid Cells. *Cold Spring Harb Perspect Biol*. 2014 Jun 1;6(6):a021857–a021857.
11. Boulakirba S, Pfeifer A, Mhaidly R, Obba S, Goulard M, Schmitt T, et al. IL-34 and CSF-1 display an equivalent macrophage differentiation ability but a different polarization potential. *Sci Rep [Internet]*. 2018 Dec [cited 2018 Sep 18];8(1). Available from: <http://www.nature.com/articles/s41598-017-18433-4>
12. Nandi S, Gokhan S, Dai X-M, Wei S, Enikolopov G, Lin H, et al. The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. *Dev Biol*. 2012 Jul;367(2):100–13.
13. Hume DA, Pavli P, Donahue RE, Fidler IJ. The effect of human recombinant macrophage colony-stimulating factor (CSF-1) on the murine mononuclear phagocyte system in vivo. *J Immunol Baltim Md 1950*. 1988 Nov 15;141(10):3405–9.
14. Giulian D, Ingeman J. Colony-stimulating factors as promoters of ameboid microglia. *J Neurosci*. 1988 Dec 1;8(12):4707–17.
15. Ushach I, Zlotnik A. Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage. *J Leukoc Biol*. 2016 Sep;100(3):481–9.

16. Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nat Med*. 2007 Nov;13(11):1359–62.
17. Boissonneault V, Filali M, Lessard M, Relton J, Wong G, Rivest S. Powerful beneficial effects of macrophage colony-stimulating factor on α -amyloid deposition and cognitive impairment in Alzheimer's disease. *Brain*. 2009 May 21;132(4):1078–92.
18. Kawanishi S, Takata K, Iteazono S, Nagayama H, Konoya S, Chisaki Y, et al. Bone-Marrow-Derived Microglia-Like Cells Ameliorate Brain Amyloid Pathology and Cognitive Impairment in a Mouse Model of Alzheimer's Disease. *J Alzheimers Dis*. 2018 Jun 19;64(2):563–85.
19. Mizuno T, Doi Y, Mizoguchi H, Jin S, Noda M, Sonobe Y, et al. Interleukin-34 Selectively Enhances the Neuroprotective Effects of Microglia to Attenuate Oligomeric Amyloid- β Neurotoxicity. *Am J Pathol*. 2011 Oct;179(4):2016–27.
20. Rivest S. TREM2 enables amyloid β clearance by microglia. *Cell Res*. 2015 May;25(5):535–6.
21. Konishi H, Kiyama H. Microglial TREM2/DAP12 Signaling: A Double-Edged Sword in Neural Diseases. *Front Cell Neurosci*. 2018 Aug 6;12:206.
22. Yin Z, Raj D, Saiepour N, Van Dam D, Brouwer N, Holtman IR, et al. Immune hyperreactivity of A β plaque-associated microglia in Alzheimer's disease. *Neurobiol Aging*. 2017 Jul;55:115–22.
23. Zheng H, Jia L, Liu C-C, Rong Z, Zhong L, Yang L, et al. TREM2 Promotes Microglial Survival by Activating Wnt/ β -Catenin Pathway. *J Neurosci*. 2017 Feb 15;37(7):1772–84.
24. Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, et al. TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer's Disease Model. *Cell*. 2015 Mar;160(6):1061–71.
25. Kawabori M, Kacimi R, Kauppinen T, Calosing C, Kim JY, Hsieh CL, et al. Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) Deficiency Attenuates Phagocytic Activities of Microglia and Exacerbates Ischemic Damage in Experimental Stroke. *J Neurosci*. 2015 Feb 25;35(8):3384–96.
26. Palomer E, Buechler J, Salinas PC. Wnt Signaling Deregulation in the Aging and Alzheimer's Brain. *Front Cell Neurosci*. 2019 May 22;13:227.
27. Shang S, Hua F, Hu Z-W. The regulation of β -catenin activity and function in cancer: therapeutic opportunities. *Oncotarget*. 2017 May 16;8(20):33972–89.
28. Valenta T, Hausmann G, Basler K. The many faces and functions of β -catenin: β -Catenin: a life by, beyond, and against the Wnt canon. *EMBO J*. 2012 Jun 13;31(12):2714–36.
29. Al-Harhi L. Wnt/ β -catenin and its Diverse Physiological Cell Signaling Pathways in Neurodegenerative and Neuropsychiatric Disorders. *J Neuroimmune Pharmacol*. 2012 Dec;7(4):725–30.
30. Otero K, Turnbull IR, Poliani PL, Vermi W, Cerutti E, Aoshi T, et al. Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAP12 and β -catenin. *Nat Immunol*. 2009 Jul;10(7):734–43.

