Conditional knockout of AIM2 in microglia ameliorates synaptic plasticity and spatial memory deficits in a mouse model of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disease, and the underlying mechanisms remain unclear. Synaptic dysfunction is a hallmark pathology of AD and is strongly associated with cognitive impairment in AD. Abnormal phagocytosis by microglia is one of the main causes of synapse loss in AD. Existing studies have revealed that inflammasomes contribute to cognitive deficits in AD. Previous studies have shown that the absent in melanoma 2 (AIM2) inflammasome was upregulated in the hippocampus of APP/PS1 mice. In this study, we identified abnormally increased expression of AIM2 in microglia in an Aβ1-42-induced AD mouse model (AD mice). Conditional knockout of microglial AIM2 rescued cognitive impairment and synaptic dysfunction in AD mice. Excessive microglial phagocytosis of synapses was decreased after knockout of microglial AIM2, which was dependent on inhibiting complement activation. These results suggest that microglial AIM2 plays a critical role in regulating synaptic plasticity and memory deficits associated with AD, providing a new direction for developing novel preventative and therapeutic interventions for this disease.

Background

Alzheimer's disease (AD) is the most common neurodegenerative disorder, characterized by progressive cognitive decline and memory loss, which are often accompanied by emotional symptoms, and is the leading cause of dementia in elderly individuals worldwide (World Alzheimer Report 2022). Extracellular amyloid-β (Aβ) deposition and intracellular neurofibrillary tau tangles are the neuropathological hallmarks of AD(1). Accumulating evidence suggests that Aβ accumulates in toxic forms and damages neural connections, resulting in synaptic dysfunction and the loss of synapses, which are the key pathological correlates of cognitive decline in AD(2). Several studies used positron emission tomography (PET) and showed reductions in synaptic vesicle glycoprotein 2A in the hippocampus of APP/PS1 mice and early AD patients, suggesting reduced synaptic density(3–5). Moreover, neural network activity and consequent cognitive function are disrupted over time, which is attributed to Aβ-mediated synaptic dysfunction in AD(6). Thus, understanding the underlying mechanisms of synaptic impairment in AD will provide novel insights into therapeutic interventions for AD.

Multiple lines of evidence indicate a central role of microglia and associated synaptic abnormalities in AD(7). Microglia are the resident immune cells in the central nervous system (CNS) and are responsible for the maintenance of CNS homeostasis. Under physiological conditions, microglia are involved in tissue repair, phagocytic removal of apoptotic cells and debris and synaptic remodeling(8). Microglia play a distinct role in synaptic remodeling by devouring eliminated synapses and remodeling the extracellular matrix (ECM) in the normally developing brain(9, 10). Recently, evidence has indicated that microglia-mediated synaptic pruning is involved in synapse loss in AD and correlates with cognitive deficits (11–13). Studies have shown that the complement system and synaptic pruning by microglia can be excessively activated, resulting in synapse loss in AD mouse models (13). Treatment with the metabotropic glutamate receptor 5 (mGluR5) silent allosteric modulator (SAM) BMS-984923 reversed these changes(11). In addition, neuronal CD47 is believed to protect synapses from excessive pruning by
producing a “do not eat me” signal through interactions with its receptor signal regulatory protein α (SIRPα), and specific deletion of microglial SIRPα leads to an increase in synaptic loss mediated by microglia phagocytosis and subsequent cognitive impairment in AD mice(12, 14).

Several studies have confirmed that microglial absent in melanoma 2 (AIM2) participates in the activation of microglia(15, 16). AIM2, a PYHIN (pyrin and HIN domain-containing protein) family member, is an essential component of the inflammasome and plays a central role in host immune responses to infections or sterile injuries. AIM2 is activated following the direct recognition of double-stranded DNA (dsDNA) and interacts with ASC to induce the activation of caspase-1 and the secretion of bioactive interleukin 1β (IL-1β) and interleukin 18 (IL-18)(17). AIM2 has been reported to be involved in a variety of diseases of the central nervous system, including stroke, vascular dementia and neurodegenerative disorders(15, 18-22). Our previous studies indicated that AIM2 expression was elevated in the hippocampus of APP/PS1 mice, and complete deletion of AIM2 improved synaptic plasticity and spatial memory in mice, but the detailed mechanism is still unclear(21).

