Journal of Neuroimmune Pharmacology (2019) 14:519–529
https://doi.org/10.1007/s11481-019-09861-2

ORIGINAL ARTICLE

Overexpression of TIPE2, a Negative Regulator of Innate and Adaptive Immunity, Attenuates Cognitive Deficits in APP/PS1 Mice

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Received: 14 December 2018 / Accepted: 30 June 2019 / Published online: 8 July 2019

Abstract

Neuroinflammation plays an early and prominent role in the pathology of Alzheimer's disease (AD). Tumor necrosis factor-α-induced protein 8-like 2 (TIPE2) has been identified as a negative regulator of innate and adaptive immunity. However, whether TIPE2 affects cognitive functions in AD-like mouse models remains unknown. In this study, we compared the gene and protein expressions of TIPE2 between the APP/PS1 mice and the age-matched wild type (WT) mice at different stages of development using western blot and RT-qPCR. The hippocampal expression of the TIPE2 mRNA and protein in APP/PS1 mice was higher than that of the WT mice starting from 6 months to 10 months. However, the difference of the TIPE2 expression between the APP/PS1 mice and the WT mice declined in a time-dependent manner. The spatial learning and memory deficit from the 8-month-old APP/PS1 mice was observed in the Y-maze test and fear conditioning task. Interestingly, overexpression of TIPE2 by intra-hippocampal injection of AAV-TIPE2 into APP/PS1 mice resulted in an improvement of learning and memory and reduced expression of inflammatory cytokines, such as TNF-α, IL-6 and IL-1β, and increased expression of anti-inflammatory cytokines, such as IL-10 and Arg-1. Taken together, our findings show that the TIPE2 expression level was negatively correlated with the pathogenesis of Alzheimer's disease, and overexpression of TIPE2 attenuates cognitive deficits in APP/PS1 mice, suggesting TIPE2 is a potential target for pharmacological intervention and improvement of cognitive deficits.

Keywords Alzheimer's disease · TIPE2 · APP/PS1 transgenic mice · Y-maze · Hippocampus

Introduction

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disease of the brain that impairs cognitive and memory functions, and causes psychiatric symptoms and behavioral issues (Pozueta et al. 2013; Ossenkoppele et al. 2015). The cellular pathology of AD is characterized by two major hallmarks of extracellular β-amyloid (Aβ) deposits and neurofibrillary tangles (NFTs) in the brain (Masters et al. 2015), where an innate immune response is initiated, suggesting the involvement of immunological mechanisms in the neurodegeneration of AD (Spangenberg and Green 2017). The fundamental role of neuroinflammation in the pathogenesis of AD is supported by findings that activated microglia are able to bind Aβ fibrils via immune receptors such as CD33, CD14 and Toll-like receptors (Bradshaw et al. 2013; Griciuc et al. 2013; Guerreiro et al. 2013). Therefore, controlling the interactions between the immune system and the nervous system may be an effective route to prevent or attenuate the pathogenic process of AD (Heneka et al. 2015).

Tumor necrosis factor-α-induced protein 8-like 2 (TIPE2) is a negative regulator of innate and adaptive immunity, and plays an essential role in the maintenance of immune homeostasis by negatively regulating T cell receptor and Toll-like receptor (TLR) signaling (Sun et al. 2008). It has been shown that TIPE2 is a cytoplasmic protein expressed preferentially in lymphoid tissues; TIPE2 is also detectable in endocrine tissues, skeletal

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muscle (Zhang et al. 2010), hippocampus, cerebral cortex, and especially microglia (Zhang et al. 2012). TIPE2 deficiency in mice causes fetal inflammatory diseases and high levels of inflammatory cytokines such as IL-1, IL-6, IL-12 and TNF-α (Sun et al. 2008). TIPE2 is markedly up-regulated in post-ischemic brains and exhibits a protective effect on cerebral ischemia-reperfusion injury and stroke (Zhang et al. 2012, 2015). Because TIPE2 is highly expressed in the hippocampus and cerebral cortex of the brain, which are critical for learning and memory, we hypothesize that the expression level of TIPE2 in the brain may play a important role in neurodegenerative and neuroinflammative diseases, such as AD.

To test this hypothesis, we firstly compared the expression of TIPE2 in APP/PS1 mice and age-matched wild type (WT) mice at different stages of development. Secondly, overexpression of TIPE2 was performed to investigate the effects of TIPE2 on the cognitive behaviors of the APP/PS1 mice and the expression levels of inflammatory and anti-inflammatory cytokines. Finally, we studied the effect of TIPE2 on microglia by using microglial marker Iba1.

Materials and Methods

Animals

APP/PS1 double transgenic mice and their age-matched wild-type (WT) C57BL/6 J mice were obtained from Model Animal Research Center of Nanjing University. All mice were genotyped and identified by PCR amplification of the genomic DNA and kept in a temperature-controlled environment (22–25 °C) with daylight between 8:00–22:00 and free access to food and water. All experiments were performed under the guidelines and regulations of Qingdao University Experimental Animal Care and Use Committee.

Behavioral Assessments

Y-Maze Test

The short-term spatial memory was tested by the Y-Maze Spontaneous Alternation assay in mice at different ages. Mice were placed into a radically symmetric Y-shaped maze with three arms separated by 120° from each other. After placed in the center of the maze, the mouse was allowed to freely explore the three arms. The number and sequence of arm entries were scored during an 8-min period. Alternations were calculated when a mouse consecutively traveled to the three arms without re-entering the previously visited arms. The percentage of alternation is the ratio of the number of alternations to the total number of arm entries minus two (Suryavanshi et al. 2014).

Fear Conditioning Task

The experiment consists of two phases: training and test. During the training phase, a mouse was placed into the shock chamber and allowed to freely explore the environment for 2 min before exposure to the conditioned stimulus (CS) with a 75-dB sound tone at 2.8 KHz for 30 s. In the last 2 s of the tone stimulus, the unconditioned stimulus (US) with 0.5 mA mild footshock for 2 s was delivered to the mouse. After 5 CS/US pairings with a one-min inter-trial interval, the mouse was allowed to return to its cage. The test phase was carried out 24 h after the training. The mice were placed into the shock chamber individually, and a tone identical to the CS was delivered for 3 min in the absence of electrical stimuli. During the training phase, the freezing behavior was measured as the percentage of time engaged in freezing responses during the initial 140 s of 5 tones without footshock. In the test phase, the freezing behavior was measured as the percentage of time engaged in freezing responses during the initial 180 s of tones without footshock (Dai et al. 2008; Zhang and Rosenkranz 2013).