31. Marzo A, Galli S, Lopes D, McLeod F, Podpolny M, Segovia-Roldan M, et al. Reversal of Synapse Degeneration by Restoring Wnt Signaling in the Adult Hippocampus. *Curr Biol*. 2016 Oct;26(19):2551–61.
32. Fortress AM, Schram SL, Tuscher JJ, Frick KM. Canonical Wnt Signaling is Necessary for Object Recognition Memory Consolidation. *J Neurosci*. 2013 Jul 31;33(31):12619–26.
33. Pons V, Laflamme N, Préfontaine P, Rivest S. Role of Macrophage Colony-Stimulating Factor Receptor on the Proliferation and Survival of Microglia Following Systemic Nerve and Cuprizone-Induced Injuries. *Front Immunol*. 2020 Jan 29;11:47.
34. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process*. 2012 May;13(2):93–110.
35. Brenowitz WD, Nelson PT, Besser LM, Heller KB, Kukull WA. Cerebral amyloid angiopathy and its co-occurrence with Alzheimer's disease and other cerebrovascular neuropathologic changes. *Neurobiol Aging*. 2015 Oct;36(10):2702–8.
36. Rosin JM, Vora SR, Kurrasch DM. Depletion of embryonic microglia using the CSF1R inhibitor PLX5622 has adverse sex-specific effects on mice, including accelerated weight gain, hyperactivity and anxiolytic-like behaviour. *Brain Behav Immun*. 2018 Oct;73:682–97.
37. Gerber YN, Saint-Martin GP, Bringuier CM, Bartolami S, Goze-Bac C, Noristani HN, et al. CSF1R Inhibition Reduces Microglia Proliferation, Promotes Tissue Preservation and Improves Motor Recovery After Spinal Cord Injury. *Front Cell Neurosci* [Internet]. 2018 Oct 16 [cited 2019 Jun 27];12. Available from: <https://www.frontiersin.org/article/10.3389/fncel.2018.00368/full>
38. Ali S, Mansour AG, Huang W, Queen NJ, Mo X, Anderson JM, et al. CSF1R inhibitor PLX5622 and environmental enrichment additively improve metabolic outcomes in middle-aged female mice. *Aging*. 2020 Feb 2;12(3):2101–22.
39. Casali BT, MacPherson KP, Reed-Geaghan EG, Landreth GE. Microglia depletion rapidly and reversibly alters amyloid pathology by modification of plaque compaction and morphologies. *Neurobiol Dis*. 2020 Aug;142:104956.
40. Riquier AJ, Sollars SI. Astrocytic response to neural injury is larger during development than in adulthood and is not predicated upon the presence of microglia. *Brain Behav Immun - Health*. 2020 Jan;1:100010.
41. Nissen JC, Thompson KK, West BL, Tsirka SE. Csf1R inhibition attenuates experimental autoimmune encephalomyelitis and promotes recovery. *Exp Neurol*. 2018;307:24–36.
42. Spangenberg EE, Green KN. Inflammation in Alzheimer's disease: Lessons learned from microglia-depletion models. *Brain Behav Immun*. 2017 Mar;61:1–11.
43. Zulfiqar S, Tanriöver G. β -Catenin Pathway Is Involved in TREM2-Mediated Microglial Survival. *J Neurosci*. 2017 Jul 26;37(30):7073–5.
44. Parker TW, Neufeld KL. APC controls Wnt-induced β -catenin destruction complex recruitment in human colonocytes. *Sci Rep*. 2020 Dec;10(1):2957.