Therefore, in the present study, we investigated the specific role of AIM2 in regulating microglial function in AD-associated learning and memory impairment and explored the underlying mechanisms. Our findings suggest that AIM2 modulates microglial phagocytosis of synapses, which is accompanied by the activation of the complement system via the classical pathway, thus leading to cognitive impairment in Aβ1−42-induced AD mice. These results reveal the precise molecular effect of AIM2 in the regulation of neural synaptic function and provides a direction for potential therapeutic targets of AD.

Materials And Methods

Mouse models and treatment

C57BL/6 mice (8 weeks old), Cx3cr1Cre mice and AIM2fl/fl were all male and provided from the Model Animal Research Center of Nanjing University. AIM2fl/fl mice were crossed with the Cx3cr1Cre transgenic mice to generate AIM2-cKO mice. The construction of AIM2 overexpression lentivirus as well as control lentivirus was completed by GeneChem Corporation (Shanghai, China). For virus injection into the hippocampal region, mice were anaesthetized and placed in a stereotaxic frame. The coordinates were 1.82 mm posterior to the bregma, 1.13 mm lateral to the midline and 1.25 mm below the surface of the skull. Human Aβ1−42 (Millipore, Darmstadt, Germany) was prepared as previously described and 4 µg Aβ1−42 was injected into the hippocampal region of AIM2-cKO mice and AIM2fl/fl mice using stereotaxic apparatus. Behavioral experiments and electrophysiological recordings were performed 2 weeks after injection. All experiments related to animals were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing University.

Behavioural Experiments
Open field

The open-field test for the assessment of mobility and anxiety was performed as previously described. Each individual mouse from the different groups was placed in a 48 cm × 48 cm ×36 cm open field box that was divided into 16 squares of equal area and recorded for 10 min. The open field area was cleaned with 75% ethanol to minimize olfactory cues. Locomotor activity measurements and time spent in the center and corner zone were quantified by ANY-maze software (Stoelting, USA).

New Object Recognition (Nor)

Novel object recognition (NOR) test was conducted to measure the recognition memory of mice in a nontransparent box measuring 30 × 30×45 cm high. Prior to testing, mice were habituated to the behavioral testing environment for 3 consecutive days (10 min per day). The mice were placed in the box containing two identical objects (A and B) placed symmetrically during the 10-min training session. During the test session, one of the two identical objects (B) was replaced with a novel object (C) and the mice were allowed to freely explore the objects for 5 min. The time spent in exploring the novel object was analyzed and the discrimination index was calculated as time spent in exploring the novel object / total time spent in exploring objects during the test phase.

Morris Water Maze

The Morris water maze test was performed to evaluate spatial learning and memory of the mice as previously described. Briefly, the mice were trained to find the hidden platform submerged 1cm below the surface for 5 consecutive days. The latency in the training stage was recorded and analyzed using ANY-maze software. For probe trials, the mice were allowed to swim for 60 s freely with the platform removed. Then the swimming speed, platform crossings, the escape latency and time spent in the target quadrant were recorded.

