High Plasma Llevels of Aβ1-42 Affect Monocytes and Macrophages via Biphasic Effects on Myeloid-Derived Suppressor Cells and Granulocyte-Monocyte Progenitors in Mouse Models of Alzheimer's Disease

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Research article

Keywords: Alzheimer's disease, Aβ1-42, plasma, monocyte, macrophage, interleukin-6, tumor necrosis factor-α, myeloid derived suppressor cell, granulocyte-monocyte progenitor, bone marrow-derived macrophage.

Posted Date: July 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-706739/v1

Abstract

Backgroud: Microglia play a crucial role in the pathogenesis of Alzheimer's disease (AD). Plasma $A\beta_{1\text{-}42}$ levels significantly increase 15 years before the onset of dominantly inherited AD. The effects of high plasma levels of $A\beta_{1\text{-}42}$ on monocytes and macrophages, the hematogenous counterparts of microglia, remain unclear.

Methods: We investigated the effects of plasma $A\beta_{1\text{-}42}$ on peripheral monocytes and macrophages in three animal models, including 7- and 11-month-old female APPswe/PS1dE9 (APP/PS1) transgenic (Tg) mice, wild-type (Wt) parabiotic with Tg (paWt(Wt-Tg)) mice, and Wt mice.

Results: We found that high plasma levels of $A\beta_{1\text{-}42}$, in younger (7-month) AD mice significantly decreased the amounts of pro-inflammatory macrophages, myeloid derived suppressor cells (MDSCs), granulocyte-monocyte progenitors (GMP), and the plasma levels of interleukin-6 (IL-6). In older (11-month) AD mice, high plasma levels of $A\beta_{1\text{-}42}$ significantly increased the amounts of pro-inflammatory macrophages, MDSCs, GMPs, the plasma levels of IL-6 and TNF-α, and the brain infiltration of bone marrow-derived macrophages (BMDMs). However, high plasma levels of $A\beta_{1\text{-}42}$ consistently increased the amounts of monocytes and the proliferation of bone marrow cells (BMCs) without affecting the phagocytic function of macrophages on $A\beta_{1\text{-}42}$.

Conclusion: The response of mouse AD model suggests that a high plasma level of $A\beta_{1\text{-}42}$ affects monocytes and macrophages via its biphasic effects on MDSCs and GMPs. We suggest that intervening in the effects of plasma $A\beta_{1\text{-}42}$ on monocytes and macrophages might offer a new therapeutic approach to AD.

1. Backgroud

Alzheimer's disease (AD), the most common neurodegenerative disorder with progressive memory and cognitive loss, is affecting almost 50 million people worldwide, and the incidence of AD is increasing rapidly with the ageing of the world population [1]. The medical care and nursing cost of AD is enormous [2–4].

The main neuropathological features of AD are neuroinflammation, neurofibrillary tangles (NFTs) formed by intracellular accumulation of hyperphosphorylated Tau (p-Tau) protein, neuritic plaques formed from amyloid-$\beta_{1-42/1-40}$ ($A\beta_{1-42/1-40}$), synapse and neuronal loss, and astroglial proliferation. Major pathogenic hypotheses for AD focus on the Aβ cascade and p-Tau accumulation. However, clinical trials using designed to test the effects of inhibiting $A\beta_{1-42}$ production by β-secretase inhibitor, clearing $A\beta_{1-42}$ by monoclonal antibodies, and inhibiting p-Tau by leuco-methylthioninium bis (hydromethanesulfonate; LMTM), have all failed to demonstrate clinical efficacy [1, 5–7]. Thus, the hypotheses of Aβ cascade and p-Tau have been challenged [5–11].

Accumulating studies suggests that neuroinflammation plays an early and crucial role in the genesis of AD pathology [12]. Microglia, the main resident immune cells in the central nervous system (CNS) act as vigilant housekeepers in the adult brain; they activate immediately if the blood-brain barrier (BBB) is disrupted, and they switch their behavior from patrolling to shielding the injured site [13]. Misfolded and aggregated $A\beta_{1-42}$ and p-Tau, which can bind to pattern recognition receptors (PRRs) on microglia and astrocytes, trigger innate immune response to release inflammatory mediators, which in turn contribute to disease progression and severity [12, 14–18]. Genome-wide analysis suggests that several genes, encoding for glial clearance of $A\beta_{1-42}$ and the inflammatory reaction, increase the risk of sporadic AD [19–20]. In the pathogenesis of AD, microglia might have a double-edged sword role. At the early stage of AD, microglia protect the brain from the toxic effects of $A\beta_{1-42}$ by phagocytizing and clearing Aβ [21]. While, as AD progresses, microglia lose their Aβ-clearing capabilities, the expression of microglial Aβ receptors and Aβ-degrading enzymes with persistent production of pro-inflammatory cytokines, results in Aβ deposition. Moreover, complement and microglia mediate the early loss of synapses AD mouse models [22].

While the brain traditionally has been regarded as an immune-privileged organ protected by the BBB, there are interactions between the brain and peripheral organs that have a significant role in the development and progression of AD [23]. Neuroinflammation is closely related to peripheral immunity, especially in the late stage of AD, because the BBB is impaired and, hence, peripheral immune cells and inflammatory molecules enter the brain parenchyma [24, 25]. There is evidence that circulating neutrophils can extravasate and surround Aβ deposits, where they secrete interleukin-17 (IL-17) and neutrophil extracellular traps (NETs). Moreover, inhibiting neutrophil trafficking or depleting these cells reduces AD-like neuropathological changes and improves memory in AD Tg mice [26].

In recent years, the role of peripheral innate immunity in the pathogenesis of AD has gained more attention [27, 28]. Several studies have found that circulating bone marrow-derived macrophages (BMDMs) can enter brain tissue, where they serve as bone marrow-derived microglia that more efficiently phagocytize $A\beta_{1-40/1-42}$ compared to resident microglial cells [29, 30]. Selective ablation of bone marrow-derived dendritic cells increases amyloid plaques in AD mouse models [31]. Increased cerebral infiltration of monocytes, either by elevating the level of circulating monocytes or by weekly treatment with glatiramer acetate (which simulates myelin basic protein), substantially attenuated disease progression in an AD mouse model [32]. Indeed, prior to these observations, monocytic cells derived from bone marrow stem cells had been used to treat AD [33], while long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) prior to the onset of AD offered protection against AD [34–36]. In November of 2019, sodium oligomannate (GV-971), a marine algae-derived oral oligosaccharide, was approved for AD treatment by the Chinese Food and Drug Administration based on its ability to alleviate neuroinflammation by regulating gut microbiota and inhibiting the brain infiltration of peripheral T helper (Th) 1 cells [37].

In dominantly inherited AD and Down syndrome, plasma $A\beta_{42}$ levels increased significantly 15 years before the onset of symptoms compared with normal people [38, 39]. Moreover, in the parabiosis animal model of APPswe/PS1dE9 (APP/PS1) transgenic (Tg) mice and wild-type (Wt) mice and the mice model of transplanting bone marrow cells (BMCs) from APP/PS1 Tg mice into Wt mice, the $A\beta_{42}$ from Tg mice plasma or BMCs of Tg mice could significantly increase the plasma $A\beta_{1-42}$ levels of Wt mice and enter into Wt mice brain to form cerebral amyloid angiopathy (CAA) and Aβ plaques similar to that of Tg mice brain [40, 41]. The present study employed three mouse models of AD to examine the effects of high plasma levels of $A\beta_{1-42}$ on monocytes and macrophages.

2. Materials And Methods

All experiments involving mice were approved by the Laboratory Animal Welfare and Ethics Committee of Shenzhen Luohu Hospital Group (SLHG), Shenzhen, China. Detailed materials and methods are presented in the supplementary information. Briefly, 6–8 week-old female pathogen-free APP/PS1 mice and C57BL/6 wild-type (Wt) mice were purchased from Shanghai Model Organisms Center, Inc (SMOC, Shanghai, China). Animals acclimatized for 2–5 days in the pathogen-free animal facility of the SLHG Precision Medicine Research Institute. Mice were housed in a room with a 12- hour light-dark cycle and provided with food and water adlibitum. Parabiosis surgery resulting in shared blood circulation was performed on pairs of mice after they had adapted to each other by living together in a cage for 1 month. Surgery was performed at 3 months of age on female Tg-Wt littermates and female Wt-Wt littermates (n = 6, per pair) according to the procedure from a previous study [40]. Parabiosis was maintained for 4 and 8 months, while age and weight-matched female Wt mice (n = 6, per group) and female Tg mice (n = 6, per group) without parabiosis were used as controls (Fig. 1a).