45. Tapia-Rojas C, Inestrosa NC. Wnt signaling loss accelerates the appearance of neuropathological hallmarks of Alzheimer's disease in J20-APP transgenic and wild-type mice. *J Neurochem*. 2018 Feb;144(4):443–65.
46. Obst J, Simon E, Martin-Estebane M, Pipi E, Barkwill LM, Gonzalez-Rivera I, et al. Inhibition of IL34 unveils tissue-selectivity and is sufficient to reduce microglial proliferation in chronic neurodegeneration [Internet]. *Neuroscience*; 2020 Mar [cited 2020 Sep 18]. Available from: <http://biorxiv.org/lookup/doi/10.1101/2020.03.09.976118>
47. Lelios I, Cansever D, Utz SG, Mildenerberger W, Stifter SA, Greter M. Emerging roles of IL-34 in health and disease. *J Exp Med*. 2020 Mar 2;217(3):e20190290.
48. Sauter KA, Pridans C, Sehgal A, Tsai YT, Bradford BM, Raza S, et al. Pleiotropic effects of extended blockade of CSF1R signaling in adult mice. *J Leukoc Biol*. 2014 Aug 1;96(2):265–74.
49. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-Derived Interleukin-34 Controls the Development and Maintenance of Langerhans Cells and the Maintenance of Microglia. *Immunity*. 2012 Dec;37(6):1050–60.
50. Ségaliny AI, Brion R, Brulin B, Maillason M, Charrier C, Téletchéa S, et al. IL-34 and M-CSF form a novel heteromeric cytokine and regulate the M-CSF receptor activation and localization. *Cytokine*. 2015 Dec;76(2):170–81.
51. Fan Y, Ma Y, Huang W, Cheng X, Gao N, Li G, et al. Up-regulation of TREM2 accelerates the reduction of amyloid deposits and promotes neuronal regeneration in the hippocampus of amyloid beta1-42 injected mice. *J Chem Neuroanat*. 2019 Apr;97:71–9.
52. Garcia A, Udeh A, Kalahasty K, Hackam A. A growing field: The regulation of axonal regeneration by Wnt signaling. *Neural Regen Res*. 2018;13(1):43.
53. Guglielmotto M, Repetto IE, Monteleone D, Vasciaveo V, Franchino C, Rinaldi S, et al. Stroke and Amyloid- β Downregulate TREM-2 and Uch-L1 Expression that Synergistically Promote the Inflammatory Response. *J Alzheimers Dis*. 2019 Oct 1;71(3):907–20.
54. Jellinger KA. Alzheimer disease and cerebrovascular pathology: an update. *J Neural Transm*. 2002 May 1;109(5–6):813–36.
55. Zhang C, Qin H, Zheng R, Wang Y, Yan T, Huan F, et al. A new approach for Alzheimer's disease treatment through P-gp regulation via ibuprofen. *Pathol - Res Pract*. 2018 Nov;214(11):1765–71.
56. ElAli A, Rivest S. The role of ABCB1 and ABCA1 in beta-amyloid clearance at the neurovascular unit in Alzheimer's disease. *Front Physiol* [Internet]. 2013 [cited 2020 Mar 26];4. Available from: <http://journal.frontiersin.org/article/10.3389/fphys.2013.00045/abstract>
57. Vogelgesang S, Warzok R, Cascorbi I, Kunert-Keil C, Schroeder E, Kroemer H, et al. The Role of P-glycoprotein in Cerebral Amyloid Angiopathy; Implications for the Early Pathogenesis of Alzheimers Disease. *Curr Alzheimer Res*. 2004 May 1;1(2):121–5.
58. Lengfeld JE, Lutz SE, Smith JR, Diaconu C, Scott C, Kofman SB, et al. Endothelial Wnt/ β -catenin signaling reduces immune cell infiltration in multiple sclerosis. *Proc Natl Acad Sci*. 2017 Feb 14;114(7):E1168–77.

59. Corrêa S, Binato R, Du Rocher B, Castelo-Branco MT, Pizzatti L, Abdelhay E. Wnt/ β -catenin pathway regulates ABCB1 transcription in chronic myeloid leukemia. *BMC Cancer*. 2012 Dec;12(1):303.
60. Kania KD, Wijesuriya HC, Hladky SB, Barrand MA. Beta amyloid effects on expression of multidrug efflux transporters in brain endothelial cells. *Brain Res*. 2011 Oct;1418:1–11.
61. Thériault P, ElAli A, Rivest S. The dynamics of monocytes and microglia in Alzheimer's disease. *Alzheimers Res Ther*. 2015 Dec;7(1):41.
62. Cheng Y, Tian D-Y, Wang Y-J. Peripheral clearance of brain-derived A β in Alzheimer's disease: pathophysiology and therapeutic perspectives. *Transl Neurodegener*. 2020 Dec;9(1):16.
63. Michaud J-P, Halle M, Lampron A, Theriault P, Prefontaine P, Filali M, et al. Toll-like receptor 4 stimulation with the detoxified ligand monophosphoryl lipid A improves Alzheimer's disease-related pathology. *Proc Natl Acad Sci*. 2013 Jan 29;110(5):1941–6.
64. Deacon RM. Assessing nest building in mice. *Nat Protoc*. 2006 Aug;1(3):1117–9.
65. Courchesne E, Mouton PR, Calhoun ME, Semendeferi K, Ahrens-Barbeau C, Hallet MJ, et al. Neuron Number and Size in Prefrontal Cortex of Children With Autism. *JAMA*. 2011 Nov 9;306(18):2001.