Quantitative Real-time Pcr

The Total RNA from treated cells and tissue was extracted using Trizol reagent kit (Invitrogen, USA) according to the standard protocol. The cDNA was synthesized from total RNA by PrimeScript RT Reagent kit (Takara). Quantitative PCR analysis was performed using an ABI 7500 PCR instrument (Applied Biosystems) with the SYBR Green PCR kit (Takara). The relative expression levels of each gene shown were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers used were as follows:
<table>
<thead>
<tr>
<th>Gene</th>
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| GAPDH | F: GCCAAGGCTGTGGGCAAGGT  
R: TCTCCAGGCGGCACGTAGA |
| AIM2 | F: CTCAGGAAGGAAGACAAGA  
R: GATTCAACATCAAACCACAAC |
| C1Q | F: CACCGTGCTTCAGCTGCGACGAG  
R: TTGCGGGGTCCTTTTGATCCAC |
| C3 | F: ACTGTGGACAAACACCTACTGC  
R: GCATGTTCTGAAAAGGCTCGG |

**Western Blotting**

Western blotting

Brain tissues were lysed with RIPA buffer plus protease inhibitor. The protein concentration was measured using the BCA Assay (Thermo Fisher Scientific). Equal amounts of protein samples were subjected to 10% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane (EMD Millipore). Membranes were blocked for 2 h at room temperature using 5% non-fat milk in Tris-Buffered Saline Tween 20 (TBST), subsequently incubated overnight at 4°C with the following primary antibodies: mouse anti-MAP 2 (1:1000, Abcam, ab11267), rabbit anti-MAP 2 (1:1000, Bioworld, BS3487), rabbit anti-PSD 95 (1:1000, Abcam, ab18258), mouse anti-PSD 95 (1:1000, Abcam, ab2723), rabbit anti-β-actin (1:1000, Bioworld, AP-0060), and rabbit anti-AIM2 (1:500, Abcam, ab119791). The membranes were then incubated for 2 h at room temperature with HRP-conjugated secondary antibodies (1:5000). Bands of western blotting were visualized in a Gel-Pro system (Tanon Technologies, Shanghai, China), and protein density was analyzed and quantified using ImageJ software.

**Immunofluorescence Staining**

For sectioning, the tissues were embedded in OCT and sectioned coronally at 20µm thickness. Brain sections were permeabilized using PBS containing 0.25% triton X-100 (PBST) and blocked with 2% BSA at room temperature for 2 h. Subsequently, the brain sections were incubated with primary antibodies as follows at 4°C overnight: rabbit anti IBA-1 (1:500, Abcam, ab178846); mouse anti-AIM2 (1:200, Santa Cruz Biotechnology, sc-515514); rat anti-CD68 (1:500, Abcam, ab53444); mouse anti-PSD 95 (1:1000, Abcam, ab2723); chicken anti-MAP 2 (1:1000, Abcam, ab5392); rat anti-C1q (1:500, Abcam, ab11861); rat anti-C3 (1:500, Abcam, ab11862). The sections were then incubated with secondary antibodies (Invitrogen, 1:500) at room temperature for 2 h and counterstained with DAPI (1:1000, Bioworld, Louis Park, MN, USA) for 20 min. The images were captured using a fluorescence microscope (Olympus IX73) or confocal laser-
scanning microscope (Olympus FV3000) and analyzed with Image J software. Three-dimensional (3D) reconstruction was obtained using the Imaris software (Bitplane).

**Electrophysiology**

Acute hippocampal slices (300 µm) were prepared as described previously. Before recordings, the slices were incubated in circulating artificial cerebrospinal fluid (ACSF) gassed with 95% O$_2$ and 5% CO$_2$ at room temperature for at least 2 h. Slices were then transferred into the microelectrode array continuously perfused with oxygenated ACSF (32°C) at a flow rate of 2 ml/min. Field excitatory post-synaptic potentials (fEPSPs) from the stratum radiatum of CA1 were recorded using MEA-2100-60-System (Multi Channel Systems, Reutlingen, Germany). To evaluate the input-output relationships, the slope of fEPSPs was recorded. For LTP experiments, half of the maximum evoked response was utilized as the stimulation intensity. After 30 min of stable baseline fEPSPs, the LTP was induced with high-frequency stimulation (100 Hz, three trains, 1-s duration, 10-s interval). We measured the initial slopes of fEPSP and normalized them to the average fEPSP slope during baseline period. Data acquisition was done on the LTP-Director software and data analysis with the LTP-Analyzer software.

