

Article

Amelioration of Experimental Autoimmune Encephalomyelitis in Alzheimer's Disease Mouse Models: A Potential Role for A β

Changjie Shi ^{1,†}, Jiaxue Cha ^{1,†}, Junyuan Gong ¹, Shaodeng Wang ¹, Peng Zeng ¹, Junjiang Lian ¹, Bowen Zhang ¹, QiuHong Hua ¹, Jie Lv ¹, Changsheng Du ¹, Xin Xie ² and Ru Zhang ^{1,*}

- ¹ Shanghai Key Laboratory of Signaling and Disease Research, Laboratory of Receptor-Based Bio-Medicine, Collaborative Innovation Center for Brain Science, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China; 11006@tongji.edu.cn (C.S.); chajiaxue@tongji.edu.cn (J.C.); 1831520@tongji.edu.cn (J.G.); shaodengwang@tongji.edu.cn (S.W.); 1410778@tongji.edu.cn (P.Z.); 1710547@tongji.edu.cn (J.L.); 1810545@tongji.edu.cn (B.Z.); 96811@tongji.edu.cn (Q.H.); 18310109@tongji.edu.cn (J.L.); duchangsheng@tongji.edu.cn (C.D.)
- ² CAS Key Laboratory of Receptor Research, The National Center for Drug Screening, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; xxie@simm.ac.cn
- * Correspondence: ru.zhang@tongji.edu.cn; Tel.: +86-216-598-6852
- † These authors contributed equally to this work.



Citation: Shi, C.; Cha, J.; Gong, J.; Wang, S.; Zeng, P.; Lian, J.; Zhang, B.; Hua, Q.; Lv, J.; Du, C.; et al. Amelioration of Experimental Autoimmune Encephalomyelitis in Alzheimer's Disease Mouse Models: A Potential Role for A β . *Cells* **2022**, *11*, 1004. <https://doi.org/10.3390/cells11061004>

Academic Editors: Adrian Israelson and Frédéric Checler

Received: 20 January 2022

Accepted: 14 March 2022

Published: 16 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Emerging data have highlighted the coexistence of multiple sclerosis (MS) and Alzheimer's disease (AD), both of which are common central nervous system degenerative diseases with a heavy burden on patients, their families, and society. However, it is unclear how MS progresses under an AD pathological background. We aimed to address the question of how MS progresses under an AD pathological background. We induced the experimental autoimmune encephalomyelitis (EAE) model of MS in two types of AD mouse models, Tg6799 and APP/PS1 mice. We found that, compared with wild-type mice, the clinical symptoms of EAE were significantly ameliorated in APP/PS1 mice but not in Tg6799 mice. Moreover, a much lower level of serum A β was observed in Tg6799 mice. EAE clinical symptoms in Tg6799 and C57BL/6J mice were ameliorated by intraperitoneal injection of A β 42. Peripheral administration of A β 42 peptides was able to inhibit Th17 development in vivo, which is likely to occur through the inhibition of IL-6 production in dendritic cells. Our findings revealed that AD and EAE could coexist in the same mouse, and A β residing in peripheral circulation likely plays an anti-inflammatory role in preventing EAE progression. These findings reveal the potential benefit of A β , one of the supervillains of AD, at least in certain contexts.

Keywords: amyloid- β peptide; experimental autoimmune encephalomyelitis; multiple sclerosis; Alzheimer's disease; Th17

1. Introduction

Multiple sclerosis (MS) is a chronic neuroinflammatory and demyelinating autoimmune disorder of the central nervous system characterized by damage to neurons and their protective myelin sheath in both the brain and spinal cord. This disorder often causes disability in young people. MS is a predominantly T-cell mediated autoimmune disorder, and CD4+ T cells, especially the Th17 and Th1 subgroups, have been suggested to cause the early initiation of the disease [1]. Experimental autoimmune encephalomyelitis (EAE) is one of the widely used animal models of MS [2,3], which shares many clinical, pathological, and histological similarities with MS, such as inflammation and axonal degeneration [4].

Alzheimer's disease (AD) is the most common form of dementia, with extracellular amyloid plaque deposition and neuron damage as the primary pathological hallmarks. It is widely accepted that amyloid β (A β) accumulation in hippocampal and cortical regions of the brain is critical to neurodegeneration and inflammation in AD [5–7]. A β peptides, mainly A β 40 and A β 42, are derived from the sequential processing of the transmembrane

amyloid precursor protein (APP) by β - and γ -secretase [8–10]. A β accumulation is generally considered detrimental to human health; however, a few reports have indicated various physiological functions of A β [11]. Interestingly, previous reports showed that A β expression could protect against microbial infection in AD mice [12], and peripheral administration of A β led to a reversal of paralysis and reduced inflammation in a multiple sclerosis mouse model [13]. The latter raises the question of whether MS and AD can coexist.