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (RT- qPCR)

For RT-qPCR and western blot analyses, the animals were sacrificed by decapitation, and the brains were removed from the skull immediately. The hippocampus was dissected and mixed with Trizol at a ratio of 1 ml Trizol (Thermo Scientific, USA) per 100 mg tissue. The hippocampus was grinded by a High flux tissue grinder. Total RNAs were extracted according to the manufacturer's instructions. The RNA concentration was measured by a spectrophotometer. A total of 1 μg RNA was reversely transcribed to cDNA by using GoScript™ Reverse Transcriptase (Promega, USA) according to the manufacturer's protocol. Primer sequences are shown in Table 1. All RT-qPCRs were carried out in duplication.

Western Blot Analysis

The hippocampus was homogenized in 500 μl protein lysate (100:1 protein lysate and protease) by a High flux tissue grinder. After incubation at 4 °C for 1 h, the lysate was centrifuged at 15,000 rpm for 15 min at 4 °C, and the supernatant was collected. The protein concentration of each sample was determined using a BCA Protein Assay Reagent Kit (Thermo Scientific, USA). The protein sample (60 μg) was loaded and separated by a 12% SDS-acrylamide gel electrophoresis and transferred to polyvinylidene fluoride blots at the current of 150 mA for 1.5 h. The membrane was blocked by fresh blocking

Table 1 Primer sequences for RT-qPCR

Gene	Primer sequences
TIPE2	F-5'-TCAGAAACATCCAAGGCCAGAC-3′
	R-5'-CGGACCGACCAGCCATTTTAC-3′
TNF-α	F-5'-ACTCCAGGCGGTGCCTATAT-3′
	R-5'-GTGAGGGTCTGGGCCATAGAA-3′
IL-6	F-5'-CCACTTCACAAGTCGGAGGCTTA-3′
	R-5'-GCAAGTGCATCGTTGTTCATAC-3′
IL-1β	F-5'-TGGTGTGTGACGTTCCCATT-3′
	R-5'-TCGTTGCTTGGTTCTCCTTG-3′
IL-10	F-5'-CGACTGTTGCCTCTCGTACA-3′
	R-5'-AGGAGGTTCACAGCCCTTTT-3′
Arg-1	F-5'-CGCCTTTCTCAAAAGGACAG-3′
	R-5'-CCAGCTCTTCATTGGCTTTC-3′
Actin	F-5'-CATTGCTGACAGGATGCAGAAGG-3′
	R-5'-TGCTGGAAGGTGGACAGTGAGG-3′

buffer (tris-buffered saline, containing 5% nonfat dry milk and 0.1% Tween-20) at room temperature for 1 h. The primary antibodies for anti-TIPE2 (1:1000, Proteintech Group Inc., USA), anti-TNF-α (1;500, Abcam, UK), anti-IL-6 (1;1000, Abcam, UK) and anti-IL-1β (1;1000, Abcam, UK), anti-IL-10 (1:1000, Abcam, UK), anti-Arg-1 (1:1000, Cell Signalling Technology, USA) were incubated with the membrane overnight on a shaker at 4 °C. After three washes with Tris-buffered saline-Tween-20 (TBST), the membrane was incubated with proper secondary antibodies (1:5000 in a 5% nonfat dry milk) for 1 h at room temperature. The membrane was finally washed with TBST. The proteins were detected with a SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Scientific, USA) according to the manufacturer's instruction.

Immunofluorescence

The mice were given an overdose of 8% chloral hydrate 0.005 ml/g and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA) in 0.1 mol/L phosphate-buffer (PB). The brains were removed and postfixed in 4% PFA for 8 h before immersed in 20% and 30% sucrose solution until the brains sunk down to the bottom. Coronal sections at 16 μm were then cut on a cryostat microtome in optimal cutting temperature (OCT) compound, and the sections were stored at −20 °C. The sections were removed from −20 °C and allowed to dry at room temperature before washed with PBS three times and postfixed in PFA for 20 min. Then, the sections were permeabilized with 0.3% Triton X-100. After blocking with 1% BSA in 0.3% Triton X-100, the sections were incubated with the primary antibody for anti-TIPE2 (1:100, Proteintech Group Inc., USA) or anti-Iba1 (1:1000, Abcam, UK) overnight at 4 °C in a wet box. After three washes with PBS, the sections were incubated with the secondary antibody Alexa fluor 568 at room temperature for 1 h. After three washes with PBS, the sections were incubated with DAPI at room temperature for 30 min. The sections were observed and photographed under a confocal laser scanning microscopy.

Stereotaxic Injection of AAV into Bilateral Hippocampi in the Brain

7-month old mice were intraperitoneally injected of 8% chloral hydrate (0.005 ml/g) and placed in a stereotaxic apparatus. Bilateral hippocampus (before the cranial 2.3 mm; side, 1.8 mm; depth, 2.0 mm) were injected with adeno-associated virus overexpressing TIPE2 at the rate of 0.2 μl/min for 4 min. The same dose of the AAV without TIPE2 was administered to WT and APP/PS1 mice at the same age. After injection at 3.5 months, the expression of TIPE2 in the hippocampus was tested by immunofluorescence and western blot. Y-maze test was carried out to assess the animal's learning and memory activity.

Data Analyses

We used the immunostained sections of the dentate gyrus of hippocampus to quantitatively analyze the Iba1-labled microglia. The number of Iba1-labled microglia was identified by typical cellular morphology (40×,3 images/mouse, $n = 3$ mice/group). Cell body areas and the fluorescence intensity of microglia were measured by using the NIS-Elements D software. Fluorescence intensity on the images was corrected for background noise which was determined from sections stained with secondary antibody only and set as threshold. Cell body area and fluorescence intensity data were normalized using WT-AAV data which were set as "1".(Freire et al. 2018). The mRNA expression was measured with the comparative threshold cycle (Ct) technique. The Ct value of β-actin was substracted from the Ct value of the target gene to obtain the ΔCt. The normalized fold change in the target mRNA expression was shown as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct_{target\ gene} - \Delta Ct_{control\ gene}$. Densitometry data for western blot were analyzed using the Image J software.