**Golgi Staining And Sholl Analysis**

Golgi staining was performed with a FD Rapid Golgi stain kit (FD Neurotechnologies, Columbia, USA). The brains were immersed in a 1:1 mixture of solutions A and B at room temperature in the dark for 14 days and then transferred into solution C for at least 3 days. Afterwards, coronal brain slices (100 µm) were sectioned with cryostat microtome (Leica, Wetzlar, Germany) and stained according to manufacturer's protocol. Images were acquired using Olympus IX73 and analyzed with ImageJ software (Fiji, NIH).

**Statistical analysis**

All statistical analysis results were presented as means ± SEM, and the analysis was performed with SPSS 17.0 software (SPSS, Chicago, IL, USA). An unpaired Student’s t test was employed to compare the two datasets, while one-way or two-way analysis of variance (ANOVA) with the Bonferroni post hoc test was used for comparisons between more than two groups. The statistical significance was assumed when the P-value was < 0.05.

**Results**

**Increased AIM2 expression in the microglia of Aβ$_{1–42}$-induced AD mice**
Our previous study demonstrated that AIM2 expression was upregulated in the hippocampus in 6-month-old APP/PS1 mice compared to wild-type littermates (21). In this study, we used an Aβ₁-₄₂-induced AD mouse model (AD mice). Consistent with our prior study, the qPCR (Fig. 1a) and WB (Fig. 1b-c) data showed that the level of AIM2 was significantly increased in the hippocampus of AD mice compared with the control group. In addition, the immunofluorescence results revealed elevated AIM2 staining predominantly in microglia (Fig. 1d). These results indicated that the expression of AIM2 in microglia was significantly increased in AD mice.

Aim2 Deficiency In Microglia Rescued Cognitive Impairment In The Aβ-induced Ad Model

To investigate the role of AIM2 in microglia in AD, we constructed microglial AIM2 conditional knockout mice (AIM2-cKO mice) (Fig. 2a and Additional file 1: Fig. S1). We injected Aβ₁-₄₂ into AIM2-cKO mice to induce the AD model (AIM2-cKO-AD mice) and then performed open field, Y maze, new object recognition (NOR) and Morris water maze (MWM) tests to explore the cognitive function of AIM2-cKO-AD mice. In the open field test, the movement speed and time spent in the corner or center were not significantly different, which suggested similar motor abilities and no anxiety-related behaviors in each group of mice (Additional file 1: Fig. S2). During the recognition phase of the NOR test, AIM2-cKO-AD mice spent significantly more time exploring the novel object than AD mice (Fig. 2b and 2c). The escape latency of the MWM test in the training sessions was increased in AD mice compared with control mice, whereas it was significantly reduced after microglia-specific AIM2 deletion (Fig. 2d). No differences were observed in each group regarding swimming speed or time in the target quadrant, while AIM2-cKO-AD mice exhibited increased numbers of platform crossings and decreased latency to reach the hidden platform and the target quadrant during the probe trial test compared with AD mice (Fig. 2e-2j). The above results suggested that AIM2 deficiency in microglia attenuated the impairment in learning and memory in AD mice.