Exogenous $A\beta_{1-42}$ peptide labeled with HiLyte Fluor 488 (AnaSpec, Fremont, CA, USA) 200ul of 100uM in phosphate buffered solution was intravenously injected into 6–8 week-old female Wt mice via the tail vein (n = 6) three times in a week, and the age and weight matched female Wt mice (n = 6) were used as controls. The mice in control group received the same volume of vehicle. Bioluminescent imaging was performed using the IVIS spectrum imaging system (Caliper Life-Sciences, Hopkinton, MA, USA) 1hour (h) after tail intravenous injection. Plasma levels of $A\beta_{1-42}$ and inflammatory cytokines TGF-β were measured by enzyme-linked immunosorbent assay (ELISA) kit (cat. CEA947Hu, Cloud-Clone Corp, Wuhan, China) and (cat. SEA124Mu, Cloud-Clone Corp, Wuhan, China). The inflammatory cytokines IL-6 (cat. 558301), IL-12p70(cat. 558303), TNF-α (cat. 558299), IL-10 (cat. 558300), and IL-1β (cat. 560232) levels were detected using cytometric beads array kits (BD-Pharmingen, La Jolla, CA, USA), respectively. The amounts of monocytes and MDSCs in spleen, macrophages in abdominal cavity, hematopoietic stem cells (HSCs) in thigh-bone bone marrow, and the ability of macrophages to phagocytose $A\beta_{1-42}$ were assessed by flow cytometry (BD FACSAria™ II Cell Sorter, La Jolla, CA, USA). The Aβ plaques and BMDMs in brains of mice were assessed by immunohistochemical and immunofluorescence techniques, respectively. The proliferation of BMCs was assessed by cell counting kit-8 assays (CCK-8) (cat. FC101, TransGen Biotech, Beijing, China).

Differences among groups were tested by the rank-sum test. Differences of categorical data were tested by Chi-square. For all statistical tests, two-sided *P*-values less than 0.05 were defined as statistically significant. All analyses were carried out using Statistical Package for Social Sciences (SPSS) version 23.0 software (IBM, West Grove, PA, USA).

3. Results

3.1 The plasma levels of $A\beta_{1-42}$ and brain amyloid deposition in Tg mice and paWt(Wt-Tg) mice.

Plasma levels of $A\beta_{1-42}$ in Tg and paWt(Wt-Tg) mice at 7 and 11 months were significantly increased relative to comparable aged Wt and paWt(Wt-Wt) mice, respectively ($p < 0.01$) (Fig. 1c).

At 7 months old, Aβ plaques were found in the neocortex and hippocampus of Tg mice and paTg(Wt-Tg) mice, but they were scarce in the neocortex and hippocampus of paWt(Wt-Tg) mice (Fig. 1d). However, at 11 months old, Aβ plaques were very obvious in both the neocortex and hippocampus of Tg mice, paTg(Wt-Tg) mice, and paWt(Wt-Tg) mice (Fig. 1d).

3.2 Effects of $A\beta_{42}$ on amounts of splenic monocytes and macrophages

At the age of 7 months and 11 months, the amounts of monocytes in the spleen of Tg mice and paWt(Wt-Tg) mice were significantly increased compared with those in Wt mice and paWt(Wt-Wt) mice, respectively ($p < 0.001$ and 0.01) (Fig. 2a), while the amounts of macrophages and anti-inflammatory macrophages in the abdominal cavity among all the groups showed no significant difference ($p > 0.05$) (Fig. 2b, 2d). However, the amounts of pro-inflammatory macrophages in the abdominal cavity of Tg mice and paWt(Wt-Tg) mice were significantly decreased at the age of 7 months compared with Wt mice and paWt(Wt-Wt) mice respectively ($p < 0.01$), and were significantly increased at the age of 11 months in Tg mice and paWt(Wt-Tg) mice ($p < 0.01$) (Fig. 2c).

The IVIS spectrum imaging system identified that $A\beta_{1-42}$ peptide labeled with HiLyte Fluor 488 entered the circulation of Wt mice 1h after tail intravenous injection (Fig. 1b). Amounts of monocytes and macrophages exhibited no significant differences between Wt mice with and those without $A\beta_{1-42}$ injection (data not shown).

3.3 The effects of $A\beta 1_{1-42}$ on the infiltration of BMDMs in mice brain

BMDMs were rarely found in brain tissue of 7 month-old Wt mice, paWt(Wt-Wt) mice, Tg mice and paWt(Wt-Tg) mice (data not shown). However, the infiltration of BMDMs in 11 month-old Tg and paWt(Wt-

Tg) mouse brains was significantly higher when compared with those in Wt and paWt(Wt-Wt) mouse brains, respectively ($p < 0.001$, and 0.01) (Fig. 3a-3b).

3.4 The effects of $A\beta_{1-42}$ on phagocytosis ability of macrophages in the abdominal cavity

The phagocytic capacity of macrophages to $A\beta_{1-42}$ showed no significant difference among Tg and Wt mice, or among paWt(Wt-Tg) and paWt(Wt-Wt) mice ($p > 0.05$) (Fig. 4a-4d).

The phagocytosis ability of macrophages to $A\beta_{1-42}$ was increased after being co-cultured with $A\beta_{1-42}$ peptide *in vitro* ($p < 0.05$) (Fig. 4e).

3.5 The effects of $A\beta_{1-42}$ on secretion of inflammatory factors by macrophages in the abdominal cavity

At the age of 7 months, only the plasma levels of IL-6 were found to be significantly reduced in Tg mice and paWt(Wt-Tg) mice compared with Wt mice and paWt(Wt-Wt) mice, respectively ($p < 0.01$ and 0.05) (Fig. 5a). At the age of 11 months, however, the plasma levels of IL-6 and TNF-α were significantly increased in Tg mice and paWt(Wt-Tg) mice when compared with Wt mice and paWt(Wt-Wt) mice, respectively ($p < 0.01$ and 0.05) (Fig. 5b). The levels of interleukin-12p70 (IL-12p70), interleukin-10 (IL-10), interleukin-1β (IL-1β), and transforming growth factor-β (TGF-β) in all groups showed no significant differences ($p > 0.05$).

The levels of IL-6 in Wt mice increased significantly after tail intravenous injection with $A\beta_{1-42}$ ($p < 0.01$) (Fig. 5c). Conversely, the levels of IL-12p70, TNF-α, IL-10, IL-1β, and TGF-β exhibited no significant differences ($p > 0.05$) (Fig. 5c).

3.6 The effects of $A\beta_{42}$ on MDSCs in spleen

At the age of 7 months, the amounts of MDSCs in the spleen of Tg mice and paWt(Wt-Tg) mice decreased significantly compared with those in Wt mice and paWt(Wt-Wt) mice ($p < 0.001$), while the M-MDSCs proportions showed no alterations (Fig. 6a-6c). At the age of 11 months, the proportions of MDSCs and M-MDSCs in the spleen of Tg mice and paWt(Wt-Tg) mice increased significantly compared to the proportions in Wt mice and paWt(Wt-Wt) mice ($p < 0.001$ and 0.01) (Fig. 6a-6c). The proportions of MDSCs in the spleen of Wt mice was significantly increased following intravenous injection of $A\beta_{1-42}$ ($p < 0.05$) (Fig. 6d).

3.7 The effects of $A\beta_{42}$ on myeloid progenitor cells

The proportions of multipotent progenitors (MPP) increased significantly in 7 month-old Tg mice compared *to* Wt *mice*, while they were significantly decreased in 11 month-old Tg mice($p < 0.01$); they

remained stable in 7- and 11-month-old paWt(Wt-Tg) mice (Fig. 7a). The proportions of common myeloid progenitors

(CMP) were stable in 7- and 11-month old Tg mice, but increased in 11 month-old paWt(Wt-Tg) mice ($p > 0.05$)(Fig. 7b). The proportions of granulocyte-monocyte progenitors (GMP) were significantly decreased in 7 month-old Tg mice whereas they showed a significant increment in 11 month-old Tg mice and paWt(Wt-Tg) mice ($p < 0.01$ and 0.05) (Fig. 7c). The proportions of megakaryocyte–erythroid progenitors (MEP) remained constant in 7 months old Tg mice and paWt(Wt-Tg) mice, but showed a significant reduction in 11 month-old Tg mice and paWt(Wt-Tg) mice ($p > 0.01$)(Fig. 7d).

The proportions of MPP, CMP, GMP and MEP in Wt mice given an intravenous injection of $A\beta_{1-42}$ remained comparable with those of normal Wt mice (data not shown).