Statistical parameters, significance, and the exact n values are reported in the figure legends. All data are expressed as the means ± SEM. Multi-group comparisons were analyzed using one-way ANOVA or two-way ANOVA. A value of $P < 0.05$ was considered statistically significant.

Results

Cognitive Deficits in the APP/PS1 Double Transgenic Mice

We evaluated the short-term spatial learning and memory of APP/PS1 mice using the Y-maze Alternation Test. Compared with the age-matched WT mice, the percentage of alternation in the APP/PS1 mice was progressively decreased in an age-dependent manner. The difference of the cognitive deficits between the APP/PS1 mice and the WT mice appeared from the age of 8-month old (Fig. 1a, $P<0.05$), and the decline remained up to 14 months. In the fear conditioning task, the APP/PS1 mice also showed a decline in the acquisition of auditory-cued fear memory in an age-dependent fashion, compared with the WT mice. The difference of the percentage of freezing between the APP/PS1 mice and the age-matched WT mice started to appear from 8-month old (Fig. 1b, $P<0.001$).

Age-Dependent Decline of TIPE2 Expressions in the Hippocampus of APP/PS1 Mice

The TIPE2 mRNA and protein expressions were detected in the hippocampus of APP/PS1 mice during the period of 5 to 12 months by using RT-qPCR and western blot. The TIPE2 mRNA expression in the hippocampus of APP/PS1 mice was higher than that of the WT mice at 6-month old (Fig. 2a); the TIPE2 mRNA level gradually declined to the same level as that in the WT at 12-month old.

We also examined the TIPE2 protein expression in the hippocampus. The TIPE2 protein expression was also increased in the hippocampus of APP/PS1 mice at 6-month old compared with that in the age-matched WT mice (Fig. 2b, $P<0.05$), and then gradually decreased to the same level as the WT mice at 12-month old, consistent with the observation of the change of the TIPE2 mRNA expression in the hippocampus of APP/PS1 mice.

Attenuation of Cognitive Deficits by AAV-Mediated TIPE2 Overexpression in the Hippocampus of APP/PS1 Mice

Three and a half months after the injection of AAV-TIPE2, the TIPE2 expression in the hippocampus of APP/PS1 mice was higher than that of the APP/PS1 mice injected with the negative control AAV (Fig. 3, $P<0.05$).

The Y-maze Alternation Test was used to test the cognitive ability of APP/PS1 mice at 1, 2, 3, and 3.5 months after the injection of the AAV overexpressing TIPE2. At 1 month after the injection, the percentage alternation of the APP/PS1 mice injected with the AAV overexpressing TIPE2 had increased compared with that in the APP/PS1 mice injected with the negative control AAV (Fig. 4a, $P<0.05$), indicating the cognitive ability of the APP/PS1 mice had improved by the overexpression of TIPE2. After 2, 3, and 3.5 months, the percentage alternation of the APP/PS1 mice injected with the AAV overexpressing TIPE2 continuously increased compared with that of the APP/PS1 mice injected with the negative control AAV (Fig. 4b, $P<0.05$, 2 months later; 4c, $P<0.01$, 3 months later; 4d, $P<0.05$, 3.5 months later), indicating the overexpression of TIPE2 continuously attenuated cognitive deficits in APP/PS1 mice.

TIPE2 Reduced Inflammatory Cytokines and Reversed Morphological Changes of Microglial Cells

The inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, and anti-inflammatory cytokines including IL-10 and Arg-1, were measured by RT-qPCR and Western blot. The expression of

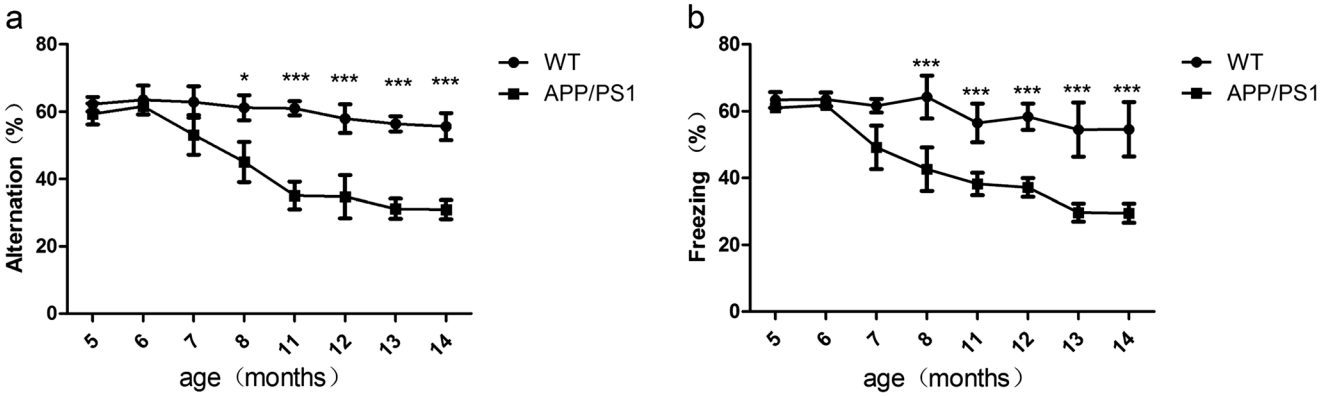

Fig. 1 Behavioral evaluation of learning and memory in APP/PS1 transgenic mice. **a** In Y-maze spontaneous alternation test, the percentage of alteration was calculated based on the number of arm entries and the number of all trials for APP/PS1 transgenic mice ($n = 8$) and WT mice ($n = 8$). **b** In Fear Conditioning task, the percentage of freezing time was determined by the percentage of time engaged in freezing responses during the initial 180 s of tones without footshock at each month of both APP/PS1 transgenic mice ($n = 8$) and WT mice ($n = 8$); two-way ANOVA was used. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; compared with WT mice