Aim2 Deficiency In Microglia Ameliorated Synaptic Dysfunction

Synaptic plasticity is considered the basis of learning and memory functions, and we next examined the impact of microglia-specific knockout of AIM2 on synaptic structure and function in the hippocampus. The WB results showed that the protein levels of PSD95 and MAP2 in AD mice were decreased compared with those in control mice, and this effect was significantly reversed in AIM2-cKO-AD mice (Fig. 3a-c). Furthermore, we performed Golgi staining and found that neuronal complexity (Fig. 3d-f) and dendritic spine density (Fig. 3g and 3h) in hippocampal CA1 neurons were rescued in AIM2-cKO-AD mice, as shown by Sholl analysis. Furthermore, we performed electrophysiological recording and analysis of the hippocampal CA1 region in each group of mice. With increasing stimulus intensity, the slope of fEPSPs was increased more in AIM2-cKO-AD mice than in AD mice (Fig. 3i). In addition, microglia-specific AIM2 deletion led to a significant increase in LTP magnitudes induced by high-frequency stimulation (Fig. 3j
and k). These results suggested that knockout of microglial AIM2 could ameliorate changes in synaptic structure and function, thereby improving memory impairment in an Aβ1−42-induced AD model.

**Aim2 Deficiency In Microglia Modulated Microglial Phagocytosis And Synaptic Elimination By Microglia**

It has been shown that microglia prune synapses by phagocytosis, thus affecting synaptic plasticity. To further evaluate the effect of conditional knockout of AIM2 on microglial function, we performed immunofluorescence staining. The results suggested an increase in Iba1+ microglia colocalized with CD68 cells in AD mice compared to control mice, and this effect was reversed in AIM2-cKO-AD mice (Fig. 4a). Furthermore, immunofluorescence and 3D reconstruction analysis revealed an increase in PSD95 immunosignals colocalized with Iba1 + microglia in AIM2+/− mice after Aβ1−42 treatment, while knockout of microglial AIM2 in the Aβ1−42-induced AD model reduced PSD95+ puncta in Iba1 + microglia (Fig. 4b). These results confirmed that microglia-specific knockout of AIM2 could inhibit the excessive engulfment of synapses through microglial phagocytosis.

**Aim2 Modulated Microglial Phagocytosis Of Synapse Elimination Via Complement Activation**

The classical complement pathway may be important for microglia-mediated synaptic pruning. To investigate the mechanism by which AIM2 regulates microglial phagocytosis in AD, we examined the distribution and relative expression levels of the complement factors C1q and C3 in the hippocampus. Our results revealed a significant increase in C1q levels (Fig. 5a) and the colocalization of C1q with IBA-1 + microglia in AD mice compared to control mice, and deletion of microglial AIM2 reversed these effects (Fig. 5b). Similarly, the increase in C3 expression (Fig. 5c) was accompanied by increased C3 deposition on synapses in AD mice, and this deposition was significantly reduced in AIM2-cKO-AD mice (Fig. 5d). Overall, these results indicated that the complement pathway was necessary for synaptic pruning by microglia.

**Discussion**

Our study has uncovered a novel role of AIM2 in the pathogenesis of AD. Here, we revealed that AIM2 expression was significantly increased in the microglia of AD mice. Conditional deletion of AIM2 in microglia ameliorated cognitive impairment and synaptic deficits in an Aβ1−42-induced AD model. Moreover, microglial activation and microglial phagocytic activity toward synapses were suppressed after knockout of AIM2, which was accompanied by inhibition of the complement system via the classical pathway. Thus, our results demonstrated that AIM2 plays an important role in AD pathogenesis and may be a novel therapeutic target for AD.
Previous studies have demonstrated that AIM2 has multiple roles in the pathogenesis of several diseases, including ischemic stroke and vascular dementia. AIM2 expression is upregulated after ischemic stimuli in a mouse middle cerebral artery occlusion (MCAO) model, while AIM2 deletion reduces cerebral infarct volume and improves motor and cognitive function (18, 20). In addition, studies have shown that AIM2 inflammasome activation can promote neuronal loss and white matter injury during cerebral hypoperfusion, leading to cognitive impairment in a mouse model of vascular dementia (VaD) (19). Our previous study showed the upregulation of AIM2 expression in the hippocampus in APP/PS1 mice, and AIM2 deletion could markedly ameliorate changes in synaptic plasticity and memory function, but the specific mechanism remains unclear (21). In this study, we first demonstrated that AIM2 mediated memory deficits in AD model mice by regulating microglial phagocytosis of neuronal synapses. We injected Aβ_1−42 into the bilateral hippocampus of C57BL/6 mice to induce AD and observed that the increase in AIM2 expression occurred predominantly in microglia. Furthermore, the behavioral results revealed that Aβ_1−42 treatment impaired synaptic function and induced memory deficits, while knockout of AIM2 in microglia rescued synaptic plasticity dysfunction and cognitive dysfunction.