MS and AD are both relatively common CNS degenerative diseases that result in a heavy burden on patients, their families, and society. However, there is limited information concerning the coexistence of MS with AD. Recently, one study analyzed PubMed citations and found that MS is frequently co-cited with Alzheimer's, indicating that MS is closely associated with Alzheimer's disease [14]. A recent review tried to explore the coexistence of MS and AD via a focused literature search to identify any reports of individuals with both MS and AD [15]. Twenty-four individuals were found with pathological features of both MS and AD in case series or reports, which provide evidence that AD and MS can coexist in the same person. However, little is known concerning how AD influences MS, or vice versa.

In this study, we aimed to address the question of how MS progresses under an AD pathological background. EAE was established in two types of AD mouse models, APP^{swe}/PSEN1 Δ E9 (APP/PS1) and 5xFAD transgenic (Tg6799) mice, and we found that the clinical symptoms of EAE were significantly ameliorated in APP/PS1 mice compared to their wild-type (WT) littermates. Unexpectedly, the alleviation of EAE was not observed in the other AD mouse model, Tg6799 transgenic mice. Interestingly, further investigations revealed that the serum A β level in Tg6799 mice was relatively low, and EAE clinical symptoms of Tg6799 mice were ameliorated by intraperitoneal injection of A β 42. Similar results were obtained in C57BL/6J mice after peripheral administration of A β 42 peptides. Our observations indicated that under an AD pathological background, the serum soluble A β level rather than insoluble senile plaques of A β in the brain had an important role in ameliorating EAE. Furthermore, we found that exogenous administration of A β 42 peptides could inhibit Th17 development and IL-6 production by DCs, which might therefore block EAE inflammation *in vivo*.

2. Materials and Methods

2.1. Animals and Reagents

APP/PS1 transgenic mice (Jackson Laboratory, Bar Harbor, ME, USA, stock number 004462) express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695^{swe}) and a mutant human presenilin 1 (PS1-dE9). 5xFAD transgenic mice (Tg6799, Jackson Laboratory) overexpress mutant human amyloid-beta precursor protein 695 (APP) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) familial Alzheimer's disease (FAD) mutations, along with human presenilin 1 (PS1) harboring two FAD mutations, M146L, and L286V.

C57BL/6J mice (Shanghai Laboratory Animal Center, Shanghai, China), MOG35-55 (MEVGWYRSPFSRVVHLYRNGK, GL Biochem, Shanghai, China), and A β 42 (purity > 95%, GL Biochem, Shanghai, China) were purchased as indicated. Human β amyloid 40/42/total ELISA kits were obtained from Shanghai ExCell Biology, Inc., Shanghai, China. Thioflavin S was obtained from Sigma, St. Louis, MO, USA. All experimental procedures were approved by the Tongji University Animal Care Committee.

2.2. EAE Induction and Intraperitoneal Injection of A β 42

EAE was induced in 4-month-old female Tg6799, APP/PS1, and C57BL/6J mice by administering 200 μ g of MOG35-55 in complete Freund's adjuvant that contained 5 mg/mL heat-killed *Mycobacterium tuberculosis* H37RA. Pertussis toxin (200 ng/mouse) was injected intraperitoneally on day 0 and day 2. EAE severity was evaluated as described in [16–18]. Briefly, EAE clinical signs were scored every day as follows: 0, normal; 1, only paralyzed tail; 2, paresis (weakness or partial paralysis of hind limbs); 3, paraplegia (totally

paralyzed hind legs); 4, paraplegia with forelimb weakness or paralysis; and 5, moribund or death. For A β 42 experimental interventions, 10-week-old C57BL/6J and Tg6799 mice received injections of 50 μ g A β 42 peptides [13] or PBS twice a day from day 5 or day 12 until the experimental endpoint. To evaluate the therapeutic effects of A β 42, A β 42 treatment was started on day 12 after MOG immunization, when EAE mice developed mild clinical symptoms. Next, to further explore the preventive and therapeutic effects of A β 42, A β 42 treatment was started on day 5 after MOG immunization, prior to the onset of clinical symptoms.

2.3. Histopathological Analysis and Thioflavin-S Staining of Senile Plaques

For histological staining, mice were anesthetized and perfused with PBS at the end of the experiment. The spinal cords and left hemibrains were carefully removed and fixed in 4% (*w/v*) paraformaldehyde overnight. The samples were then soaked in 30% sucrose (in PBS) for 24 h and embedded in optimum cutting temperature compound (OCT). Then, 14 μ m spinal cord sections were collected for staining with hematoxylin and eosin (H&E) to analyze infiltrating cells. Thioflavin-S staining was performed on 30 μ m brain sections of 4-month-old mice. Briefly, the brain sections were rinsed in PBS three times for 5 min each, immersed in 0.1% (*w/v*) thioflavin S for 8 min, and then washed in PBS and 70% alcohol for 5 min each.