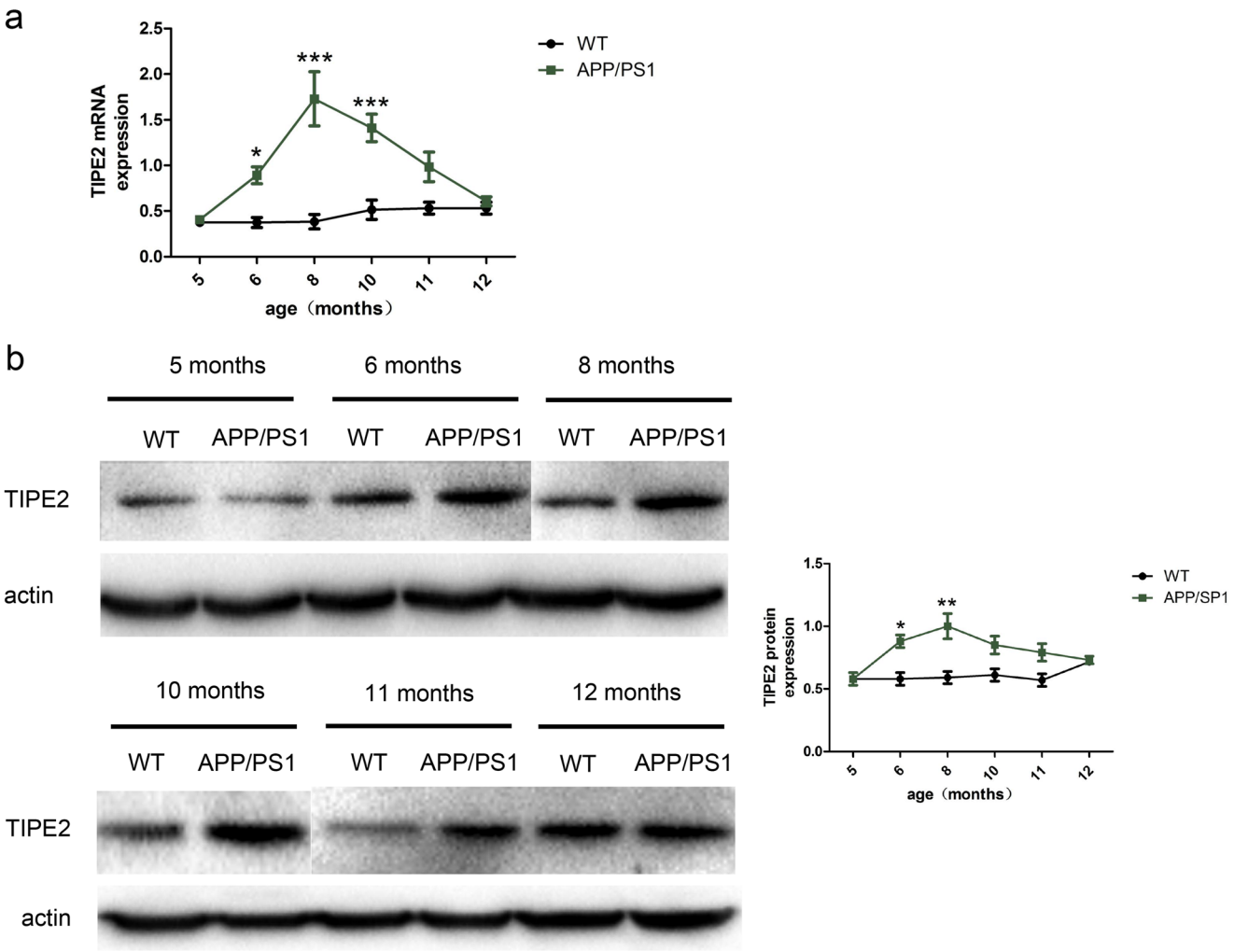

Fig. 2 Comparison of the TIPE2 mRNA and protein expression in the hippocampus between APP/PS1 and WT mice from 5- to 12-month old. The TIPE2 mRNA and protein expression was detected by RT-qPCR (**a**) and western blot assay (**b**), two-way ANOVA revealed a significant difference in TIPE2 mRNA and protein expression between WT mice and APP/PS1 mice from 6-month old ($P<0.05$). $^{*}P<0.05$; $^{**}P<0.01$, $^{***}P<0.001$; compared with WT mice. The experiment was repeated three times ($n=5$)

TNF-α, IL-1β and IL-6 were reduced, while the expression of IL-10 and Arg-1 were increased in APP/PS1 mice injected AAV-overexpressing TIPE2 compared with those in APP/PS1 mice injected with the negative control AAV (Fig. 5a, b), indicating that TIPE2 reduced the inflammatory response in the brain. In addition, immunofluorescence staining of hippocampal slices with the Iba1 antibody demonstrated morphological changes in microglia in the hippocampal of APP/PS1 mice. Microglial cells in the hippocampus of APP/PS1 mice displayed a shortened and less branched process, displaying cytoplasmic abnormalities and even fragmentation, compared with the WT mice (Fig. 6). Overexpressing TIPE2 in the hippocampus of APP/PS1 reversed the morphological changes, indicating that TIPE2 had a function of protecting microglial cells. Although, there were no significant changes in the cell body areas, the number of Iba1-positive cells and the fluorescence intensity of the Iba1-labled cell body areas between the APP/PS1-AAV mice and the APP/PS1-AAV-TIPE2 mice (Fig. 6).