As resident immune cells in the brain, microglia play a complex and key role in AD pathogenesis. On the one hand, microglial activation might be beneficial for reducing Aβ aggregation in AD. On the other hand, excessive activation of microglia is responsible for the overexpression of inflammatory factors, contributing to neuronal toxicity and synaptic dysfunction (23, 24). Microglial activation-mediated neuroinflammation has been considered to play an important role in the pathogenesis of AD. AIM2 controls microglial inflammation (25). In addition, AIM2 knockout could reduce microglial activation in different models of neurological disease, including ischemic stroke, vascular dementia, and AD (19, 26). It has been shown that complete deletion of AIM2 mitigates microglial activation but has no beneficial effects on spatial memory in 5xFAD mice (16). However, there has been no direct evidence on the role of AIM2 in regulating microglial phagocytic function. Our data revealed that conditional knockout of AIM2 in microglia could effectively suppress microglial phagocytic capacity and the phagocytosis of synapses in AD mice.

Complement cascades are involved in microglial phagocytosis and selective synaptic pruning. C1q, which is the initiating protein in the classical complement pathway, is localized at synapses, resulting in the deposition of the downstream molecule C3. Deposited C3 can activate C3 receptors, which are only expressed by activated microglia, thereby contributing to synapse phagocytosis (27, 28). It has been shown that the increased expression of C1q in AD mouse models is associated with impaired synapses and hippocampal LTP (13). Moreover, inhibiting C1q or C3 reduces the phagocytic ability of microglia and microglial synapse removal, rescuing synapse loss (29, 30). In our study, we showed that the expression of C1q and C3 was increased in the hippocampus, and these factors were deposited on synapses after Aβ_1−42 treatment, which could be reversed by microglia-specific AIM2 deletion. However, the mechanisms that regulate the complement system via AIM2 remain to be clarified.

**Limitation**
Several limitations of our mouse model should be considered. The mouse model of AD induced by the Aβ$_{1-42}$ peptide is unable to recapitulate the complex pathology of human AD. Moreover, additional critical risk factors, including neurofibrillary tangles of tau, aging, and perturbations in the vasculature, are disregarded (31). Further work is required to translate our findings into a clinical setting.

**Conclusion**

In summary, this study demonstrates that an increase in AIM2 expression mediates aberrant synaptic elimination via microglial activation and phagocytosis, and the complement system is activated through the classical pathway, contributing to impaired hippocampal synaptic plasticity and cognitive dysfunction in Aβ$_{1-42}$-induced AD mice. These results demonstrate a key role for AIM2 in regulating microglial function in the pathogenesis of AD and suggests a potential target for the treatment of AD.

**Abbreviations**

AD: Alzheimer's disease; Aβ: amyloid-β; PET: positron emission tomography; CNS: central nervous system; ECM: extracellular matrix; mGluR5: metabotropic glutamate receptor 5; SAM: silent allosteric modulator; SIRPα: signal regulatory protein α; AIM2: absent in melanoma 2; dsDNA: double-stranded DNA; IL-1β: interleukin 1β; IL-18: interleukin 18; NOR: Novel object recognition; MCAO: mouse middle cerebral artery occlusion; VaD: vascular dementia.

**Declarations**

**Ethics approval and consent to participate**

All experiments related to animals were approved by the Animal Care Committee of Nanjing University.

**Consent for publication**

All authors have consented for publication.

**Availability of data and materials**

The data support the findings of the current study are available from the corresponding author upon reasonable request.