3.8 The effects of $A\beta_{1-42}$ on the proliferation of bone marrow cells

The proliferation of BMCs in 7 and 11 month-old Tg mice and paWt(Wt-Tg) mice increased significantly compared that seen in Wt mice and paWt(Wt-Wt) mice ($p < 0.001$ and 0.01) (Fig. 8a-8b). The proliferation of BMCs significantly increased in Wt mice after intravenous injection of $A\beta_{1-42}$ ($p < 0.05$) (Fig. 8c). Moreover, the proliferation of BMCs showed significant increment after being co-cultured with $A\beta_{1-42}$ peptide *in vitro* ($p < 0.01$ and 0.05) (Fig. 8d).

4. Discussion

In the present study, the plasma $A\beta_{1-42}$ levels and Aβ plaques in paWt(Wt-Tg) mice significantly increased similar to those of Tg mice. Thus, paWt(Wt-Tg) mice might be a reliable model to investigate the effects and mechanisms of high plasma $A\beta_{1-42}$ levels on monocytes and macrophages, which could eliminate or at least attenuate the direct influence of genetic background of Tg mice. In the early stages (7 month-old) and late stages (11 month-old) of both Tg mice and paWt(Wt-Tg) mice, high levels of plasma $A\beta_{1-42}$ could consistently increase monocytes in the spleen without affecting the amount of macrophages in the abdominal cavity. However, the proportions of pro-inflammatory macrophages in the abdominal cavity of Tg mice and paWt(Wt-Tg) mice reduced significantly at 7 months, but increased significantly at 11 months of age. The proportions of anti-inflammatory macrophages in the abdominal cavity consistently remained stable both in the early and late stages of Tg mice and paWt(Wt-Tg) mice.

We further studied the effects of the high plasma levels of $A\beta_{1-42}$ on the secretion of pro-inflammatory and anti-inflammatory cytokines. To that end, we found that the high levels of plasma $A\beta_{1-42}$ inhibited the secretion of pro-inflammatory cytokines IL-6 in the early stage of both Tg and paWt(Wt-Tg) mice, whereas increased secretion of IL-6 and TNF-α was observed in the late stage of both animal groups. The secretion of anti-inflammatory factors, including IL-10 and TGF-β, showed no significant alterations at either age in any animal group. Additionally, we found that the infiltration of BMDMs into the brain was

significantly increased only in the late stages of paWt(Wt-Tg) mice and Tg mice (Fig. 9). Collectively, these results suggest that high plasma levels of $A\beta_{1-42}$ in the early stage of AD inhibit peripheral innate immunity, whereas in the late stage of AD, they over-activate peripheral innate immunity. These conclusions may be in line with a previous study showing that peripheral monocyte gene expression is pro-inflammatory throughout the course of AD, while the pro-inflammatory gene expression is suppressed at the prodromal stage of disease [42].

We found that the capacity of macrophages to phagocytose $A\beta_{1-42}$ in Tg mice and Wt mice, paWt(Wt-Tg) mice and paWt(Wt-Wt) mice, showed no significant difference both in animals aged 7 months and 11 months; however, the phagocytosis ability of macrophages was increased after *in vitro* $A\beta_{1-42}$ peptide co-culture. In a previous study, Aβ was found to be an effective stimulant of macrophage/microglia phagocytosis [43]. However, the phagocytosis ability of macrophage/microglia in AD patients is controversial and complicated. The basal levels of phagocytosis in all three subsets of monocytes, such as non-classic, intermediate and classic monocytes, were reported to be similar between healthy controls and AD patients, while a significant increase in basal phagocytosis was found in subjects with high Aβ-amyloid burden as assessed by PET scans [44]. However, in another study, $A\beta_{1-42}$ uptake by blood monocytes was reduced with ageing and AD [45]. Our study results suggest that although high plasma $A\beta_{1-42}$ can activate peripheral monocytes, leading to their infiltration into the brain, these BMDMs might not engulf $A\beta_{1-42}$ in the brain and could possibly be harmful by secreting pro-inflammatory cytokines. Thus, it might not be reasonable to expect that the activated peripheral monocytes and macrophages that infiltrate the brain parenchyma could replace the ageing microglia in AD.

We also investigated the mechanisms by which high plasma levels of $A\beta_{1-42}$ affect monocytes and macrophages. In previous studies, Aβ and Tau were found to deposit in bone marrow; simultaneous Aβ treatment promoted osteogenic differentiation via Wnt/β-catenin signaling and inhibited osteoclast differentiation via the OPG/ RANKL/RANK system. Osteoclast activation was regulated by Aβ in an age-dependent manner [46–48]. To the best of our knowledge, we are the first to demonstrate that high plasma levels of $A\beta_{1-42}$ significantly increase the proliferation of BMCs in the early and late stages of AD. In addition, we also found that MDSCs in spleen and GMP proportions in bone marrow (the precursor cells of monocytes and macrophages in the plasma) significantly decrease in the early neurodegenerative stage of Tg mice and paWt(Wt-Tg) mice, but increase in the later stage of Tg mice and paWt(Wt-Tg) mice; these results are consistent with the increase of monocytes in spleen and pro-inflammatory macrophages in abdominal cavity.

MDSCs are believed to be the most important immune modulatory cells of the innate immune system. Interestingly, in line with our findings, a significant increase in MDSCs was reported in the peripheral blood of patients with amnesic mild cognitive impairment (MCI) when compared with healthy controls or mildly affected AD patients [24]. To date, there are few studies of the changes of HSCs in AD patients and AD animal models. A previous study reported a significant decrease of short-term hematopoietic stem cells (ST-HSC) proportion in 12 month-old 3×Tg mice [49]. In our study, the proportions of long-term

hematopoietic stem cells (LT-HSC) decreased but ST-HSC proportions increased significantly at the age of 7 months in Tg mice, whereas no significant difference was observed in the proportions of LT-HSC and ST-HSC among 11 month-old Tg mice, Wt mice and paWt(Tg-Wt) mice (data not shown).

There are some limitations in our study. Firstly, the mechanisms of plasma $A\beta_{1-42}$ on monocytes, macrophages, MDSCs and HSCs remain unclear. Secondly, we did not study the effects of plasma $A\beta_{1-42}$ on the subtype of splenic monocytes, including non-classic ($CD14^{dim}CD16^{+}$) monocytes, intermediate ($CD14^{+}CD16^{+}$) monocytes and classic ($CD14^{+}CD16^{-}$) monocytes. Lastly, we also did not study the effects of plasma $A\beta_{1-42}$ on the amounts and functions of monocytes, macrophages, MDSCs and HSCs in the stages of preclinical, prodromal and clinical AD patients.

5. Conclusion

We propose that high plasma levels of $A\beta_{1-42}$ may inhibit the activation of peripheral inflammatory processes in the early stage of AD, whereas in the late stage of AD, high plasma levels of $A\beta_{1-42}$ may over-activate the peripheral and central inflammatory processes by affecting the proliferation and differentiation of HSCs. Further preclinical studies are needed to determine whether modulating the effects of plasma $A\beta_{1-42}$ on monocytes, macrophages, MDSCs and HSCs might have use as a new therapeutic strategy in AD.

Abbreviations

AD:Alzheimer’s disease; MDSCs: myeloid derived suppressor cells; GMP: granulocyte-monocyte progenitors; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α;BMDMs:bone marrow-derived macrophages; BMCs:bone marrow cells; NFTs：neurofibrillary tangles；LMTM:leuco-methylthioninium; CNS:central nervous system; BBB:blood-brain barrier; PRRs: pattern recognition receptors; NETs：neutrophil extracellular traps; IL-17:interleukin-17; NSAIDs: nonsteroidal anti-inflammatory drugs; CAA: cerebral amyloid angiopathy; ELISA: enzyme-linked immunosorbent assay; HSCs：hematopoietic stem cells；CCK-8:cell counting kit-8 assays; SPSS: Statistical Package for Social Sciences; IL-12p70: interleukin-12p70; IL-10: interleukin-10; IL-1β: interleukin-1β; TGF-β: transforming growth factor-β; MPP: multipotent progenitors; CMP: common myeloid progenitors; MEP: megakaryocyte–erythroid progenitors; MCI: mild cognitive impairment; ST-HSC: short-term hematopoietic stem cells; LT-HSC: long-term hematopoietic stem cells.

Declarations

Authors’ contributions

CL conducted the study, analyzed the data and wrote the manuscript; JZ took part in the study design and reviewed the manuscript; KL analyzed the data and reviewed the manuscript; FZ designed and

funded the study, analyzed the data, wrote and finalized the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This work was supported by Sanming Project of Medicine in Shenzhen City (SZSM201801014) and Key project of Shenzhen Science and Technology Innovation Committee (JCYJ20200109143431341).

The authors would like to thank Prof Yanjiang Wang and Dr Chi Zhu from the Department of Neurology and Centre for Clinical Neuroscience, Daping Hospital, Third Military Medical University, for establishing the parabiosis mouse models. Additionally, we thank Prof. Peter Spencer from Oregon Health & Science University, who shared his expertise on animal-based studies and English language use.