Discussion

Alzheimer disease is initially thought as a cell-autonomous neurodegenerative disease alongside extracellular amyloid beta deposition and neurofibrillary tangles (Shi and Holtzman 2018). Neuroinflammation has been observed in human AD patients and AD mouse models. A 3D human triculture system modeling neuroinflammation in vitro has also demonstrated that neuroinflammation contributes to the pathological process of AD (Park et al. 2018). These findings provide a novel spotlight on the pathological process of AD. Most of the risk-associated genes involved in the development of AD,

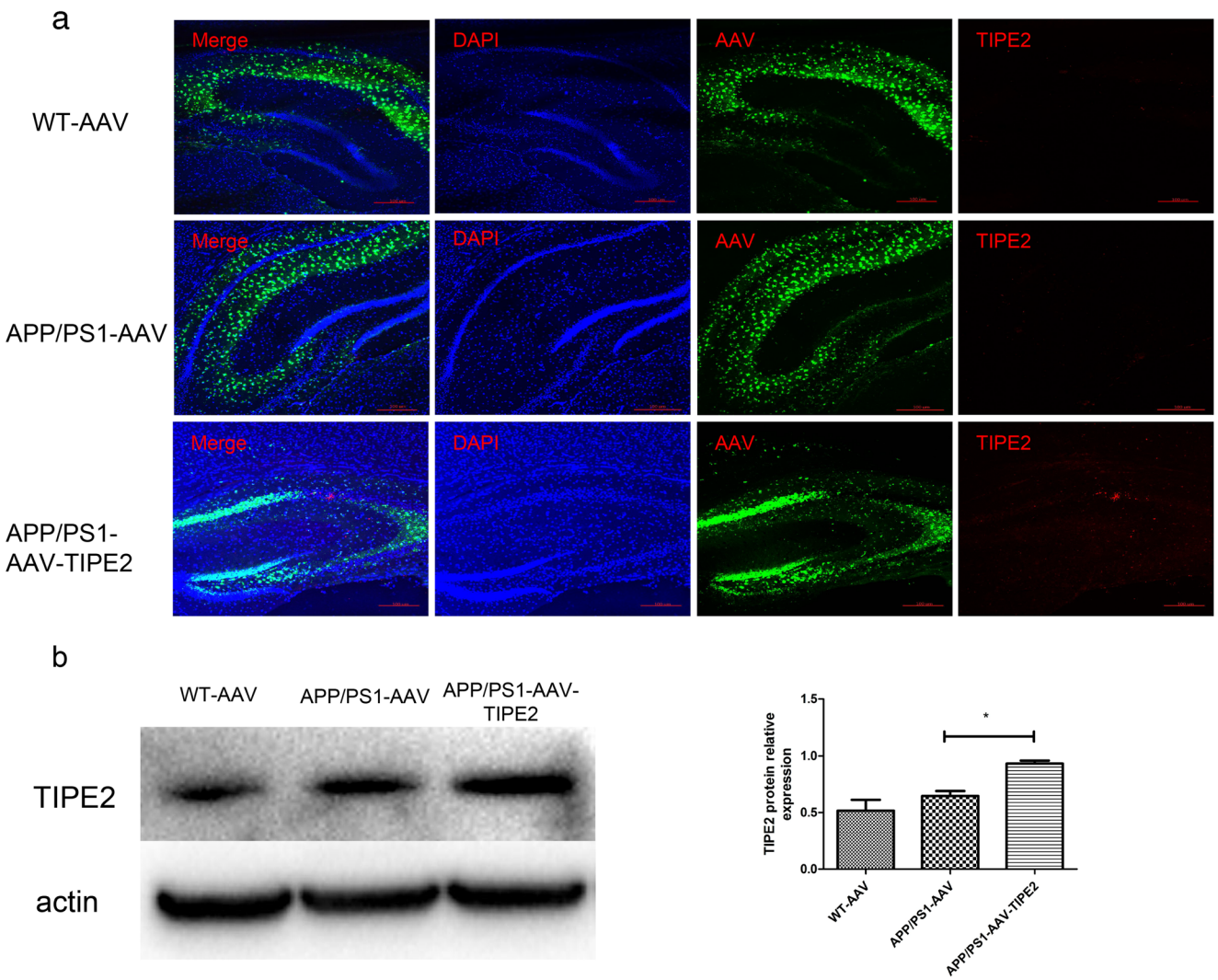

Fig. 3 The TIPE2 expression in hippocampus after stereotaxic injection of AAV into bilateral hippocampi in the brain. 7-month APP/PS1 mice received the AAV-TIPE2 injection. The TIPE2 expression was detected by immunofluorescence using hippocampal slices (**a**) and western blot assay (**b**). One-way ANOVA was used. *$P<0.05$, APP/PS1 mice with TIPE2 overexpression ($n = 5$) compared with the APP/PS1 mice with negative control AAV injection ($n = 5$) .Scale bar = 100 μm

such as CD33 (Griciuc et al. 2013), CR1 (Crehan et al. 2013) and TREM2 (Guerreiro et al. 2013), have been identified to be either expressed by microglia or associated with their reactivity (Park et al. 2018) as revealed by genome-wide association studies (Pimenova et al. 2018). The innate immune cells involved in the neuroinflammatory process of AD may contribute to disease pathogenesis, thus providing a potential therapeutic target. An increasing understanding of the physiological function of the immune system and its modulators may be a key direction to identify new pathological mechanisms or therapeutic targets of AD (Van Eldik et al. 2016).

TIPE2 is a negative regulator of innate and adaptive immunity that maintains immune homeostasis. It is highly induced in the brains from cerebral ischemia mice, which significantly down-regulates inflammatory factors caused by cerebral ischemia/reperfusion injury, and provides a neuroprotective function in stroke (Zhang et al. 2012). We found that the Aβ deposited in the hippocampus of APP/PS1 mice from 6-month old and increased gradually (data not shown). While the APP/PS1 mice did not show any cognitive deficits at 6-month old. This contradictory phenomenon suggests Aβ did not damage neurons directly because of some protective factors. Interestingly, the TIPE2 mRNA and protein levels in the hippocampus of APP/PS1 mice were markedly up-regulated at 6-month old. The results indicated that the TIPE2 over-expression induced by Aβ deposition suppressed the development of AD by inhibiting inflammatory responses. To further clarify whether TIPE2 is the protective factor for AD, we continuous monitored the cognitive ability of APP/PS1 mice, the expression of TIPE2 and the Aβ deposition. The APP/PS1 mice showed significant cognitive deficits from 8-month old, accompanying with substantial Aβ