**Competing interests**

The Authors declare that they have no competing interests.

**Funding information**

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Authors’ contributions

Shu shu, Feng Bai, Lei Ye and Mengsha Hu initiated, designed the study and wrote the manuscript. Lei Ye and Mengsha Hu conducted behavioral experiments, electrophysiological experiments, and data analysis. Lei Ye, Rui Mao, Yi Tan, Min Sun, Junqiu Jia and Siyi Xu performed molecular biology experiments. Yi Liu, Xiaolei Zhu, and Yun Xu performed the sample collection. All authors approved the manuscript prior to submission.

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References


Figures
Figure 1

The increase in AIM2 expression occurred in the microglia of Aβ1-42-induced AD mice

(a) The level of AIM2 was measured by qPCR and normalized to GAPDH mRNA. n = 6-7 for each group. \( t \) (11) = 3.507, \( p = 0.0049 \). (b and c) The expression level of AIM2 was verified by Western blotting, and the corresponding quantified results were obtained with β-actin as a loading control. n = 7 for each group.
(12) = 3.856, p = 0.0023. (d) Representative confocal images showing the colocalization of IBA1 (red) and AIM2 (green) immunofluorescence in the hippocampus of Aβ1-42-induced AD mice and control mice. The data are shown as the mean ± SEM. Unpaired two-tailed t test for a and c. **p < 0.01.

**Figure 2**
AIM2 deficiency in microglia rescued cognitive impairment in the Aβ1-42-induced AD model
(a) Schematic diagram for generating the microglial AIM2 conditional knockout mice. (b and c) The percentage of time spent exploring the same object (b) and a novel object (c) was measured in the NOR tests. n = 10-15 for each group. F(2,33) = 0.1612, AD vs. control group: p = 0.0022, AIM2-cKO-AD vs. AD group: p = 0.0053. (d) The escape latency during the training period in MWM tests was detected. n = 10-15 for each group. F (2, 33) = 4.032, AD vs. control group: p = 0.0268, AIM2-cKO-AD vs. AD group: p = 0.0459. (e-i) The swimming speed (e), latency to reach the platform (f), number of platform crossings (g), time spent in the target quadrant (h) and latency to reach the target quadrant (i) were evaluated during the probe trial in MWM tests. n = 10-15 for each group. F(2,33) = 0.6774, AD vs. Control group: p = 0.9354, AIM2-cKO-AD vs. AD group: p = 0.2441 for swimming speed; F(2,33) = 2.329, AD vs. Control group: p = 0.0006, AIM2-cKO-AD vs. AD group: p = 0.0041 for latency to platform; F(2,33) = 2.172, AD vs. Control group: p = 0.0023, AIM2-cKO-AD vs. AD group: p = 0.0142 for the number of platform crossings; F(2,33) = 0.2824, AD vs. Control group: p = 0.1053, AIM2-cKO-AD vs. AD group: p = 0.3232 for time in target quadrant; F(2,33) = 5.877, AD vs. Control group: p < 0.0001, AIM2-cKO-AD vs. AD group: p = 0.0004 for latency to target quadrant. (j) Representative locomotor traces in the MWM tests. The data are shown as the mean ± SEM. One-way ANOVA followed by Dunnett’s post hoc test for c, e, f, g, h and i. Two-way ANOVA followed by Bonferroni’s post hoc test for d. *p < 0.05, **p < 0.01, ***p < 0.001; ns no significance.
Figure 3