Compliance with ethical standards.

Conflict of interest

The authors declare no competing interests.

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Figures

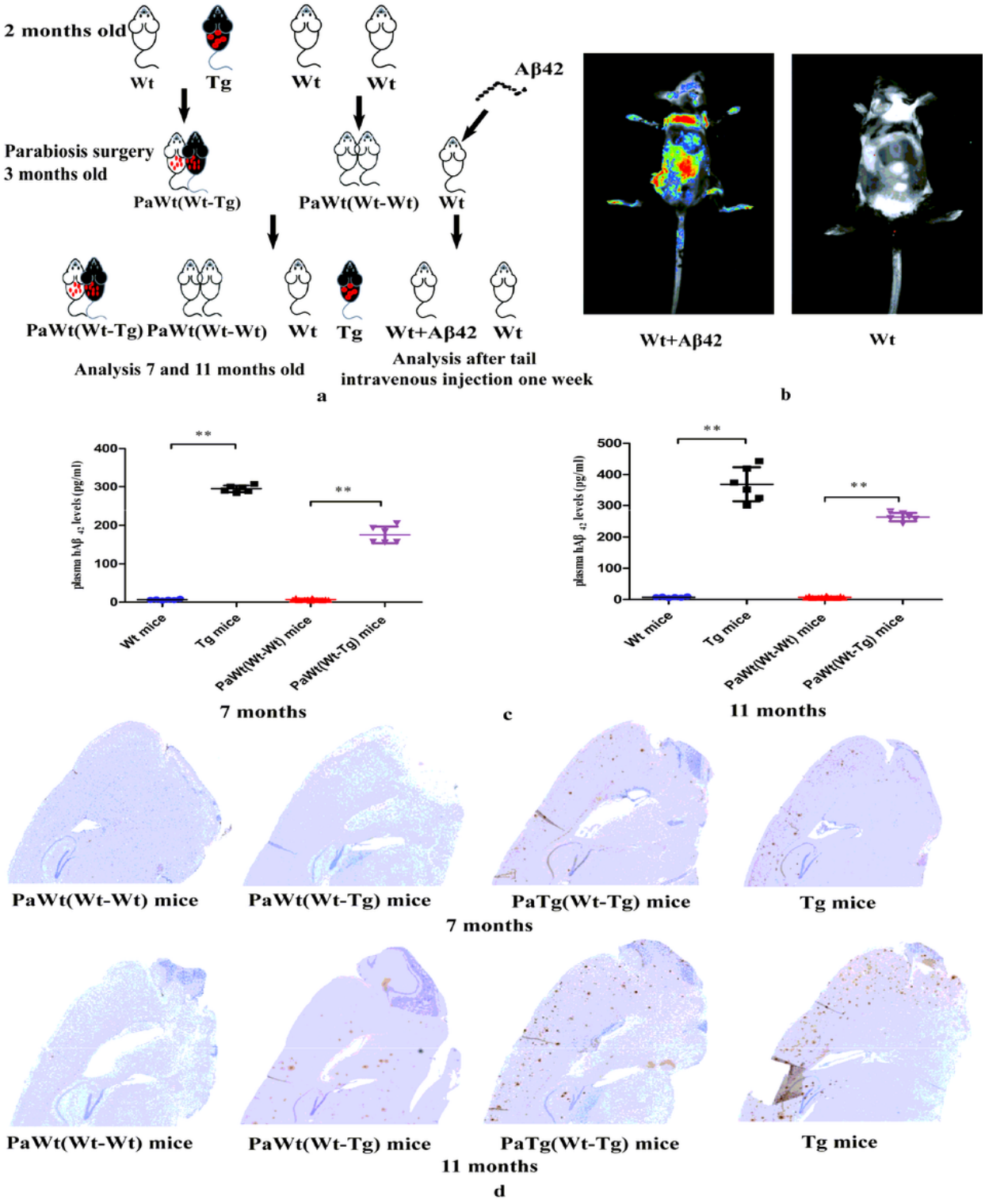


Figure 1

Wt mice showed chronic Aβ42 stimulation from Tg mice after parabiosis. a: Schematic diagram depicting the parabiotic pairings. A pair of 3 month-old female Tg mice and age-matched female Wt littermates were used for parabiosis. A pair of 3 month-old female Wt mice were used for parabiotic controls. Samples were collected for analysis at 7 and 11 months of age. Age-matched female Tg and Wt mice without parabiosis were also explored. b: Bioluminescent images of Wt mice after tail intravenous

injection with hAβ42 labeled with HiLyte Fluor 488 1h. c: Plasma Aβ42 levels in Wt, paWt(Wt-Wt), paWt(Wt-Tg), paTg(Wt-Tg), and Tg mice at the age of 7 and 11 months. d: Representative images of amyloid deposition stained with 6E10 in paWt(Wt-Wt), paWt(Wt-Tg), paTg(Wt-Tg), and Tg mice at the age of 7 and 11 months respectively. Scale bars 2500 μm. (n=6 for each group, Mean±SD, one-way analysis of variance, ***P<0.001, **P<0.01, ns denotes no statistical significance).

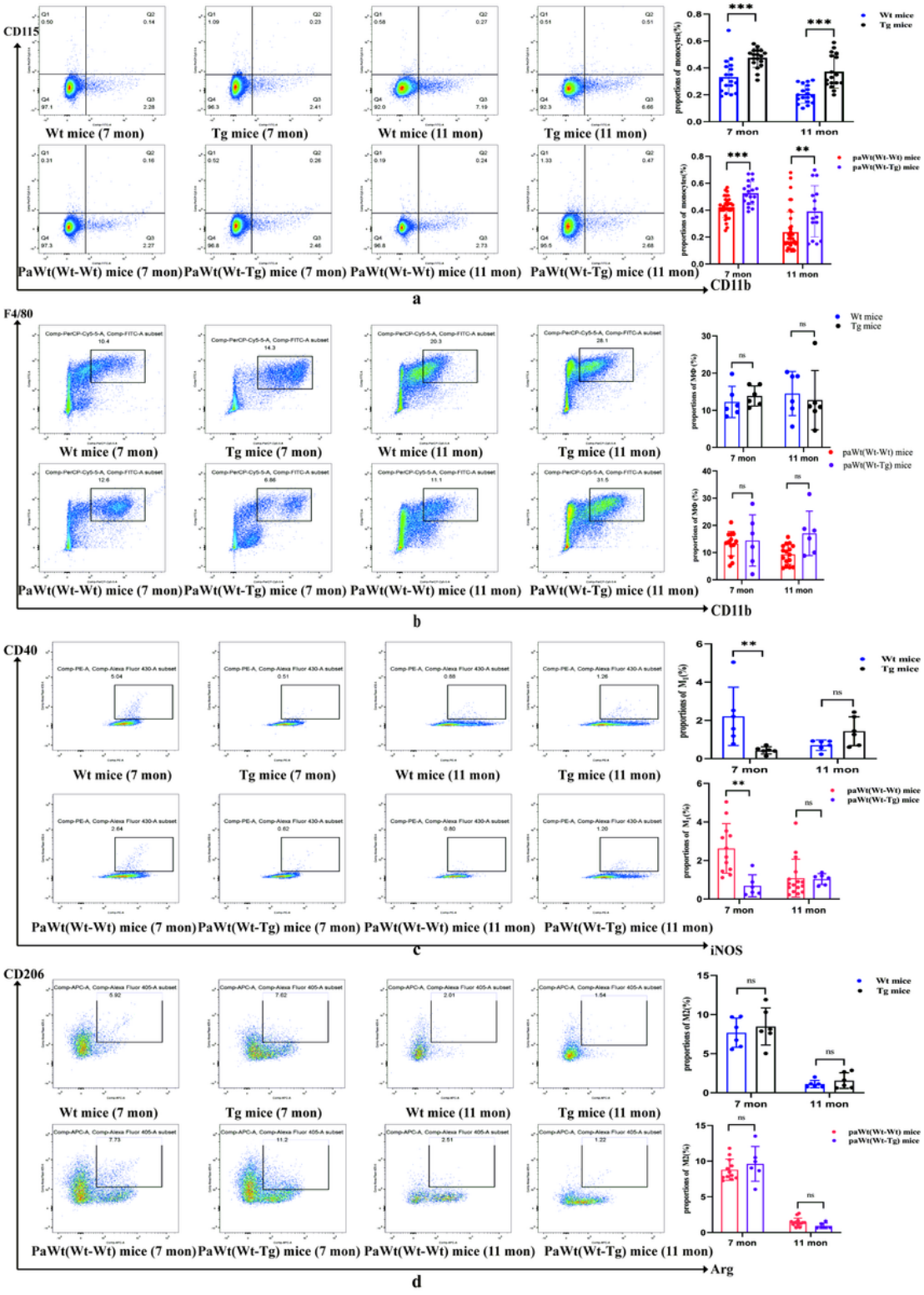


Figure 2

Alteration in the amounts of monocytes and macrophages. a: Alterations in the proportion of monocytes in Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. Monocytes were stained with CD11b+ CD115+. b: Alterations in the proportion of macrophages in Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. Macrophages were stained with CD11b+ F4/80+. c: Alterations in the proportion of pro-inflammatory macrophages in Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. The pro-inflammatory macrophages were stained with CD206+ Arg+. d: The alterations in the proportion of anti-inflammatory macrophages in Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. The anti-inflammatory macrophages were stained with CD40+ iNOS+. (n=6 for each group, Mean±SD, one-way analysis of variance, ***P<0.001, **P<0.01, *P<0.05, ns denotes no statistical significance).