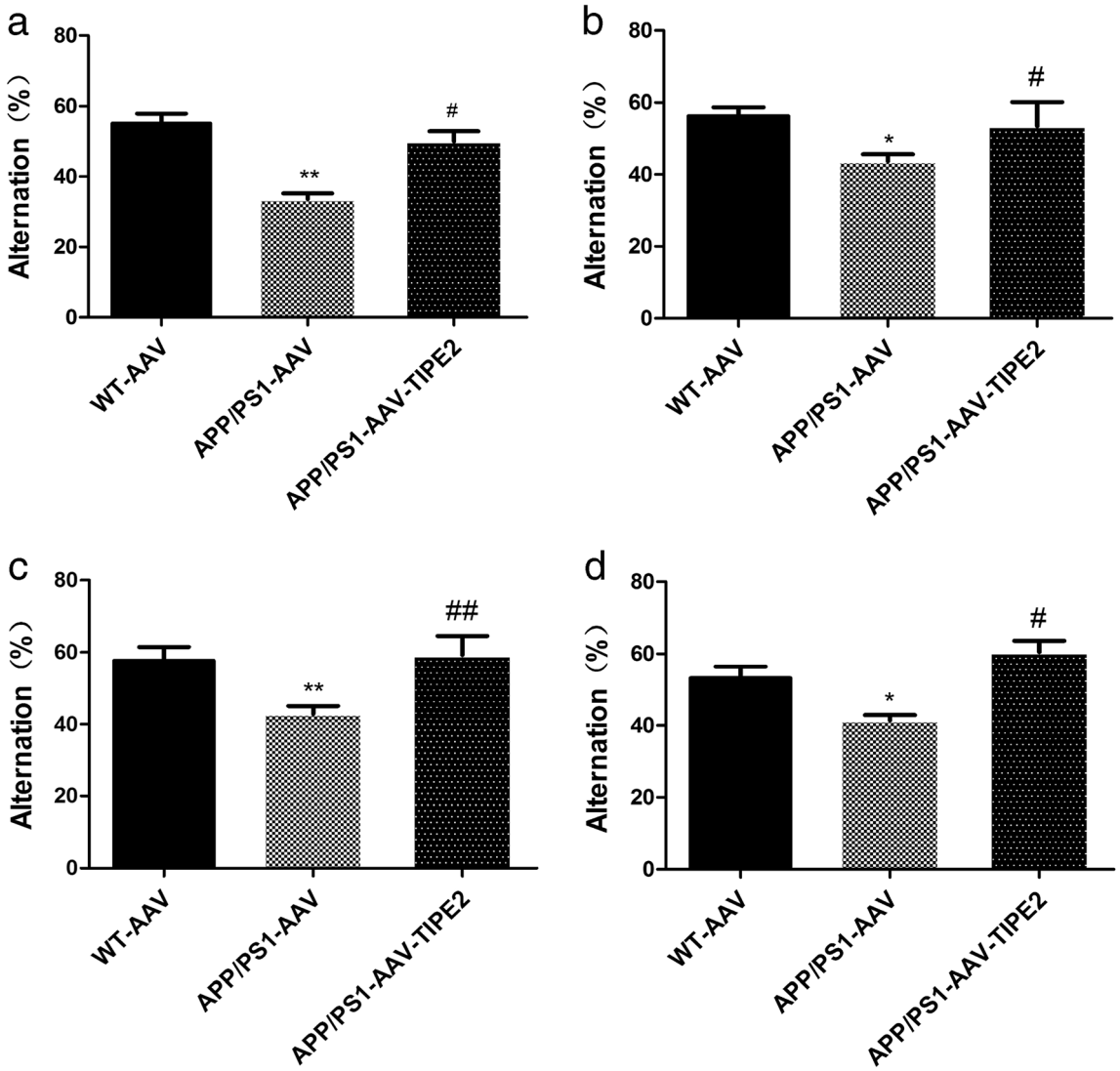

Fig. 4 The Y-maze Alternation Test after injection of the AAV overexpressing TIPE2 in the hippocampus of APP/PS1 mice. One month (**a**), 2 months (**b**), 3 months (**c**) and 3.5 months (**d**) after injection. One-way ANOVA was used, $n = 6$. *P<0.05; **P<0.01, APP/PS1 mice injected with negative control AAV compared with WT mice. $^{\#}P$<0.05; $^{\#\#}P$<0.01, APP/PS1 mice with TIPE2 overexpression compared with APP/PS1 mice injected with negative control AAV

deposition. During this period, the difference of the TIPE2 expression between the APP/PS1 mice and the WT mice gradually decreased, the TIPE2 mRNA and protein levels gradually declined from 8-month old, finally to the same levels as the WT mice at 12-month old. We speculate that there are potential relationships among the three. Aβ deposition activates microglia and astrocyte, and triggers an innate immune response characterized by the release of inflammatory mediators (Zhang and Jiang 2015). These inflammatory mediators contribute to disease progression and severity, including axonal cleavage, and neuronal dysfunction and loss (Park et al. 2018). TIPE2 was induced to limit excessive inflammation to maintain homeostasis and rescue neural damage at the beginning of the Aβ deposition. Sustained Aβ deposition not only triggers the release of inflammatory cytokines, but also damages microglia, including microglial dysfunction and degeneration (Navarro et al. 2018), and gained toxic function because of clustering around neuritic plaque (Jay et al. 2015). Microglia degeneration and gained toxic function may account for the decline of the TIPE2 expression in the hippocampus of the APP/PS1 mice from 8-month old. The decreased TIPE2 expression cannot effectively reduce the level of inflammatory cytokines and prevent neuronal damage, eventually leading to the occurrence of cognitive deficits in the APP/PS1 mice.