Knockout of microglial AIM2 ameliorated synaptic dysfunction

(a) The protein expression of PSD95 and MAP2 was determined by Western blotting. (b and c) Quantitative analysis of PSD95 (b) and MAP2 (c) protein levels normalized to β-actin. n = 6 for each group. F(2, 15) = 1.624, AD vs. control group: p = 0.0006, AIM2-cKO-AD vs. AD group: p = 0.0015 for
PSD95; F(2, 15) = 5.693, AD vs. control group: p = 0.0043, AIM2-cKO-AD vs. AD group: p = 0.0050 for MAP2. (d) Representative Golgi staining showing an overview of hippocampal CA1 neurons. (e) Representative traces of CA1 pyramidal neurons in control, AD and AIM2-cKO-AD mice. (f) The number of Golgi-stained dendritic intersections was counted in control (n = 8 neurons, 3 mice), AD (n = 6 neurons, 3 mice) and AIM2-cKO-AD (n = 8 neurons, 3 mice) mice. F(2, 19) = 10.04, AD vs. control group: p < 0.0001, AIM2-cKO-AD vs. AD group: p < 0.0001. (g) Representative images of Golgi-stained apical and basal dendritic spines of CA1 pyramidal neurons in control, AD and AIM2-cKO-AD mice. (h) Quantification of dendritic spine density in CA1 pyramidal neurons from control (n = 8 spines, 3 mice), AD (n = 9 spines, 3 mice) and AIM2-cKO-AD mice (n = 9 spines, 3 mice). F(2, 23) = 8.590, AD vs. control group: p < 0.0001, AIM2-cKO-AD vs. AD group: p < 0.0001 for apical spines; F(2, 23) = 7.726, AD vs. control group: p < 0.0001, AIM2-cKO-AD vs. AD group: p < 0.0001 for basal spines. (i) The fEPSP amplitude of hippocampal slices in control (n = 9 slices, 4 mice), AD (n = 9 slices, 3 mice) and AIM2-cKO-AD mice (n = 9 slices, 4 mice). F(2, 24) = 10.30, AD vs. control group: p < 0.0001, AIM2-cKO-AD vs. AD group: p < 0.0001. (j and k) LTP induced by high-frequency stimulation in control (n = 10 slices, 4 mice), AD (n = 8 slices, 3 mice) and AIM2-cKO-AD mice (n = 11 slices, 4 mice) was evaluated in hippocampal CA1. F(2, 26) = 1.427, AD vs. control group: p = 0.0010, AIM2-cKO-AD vs. AD group: p = 0.0095. Data are shown as the mean ± SEM. One-way ANOVA followed by Dunnett’s post hoc test for b, c, h and k. Two-way ANOVA followed by Bonferroni’s post hoc test for f and i. *p < 0.05, **p < 0.01, ***p < 0.001; ns no significance.
Figure 4

Microglia-specific AIM2 deletion modulated microglial phagocytosis and synaptic elimination by microglia

(a) Immunostaining for IBA-1 (red) and CD68 (green) in the hippocampal region in control, AD and AIM2-cKO-AD mice. (b) Confocal images showing the presence of PSD-95+ (green) puncta around IBA-1+ (red)
microglia and the corresponding 3D reconstructions.

Figure 5

AIM2 modulated microglial phagocytosis of synapse elimination via complement activation
(a) The mRNA level of C1q in the hippocampal region in control, AD and AIM2-cKO-AD mice was determined by quantitative RT–PCR. n = 8-9 for each group. F (2, 22) = 2.134, AD vs. control group: p < 0.0001, AIM2-cKO-AD vs. AD group: p = 0.0004. (b) The colocalization of C1q (green) with IBA-1 (red) in the hippocampal CA1 region in control, AD and AIM2-cKO-AD mice. (c) The mRNA level of C3 in the hippocampal region in control, AD and AIM2-cKO-AD mice was determined by quantitative RT–PCR. n = 8-9 for each group. F (2, 22) = 30.36, AD vs. control group: p < 0.0001, AIM2-cKO-AD vs. AD group: p = 0.0003. (d) The colocalization of C3 (green) with PSD-95 (red) in the hippocampal CA1 region in control, AD and AIM2-cKO-AD mice. The data are shown as the mean ± SEM. One-way ANOVA followed by Dunnett’s post hoc test for a and c. ***p < 0.001.

Supplementary Files

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