2.4. ELISA Analysis

First, we isolated the cerebral cortex and hippocampus from the right hemibrains. Subsequently, A β 40 and A β 42 in the SDS and FA extracts of the brain tissues were measured by ELISA. Orbital blood was collected from the mice in each group, and serum was isolated. The serum A β levels were determined by using human total A β ELISA kits. The serum IL-6 levels were quantified by ELISA following the manufacturer's instructions.

2.5. CD4+ T Cell Separation and In Vitro Differentiation

Naive CD4+ T cells were isolated from spleens of 8-week-old female C57BL/6J mice as previously described [19]. Cells were activated with anti-CD3 (2 μ g/mL) and anti-CD28 (2 μ g/mL). For Th1 differentiation, cells were stimulated with IL-12 (10 ng/mL) and anti-IL-4 (10 μ g/mL). For Th17 differentiation, cells were treated with anti-IL-4 (10 μ g/mL) and anti-IFN- γ (10 μ g/mL), together with a cytokine mixture containing IL-6 (30 ng/mL), IL-23 (10 ng/mL), IL-1 β (10 ng/mL), TNF- α (10 ng/mL), and TGF- β 1 (3 ng/mL). Treg polarization was induced by adding anti-IFN- γ (10 μ g/mL), IL-2 (10 ng/mL), and TGF- β 1 (5 ng/mL).

2.6. DC Isolation, Stimulation, IL-6, and Cell Toxicity Measurement

Mouse CD11c+ DC cells were isolated from the lymph nodes of mice by MACS (Miltenyi Biotec, Bergisch Gladbach, Germany). Then, DCs were stimulated with anti-CD40 (10 μ g/mL). IL-6 was measured in the supernatants of cultured DCs receiving no treatment or receiving treatment with anti-CD40 alone or in combination with A β 42 (0.5, 5, or 50 ng/mL). Cell Counting Kit-8 was used to test cell toxicity.

2.7. Flow Cytometry

The percentage of CD4 + Foxp3+ Tregs was analyzed using flow cytometry (FACS). The percentage of Th1 or Th17 cells was evaluated by FACS; only cells positive for IFN- γ (IL-17-) were defined as Th1 cells, whereas cells positive for IL-17 (IFN- γ -) were considered Th17 cells.

2.8. Statistical Analysis

Data are expressed as mean \pm SEM. A two-way ANOVA test was applied to analyze the significance of EAE clinical scores. Student's *t*-test was used to assess other analyses, and *p* values less than 0.05 (*p* < 0.05) were considered statistically significant.

3. Results

3.1. Clinical Symptoms Were Ameliorated When EAE Was Induced in APP/PS1 Mice, but Not in Tg6799 Transgenic Mice

To investigate how MS progresses in AD, we examined the progression of EAE in two popular AD models, Tg6799 and APP^{swe}/PS1 Δ 9 (APP/PS1) mice. We induced EAE with MOG_{35–55} in 4-month-old AD mice and their littermates (wild type, WT) at the same age. WT mice exhibited a typical course of EAE and began to develop clinical signs on day 14 postimmunization. Compared with WT, the onset of EAE in APP/PS1 mice was delayed until day 16 postimmunization, and the peak severity and cumulative clinical score were significantly reduced in APP/PS1 mice (Figure 1a). Surprisingly, this amelioration of EAE clinical symptoms was not observed in Tg6799 AD mice (Figure 1d).