To test the above hypothesis, we examined the effects of TIPE2 overexpression on cognitive deficits by intracranial

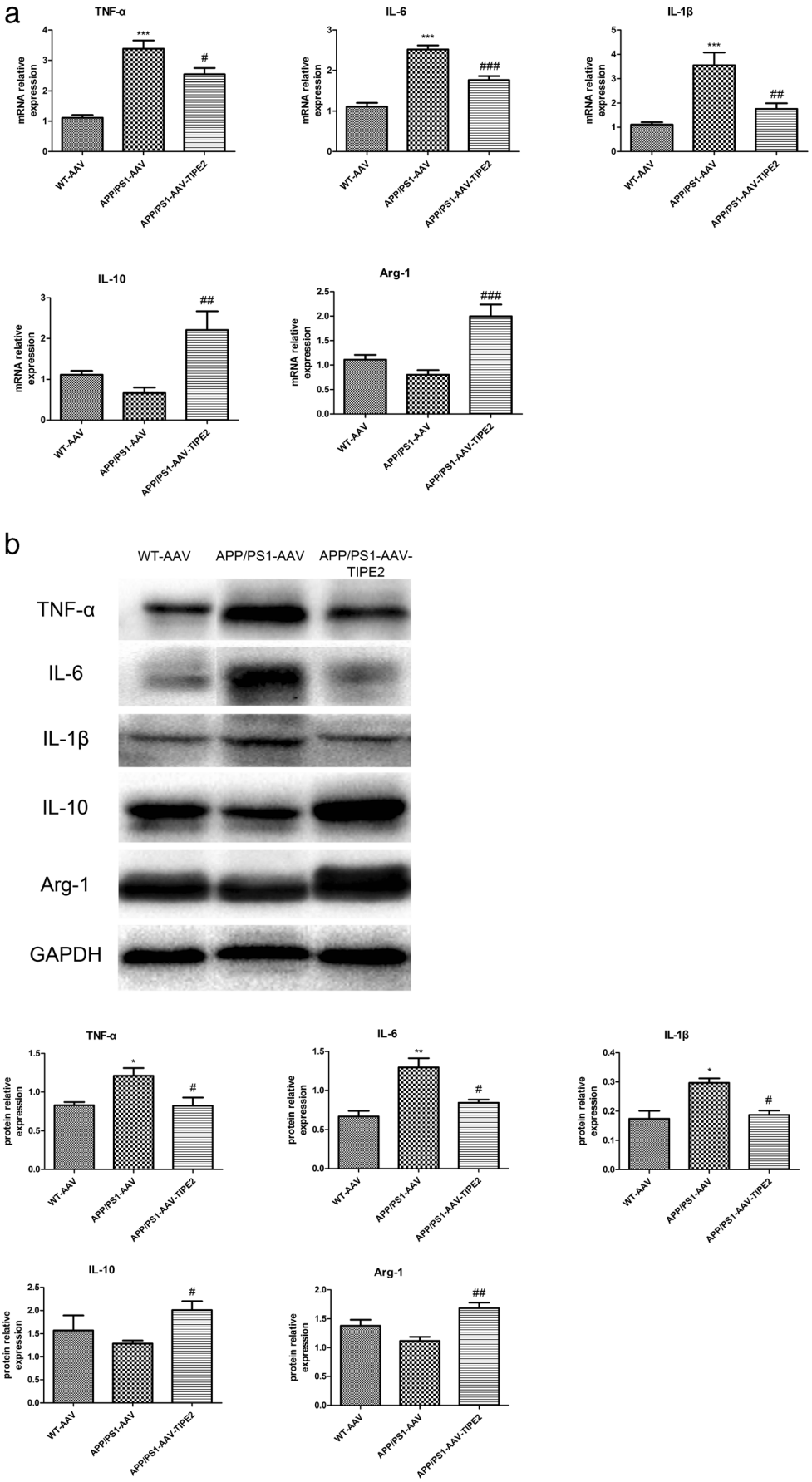

Fig. 5 Overexpressing TIPE2 in the hippocampus of APP/PS1 mice reduced inflammatory cytokines and increased anti-inflammatory cytokines. The inflammatory and anti-inflammatory cytokines were detected by RT-qPCR (**a**) and western blot assay (**b**). One-way ANOVA was used. *$P<0.05$; **$P<0.01$; ***$P<0.001$, APP/PS1 mice injected with negative control AAV compared with WT mice. $^{\#}P<0.05$; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$, APP/PS1 mice with TIPE2 overexpression compared with APP/PS1 mice injected with negative control AAV, $n = 5$