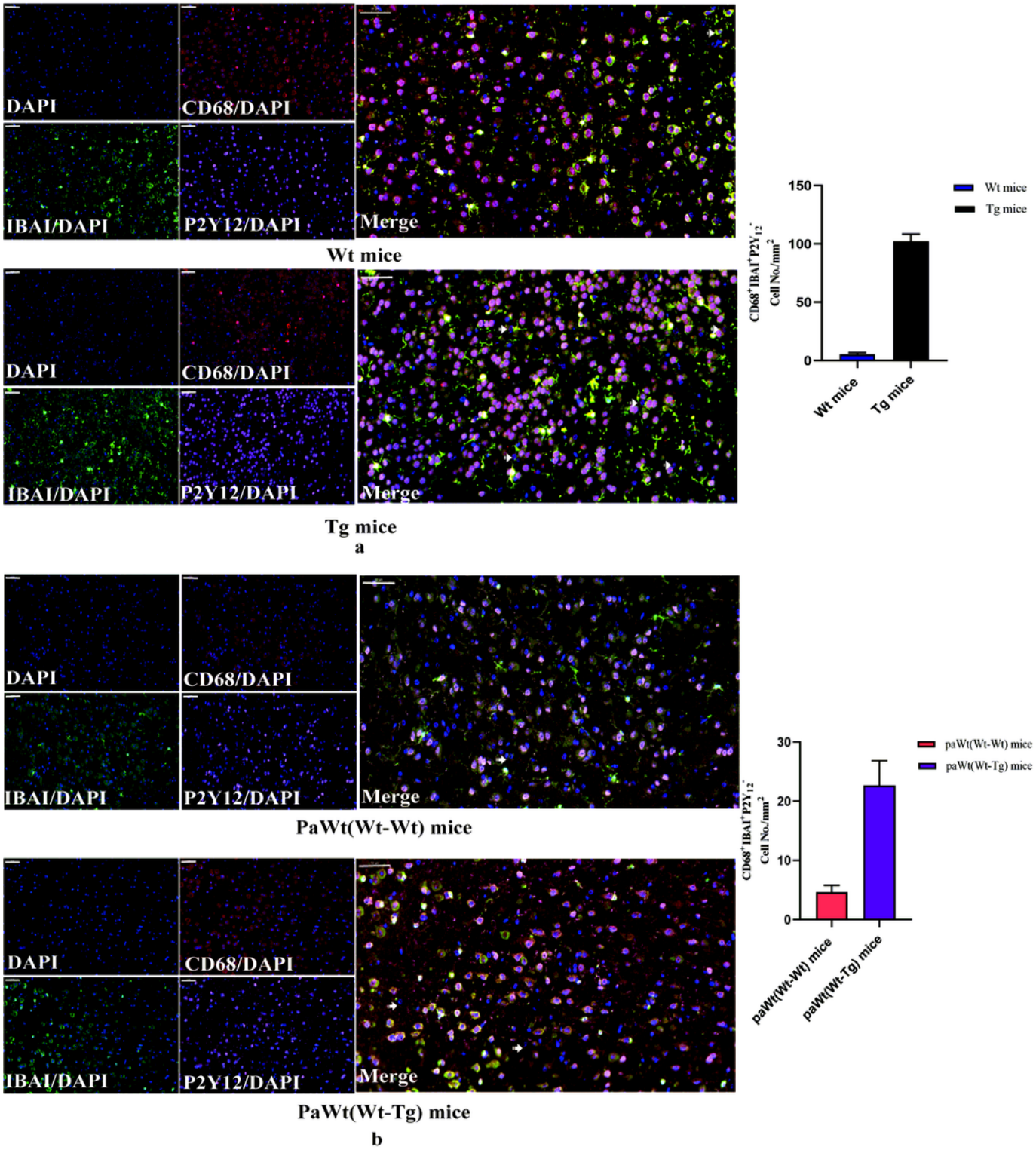


Figure 3

BMDMs entered into the brain under AD-like pathology conditions. a: Immunofluorescence image of microglia and BMDM co-stained with CD68, IBAI, and P2Y12 antibodies in Wt and Tg mice brain at the age of 11 months. Scale bars: 500 μm. b: Immunofluorescence image of microglia and BMDM co-stained with CD68, IBAI, and P2Y12 antibodies in paWt(Wt-Wt) and paWt(Wt-Tg) mice brain at the age of 11 months. Scale bars 500 μm. (***P<0.001, **P<0.01, ns denotes no statistical significance).