Figures

Figure 1

CSF1R deletion is solely deleted in microglia A experimental design. B genetic construction of Cre/Lox system. C timeline starting at 3-month-old D representative image of microglia (Iba1 green, Cre-recombination reporter gene Redtm) in WT and cKO. Scale bar = 200µm. E quantification of the average percentage of Redtm⁺/Iba1⁺ cells in RosaredTm-CSF1R-lox/CX3CR1-Cre/ER without tamoxifen (ctl cKOredtm) or with tamoxifen (cKOredtm) n=4 in each group. E representative image of CSF1R staining in

immunohistochemistry using 3-month-old WT and cKO mice. Scale bar = 200µm. F analysis of CSF1R staining at 3-month-old WT and cKO mice n=4 in each group. (****p<0.0001).

Figure 2

The deletion of CSF1R does not affect microglia survival and delay cognitive decline in APP cKO mice. A-B representative image of microglia (Iba1 green) in APPSwe/PS1(6-8-month-old) and APP cKO (6-8-month-old). Scale bar = 500µm and 20µm. C unbiased stereological count of microglia in 6-8-month-old

APP^{Swe}/PS1 and APP cKO. APP 6m, n=6, APP 8m n=4, n=6 APP cKO 6m and n=4 APP cKO 8m. D NOR with 3-time points comparison of WT, APP^{Swe}/PS1 and APP cko from 3 to 8-month-old. (###, ## Discrimination differs from 0.5, APP^{Swe}/PS1 6-month-old ***p=0.0002, 8-month-old ***p=0.0007). n=10 for all groups. E average time of observation during NOR acquisition phase. F nesting with 3-time points comparison of WT, APP^{Swe}/PS1 and APP cKO from 3 to 8-month-old. Score range from 1 (untouched square) to 5 (perfect nest) (APP^{Swe}/PS18-month-old ***p=0.0009). n=10 in all groups.

Figure 3

Long-term Knock-out reduces volume and plaque number, accompanied by cerebral amyloid angiopathy (CAA) onset. A hemisphere stitches microglia (Iba1 green) amyloid plaque (6E10 red) in 6 and 8-month-old APPSwe/PS1 and APP cKO Scale bar = 500µm. B-E Unbiased stereological analysis of plaques in hippocampus and cortex. Analysis has shown a reduction in plaque volume at 6 (hippocampus *p=0.0354, cortex *p=0.0479) and at 8-month-old (hippocampus *p=0.0320, cortex *p=0.0227) in APP cKO. A reduction in number of plaque/mm³ in cortex (*p=0.0270). APPSwe/PS1 6m, n=6, n=6 for APP cKO 6m. F Elisa Aβ₄₀ APP cKO 6 and 8-month-old and their controls. APPSwe/PS1 (6-8m) n=6 and APP cKO (6-8m) n=7 (**p=0.0098). G Western blot analysis for ABCB1 between APP cKO (6-8m) and their control. APPSwe/PS1 (6-8m) n=6, n=7 APP cKO (6-8m) (*p=0.023). H Representative images of amyloid (6E10 red) on blood vessels (CD31 green) in APPSwe/PS1 and APP cKO at 6 and 8-month-old Scale bar = 50µm.

Figure 4

TREM2, β-Catenin and IL-34 can compensate CSF1R deletion A western blot analysis of TREM2/β-Catenin pathway in WT and cKO 3-month-old. WT, n=6, n=7 for cKO. (TREM2, *p=0.0384, β-Catenin **p=0.0360, APC, **p=0.0023, Syndecan-1, *p=0.0498, IL-34, *p=0.0274 B western blot analysis on 6-month-old APP cKO and their littermate controls. APPSwe/PS1 and APP cKO 6-month-old, n=6 (TREM2, *p=0.0356, β-Catenin **p=0.0027, BDNF, **p=0.0071, Syndecan-1, *p=0.0498, IL-34, *p=0.0274, PSD-

95,*p=0.0120) C western blot analysis on 8-month-old APP cKO and their littermate controls. APPSwe/PS1 and APP cKO 8-month-old, n=6. (TREM2, *p=0.0470, β -Catenin, *p=0.0352, BDNF, *p=0.0494, Syndecan-1, *p=0.0378, Bace-1, **p=0,0010) D representative image of BACE-1 (green) and 6E10 (red) in APPSwe/PS1 and APP cKO 8-month-old Scale bar = 50 μ m.

Figure 5

The knock-out has no impact on microglia survival over the time A unbiased count of microglia in WT and cKO mice n=20 for each group. B representative image of microglia Iba+ (green) and knock-out cells (RedTm) for D2, D4, D10, D20 after tamoxifen injections. Scale bar = 100µm. n=4 per group for each time point C fictive representation of molecular expression in APP cKO, APPSwe/PS1, cKO and WT over the time.