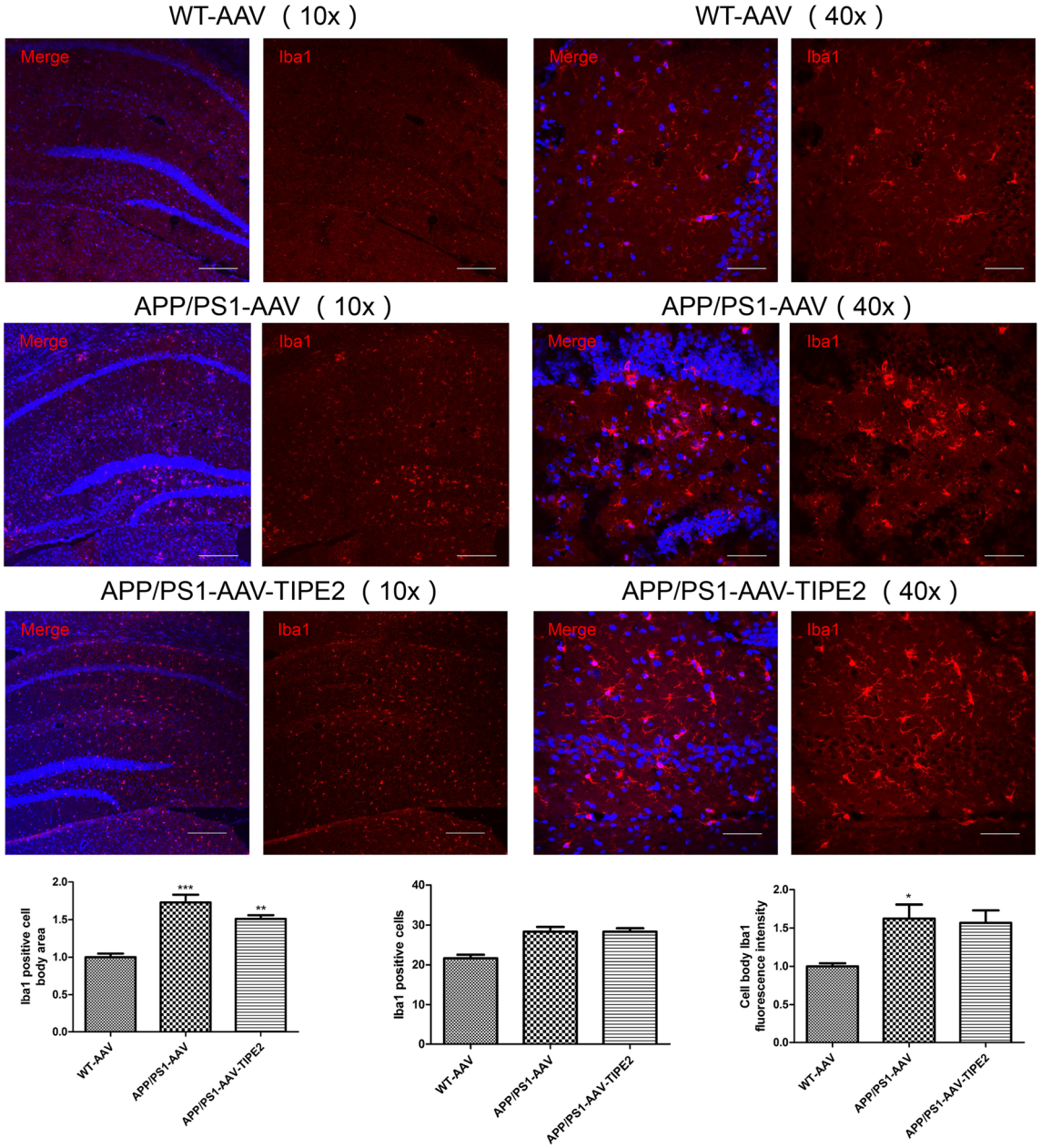

Fig. 6 TIPE2 reversed microglial morphological changes in APP/PS1 mice. The number of Iba1 positive cells, body area, and Iba1 fluorescence intensity were measured. One-way ANOVA was used. *$P<0.05$; **$P<0.01$; ***$P<0.001$, APP/PS1 mice injected with negative control AAV compared with WT mice. $^{\#}P<0.05$; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$, APP/PS1 mice with TIPE2 overexpression compared with APP/PS1 mice injected with negative control AAV. (3 images/mouse, $n = 3$ mice/group). Scale bar = 100 μm (10×). Scale bar = 50 μm (40×)

injection of AAV-TIPE2 in the hippocampus of APP/PS1 mice. The Y-maze alternation test showed the spontaneous alternation rate of the APP/PS1 mice overexpressing TIPE2 was significantly increased, indicating that overexpression of TIPE2 could attenuate cognitive impairment induced by Aβ deposition.

Microglia is a neuropathology sensor and responds to subtle changes in the brain tissues with morphological changes (Kaur et al. 2017). We used immunofluorescence staining of hippocampal slices with the Iba1 (biomarker for microglia) antibody to detect microglial morphological changes. The results showed that microglial morphology was changed in the hippocampus of APP/PS1-AAV mice evidenced by shortened and less branches compare with those in WT mice, displaying cytoplasmic abnormalities in APP/PS1 mice. The changes were reversed in APP/PS1-AAV-TIPE2 mice, indicating that overexpression of TIPE2 protected microglia. The expression of Iba1 in microglia and microglial body areas are increased when microglia is activated (Graeber 2010; Colonna and Butovsky

2017; Shoham et al. 2018). The Iba-1 immunofluorescence results showed the cell body areas and the fluorescence intensity were increased in the hippocampus of APP/PS1-AAV mice and APP/PS1-AAV-TIPE2 mice, compared with WT mice. Indicated that microglia was in activated state in the hippocampus of APP/PS1-AAV and APP/PS1-AAV-TIPE2 mice. Microglia has two major activated states, the proinflammatory (M1) phenotype which expresses cytotoxic genes including TNF-α, IL-6 and IL-1β, and the noninflammatory activated (M2) phenotype which expresses anti-inflammatory genes such as IL-10 and Arg-1 (Mantovani et al. 2004; Colton et al. 2006; Sierra-Filardi et al. 2011). The expression of inflammatory cytokines, such as TNF-α, IL-6 and IL-1β, were increased in the brain of APP/PS1 mice compared with those in WT mice, suggesting that there were more M1 microglia in the brain of APP/PS1 mice. Overexpressing TIPE2 in the hippocampus of APP/PS1 mice reduced the inflammatory cytokines and increased anti-inflammatory cytokines, such as IL-10 and Arg-1, suggested TIPE2 promoted the conversion from M1 microglia to M2 microglia. These findings suggested TIPE2 reduced inflammatory levels to rescue cognitive deficits in the APP/PS1 mice by contributing to M2 polarization of microglia.

Conclusion

Our findings demonstrate that TIPE2 was negatively correlated with the pathogenesis of AD. Overexpression of TIPE2 attenuated cognitive deficits in APP/PS1 mice. These maybe by promoting M2 polarization of microglia and reducing inflammatory cytokines to prevent microglia dysfunction. These findings suggest TIPE2 is a potential target for pharmacological intervention and improvement of cognitive deficits. More studies are needed to understand the regulatory mechanisms of TIPE2 in preventing the pathogenesis of AD, such as the mechanism of TIPE2 in the conversion of M1 to M2 microglia .

Acknowledgements This work was supported by the research fundings granted to Fang ZHANG from the Natural Science Foundation of Shandong Province of China, No. ZR2016 HM 46; Post doctoral application foundation of Qingdao Municipal Bureau of Social Sciences, No. 2016062; Qingdao Science and Technology Bureau, No. 18-6-1-75-nsh; Clinical medicine +X Project of Qingdao University Medical Department, No. 2017 M08; and Innovation and Entrepreneurship Training Program of undergraduate student in Qingdao University, No. 201711065152.

Authors' Contributions Fang Zhang, Wenjian Shao, Zhihong Yang, Lei Wang and Chuanxia Ju contributed to the experimental design. Yongzhen Miao, Zihan Xu and Ruoyu Zhang contributed to the experimental process. Yongzhen Miao wrote this article. Naidong Wang provided fund support for our supplementary experiment and revised our manuscript.

Data Availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

Ethics Approval The experiments were approved by the Qingdao University Experimental Animal Care and Use Committee.

Competing Interests The authors declare that they have no competing interests.

Informed Consent Not applicable.

Abbreviations *AD,* Alzheimer's disease; *TIPE2,* Tumor necrosis factor-α-induced protein 8-like 2; *Aβ,* β-amyloid; *Iba1,* ionized calcium binding adaptor molecule 1

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