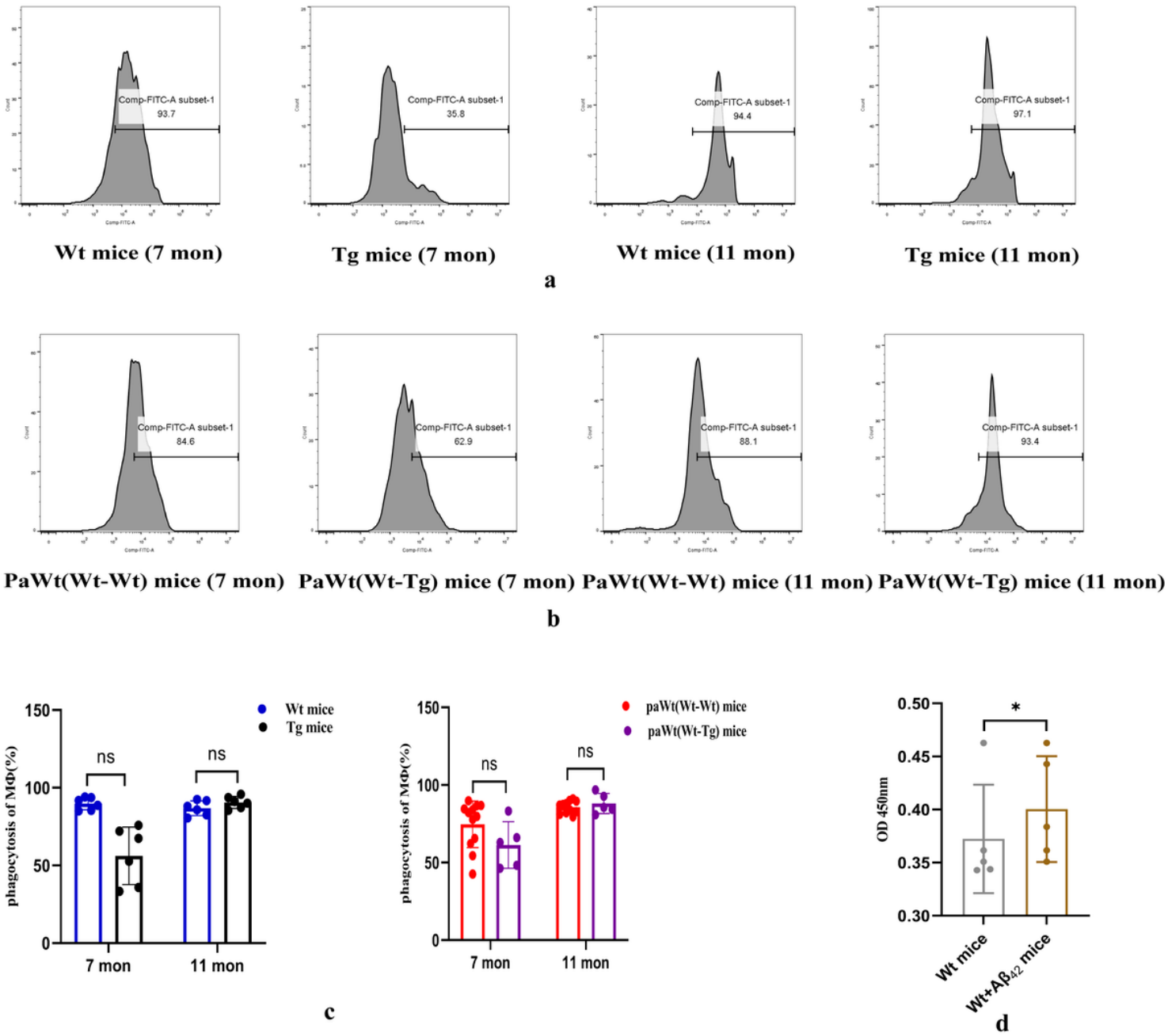
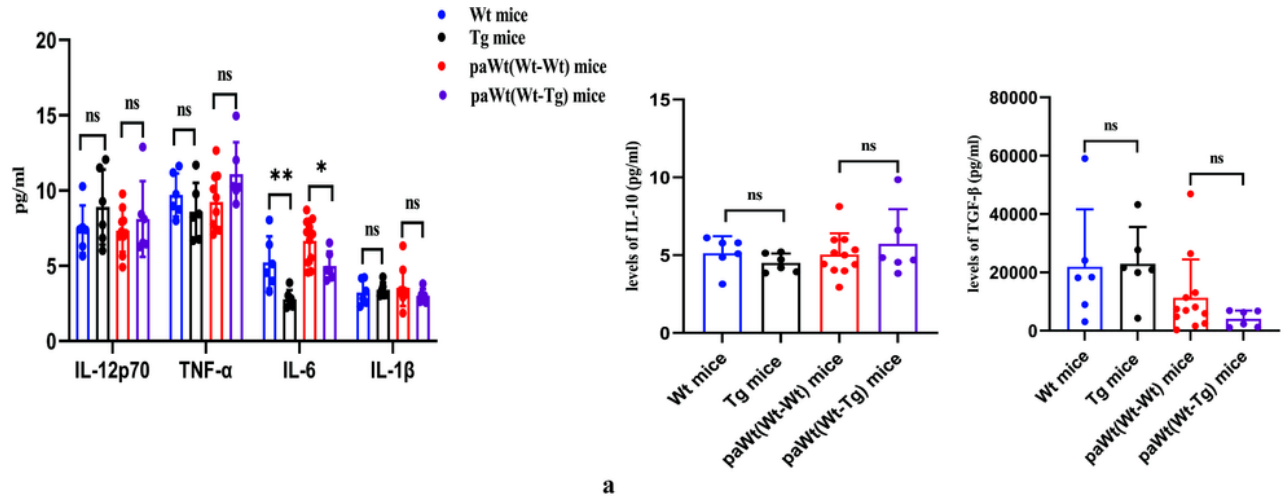
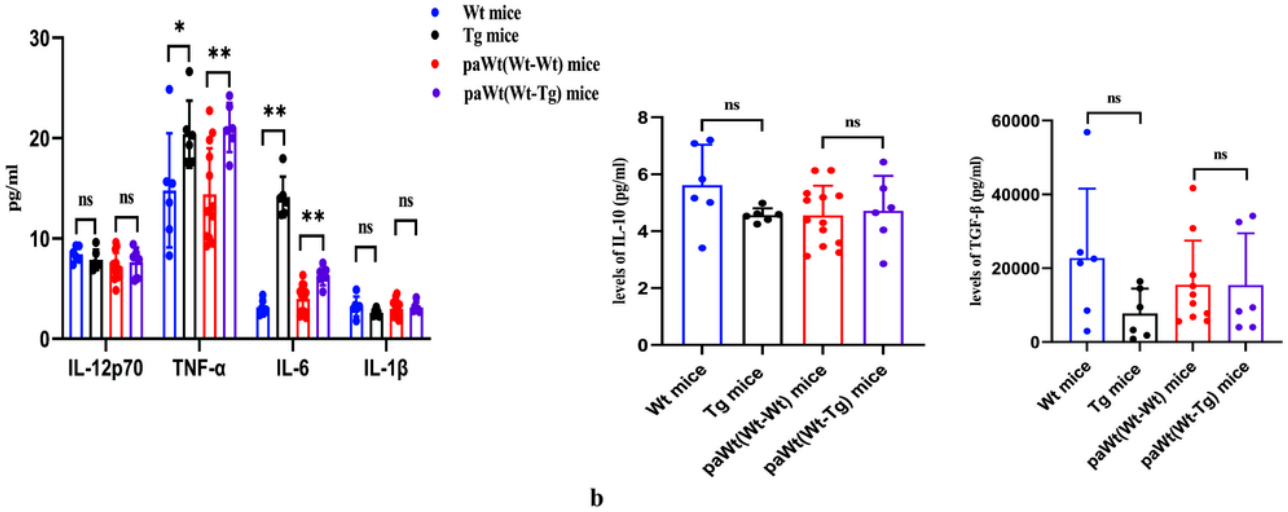


Figure 4

Changes in the phagocytosis ability of macrophages. a: Phagocytosis ability of macrophages in Wt and Tg mice. Macrophages were stained with CD11b+ F4/80+. b: Phagocytosis ability of macrophages in paWt(Wt-Wt) and paWt(Wt-Tg) mice. c: Phagocytosis ability of macrophages in Wt and Tg mice displayed no difference. d: Phagocytosis ability of macrophages in paWt(Wt-Wt) and paWt(Wt-Tg) mice showed no difference. e: Phagocytosis ability of macrophages after co-culture with Aβ42 peptide in vitro. (n=6 for each group, Mean ± SD, one-way analysis of variance, *P<0.05, ns denotes no statistical significance).





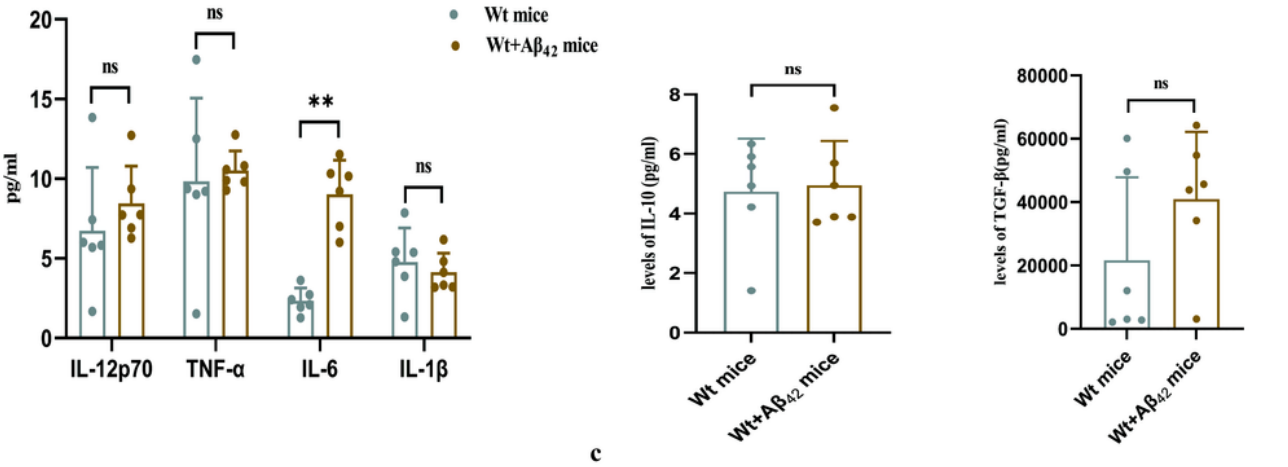


Figure 5

Changes in the secretory functions of macrophages. a: Cytokine levels in plasma of Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice at of 7 months of age. b. Plasma cytokine levels of Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice at the age of 11 months. c: Plasma cytokine levels of Wt and Wt mice after tail intravenous injection with Aβ42 peptide. (n=6 for each group, Mean ± SD, one-way analysis of variance, **P<0.01, *P<0.05, ns denotes no statistical significance).

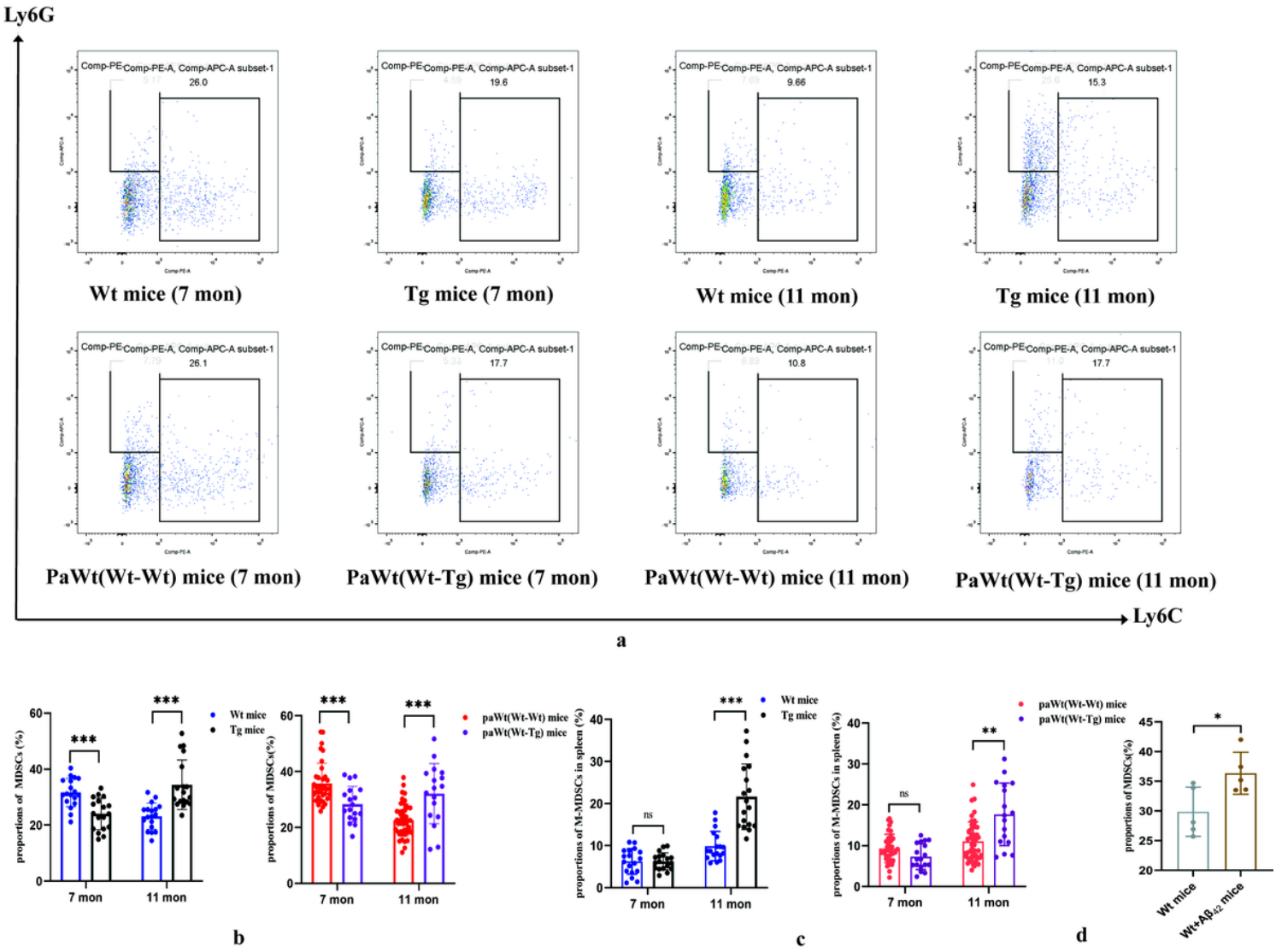


Figure 6

Alterations in the proportion of MDSCs in the spleen. a: Alterations of the proportion of MDSCs in the spleen of Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. M-MDSCs were stained with Ly6C+Ly6G-, MDSCs were stained with Ly6C-Ly6G+. b: The proportions of MDSCs in the spleen of Wt and Tg, paWt(Wt-Wt) and paWt(Wt-Tg) mice were significantly different. c: The proportions of M-MDSCs in the spleens of Wt and Tg, paWt(Wt-Wt) and paWt(Wt-Tg) mice displayed significant difference. d: The alterations in the proportions of MDSCs in the spleen after intravenous injection with Aβ42. (n=6 for each group, Mean±SD, one-way analysis of variance, ***P<0.001, **P<0.01, ns denotes no statistical significance).

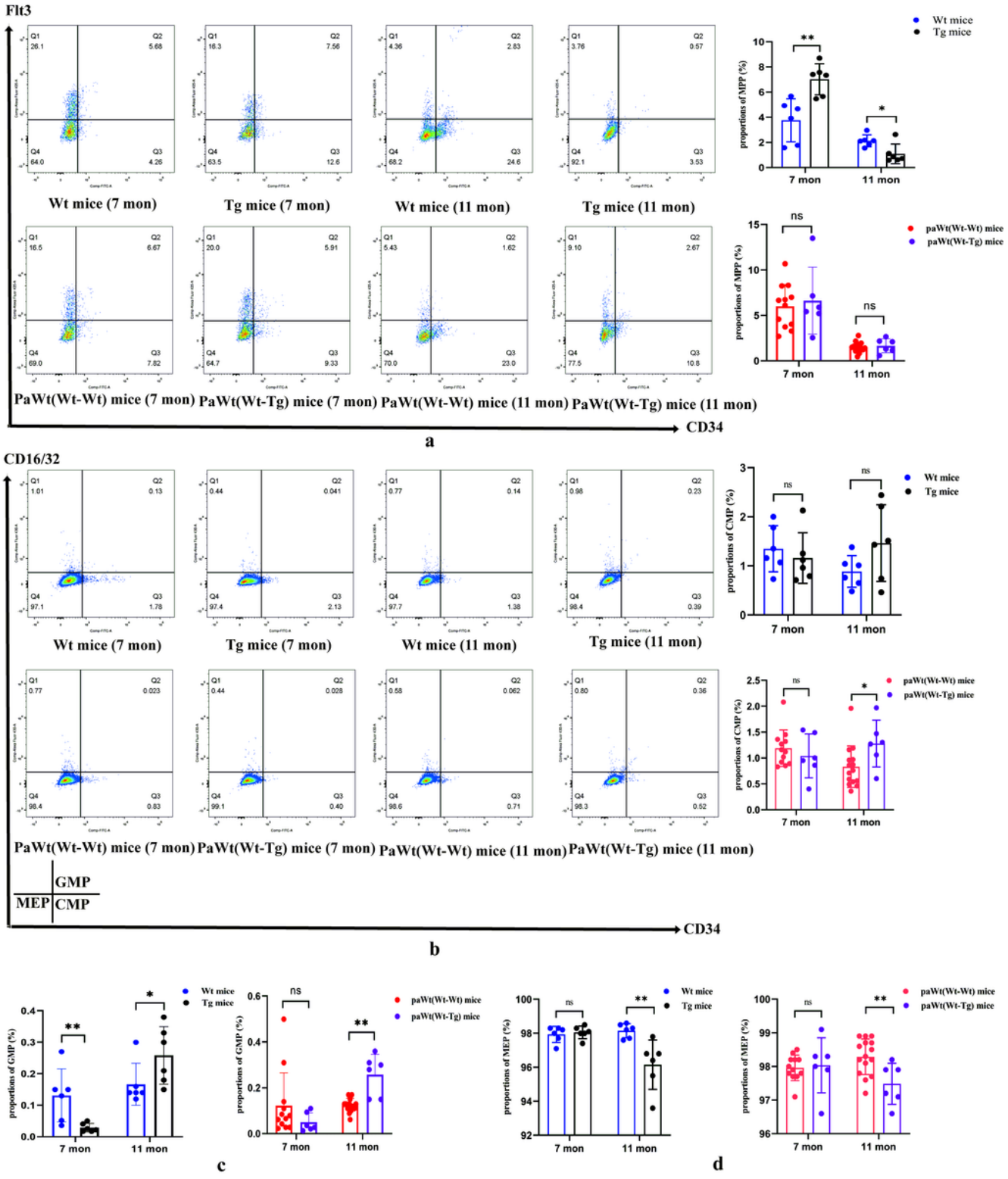


Figure 7

Alterations in the proportion of HSCs in the bone marrow. a: The proportions of MPP in the bone marrow of Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. MPP were stained with CD34+CD16/32+. b: The proportions of CMP in bone marrow of Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. CMP were stained with CD34+CD16/32-. c: The proportions of GMP in bone marrow of Wt, Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. GMP were stained with CD34+CD16/32+. d: The proportions of MEP in the bone marrow of Wt,

Tg, paWt(Wt-Wt), and paWt(Wt-Tg) mice. MEP were stained with CD34-CD16/32-. (n=6 for each group, Mean±SD, one-way analysis of variance, **P<0.01, *P<0.05, ns denotes no statistical significance).

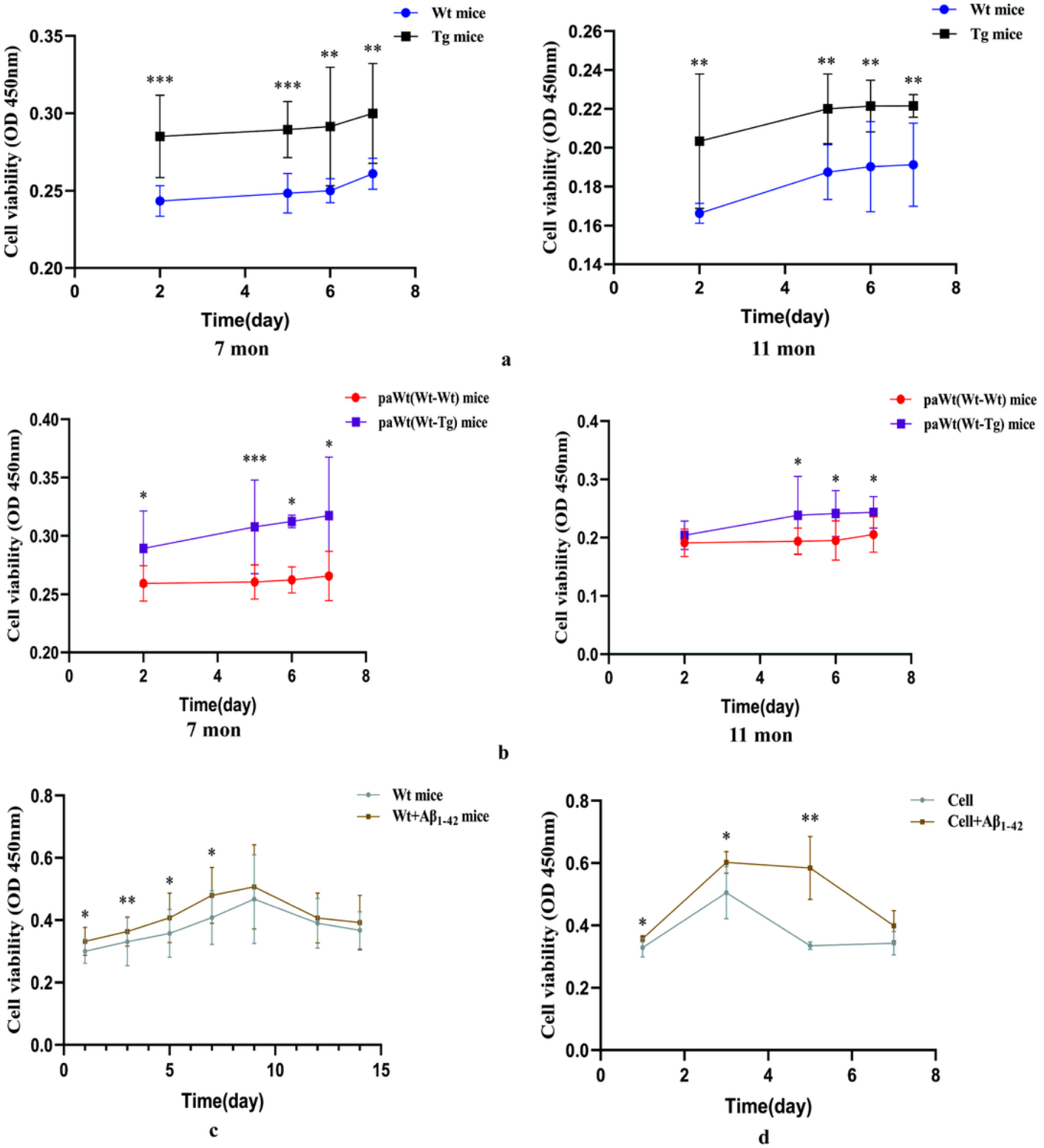


Figure 8

Changes in the proliferation of BMCs. a: The proliferation of BMCs in Wt and Tg mice at the age of 7 and 11 months. b: The proliferation of BMCs in paWt(Wt-Wt) and paWt(Wt-Tg) mice at the age of 7 and 11 months. c: The proliferation of BMCs in Wt and Wt with Aβ42 peptide mice group. d: The proliferation of

BMCs following co-culture with Aβ42 peptide in vitro. (n=6 for each group, Mean±SD, one-way analysis of variance, ***P<0.001, **P<0.01, *P<0.05, ns denotes no statistical significance).

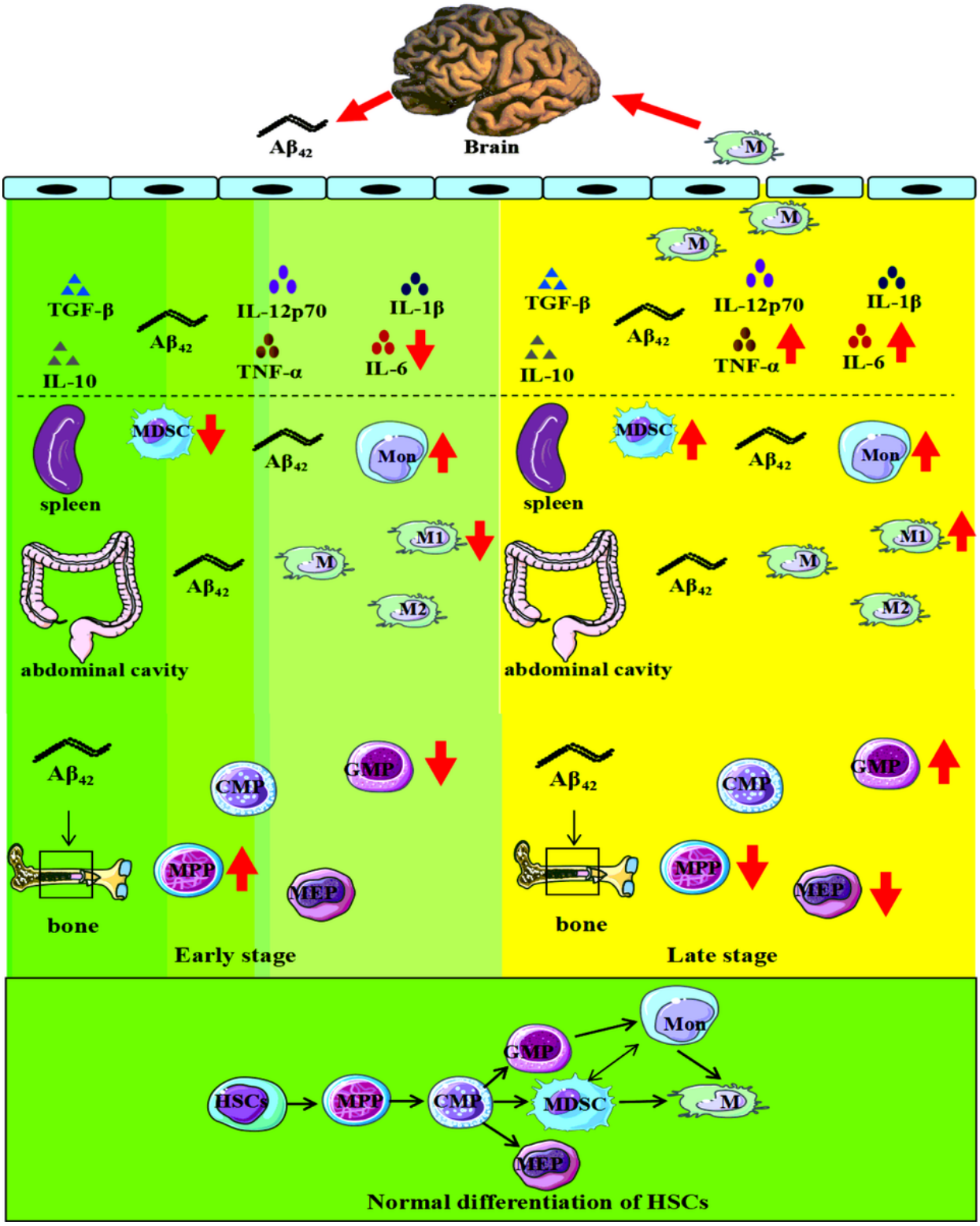


Figure 9

Schematic diagram depicting the effects of high plasma levels of Aβ42 on peripheral innate immune cells and HSCs In the early stage (7 month-old) of AD in these mice models, the high levels of plasma Aβ42 significantly decreased the amounts of peripheral pro-inflammatory macrophages and MDSCs,

GMP, as well as the plasma levels of IL-6. In the late stage (11-month-old), the high levels of plasma Aβ42 significantly increased the amounts of peripheral pro-inflammatory macrophages and MDSCs, GMP, and plasma levels of IL-6 and TNF-α, as well as the brain infiltration of BMDMs. In addition, the high plasma levels of Aβ42 consistently and significantly increased the amounts of peripheral monocytes in both the early and late stages of AD in these mice models.

Supplementary Files

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