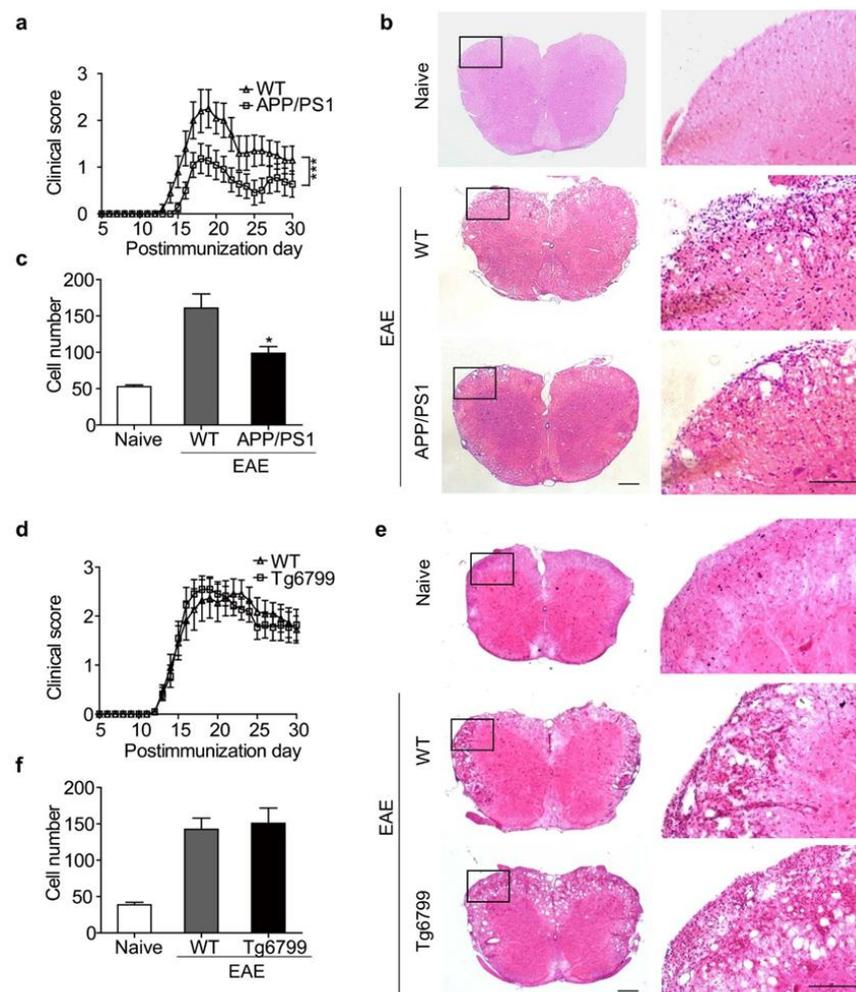


Figure 1. Clinical symptoms of EAE in APP/PS1 and Tg6799 transgenic mice. (a) Clinical scores of EAE in WT and APP/PS1 mice. Results are presented as daily mean clinical scores \pm SEM. *** $p < 0.001$, two-way ANOVA (WT, $n = 10$; APP/PS1, $n = 11$). (b) Hematoxylin and eosin (H&E)-stained slides of spinal cords from naive, WT, and APP/PS1. (c) Quantification of infiltrating cells. Data are mean \pm SEM, * $p < 0.05$ (Student's t -test). (d) Clinical scores of EAE in WT and Tg6799 mice ($n = 11$ per group). (e,f) H&E staining was performed to analyze infiltrating cells. The boxed areas in the left columns are presented enlarged on the right. Scale bars, 200 μ m.

Histological examination of the spinal cord was performed on day 30 postimmunization. The WT mice showed typical leukocyte infiltration in the spinal cord, whereas the APP/PS1 mice had much fewer infiltrating leukocytes in the spinal cord (Figure 1b,c). Consistent with the previous clinical score, Tg6799 mice showed similar cell infiltration to

WT mice (Figure 1e,f). These results indicate that some beneficial factors preventing EAE exist in APP/PS1 mice but not in Tg6799 AD mice.

3.2. Distinct A β Pathology in APP/PS1 and Tg6799 Mice

APP/PS1 and Tg6799 are both commonly used AD mouse models showing the age-related progression of A β plaque deposits in the brain and cognitive decline [20]. To gain insight into the factors responsible for their different reactions to EAE, we first performed a detailed investigation of A β pathology in these mice. Thioflavin-S staining revealed large A β plaque deposits in the cortex and hippocampus in 4-month-old Tg6799 mice (Figure 2a–c) but not in WT mice. In APP/PS1 mice, only a few A β plaque deposits in the brain were visible at this age. When using SDS and FA to lyse the brain, A β 40/42 accumulation was observed in both Tg6799 and APP/PS1 mice, but much higher A β levels were detected in Tg6799 (Figure 2d–g). This phenomenon is consistent with a previous report showing accelerated plaque development and even earlier intraneuronal A β 42 accumulation at 1.5 months of age in Tg6799 strains [21]. Furthermore, we measured the serum A β level in Tg6799 and APP/PS1 mice using ELISA (Figure 2h). Unexpectedly, the serum A β level was nearly undetectable in Tg6799 mice, whereas in APP/PS1 mice of the same age, the serum total A β reached 3355 pg/mL on average. This result raises the possibility that the serum A β , rather than that in the brain, leads to a reduction in paralysis of EAE in APP/PS1 mice. This suggestion is reminiscent of a previous report showing that peripheral administration of A β in mice could alleviate EAE [13].

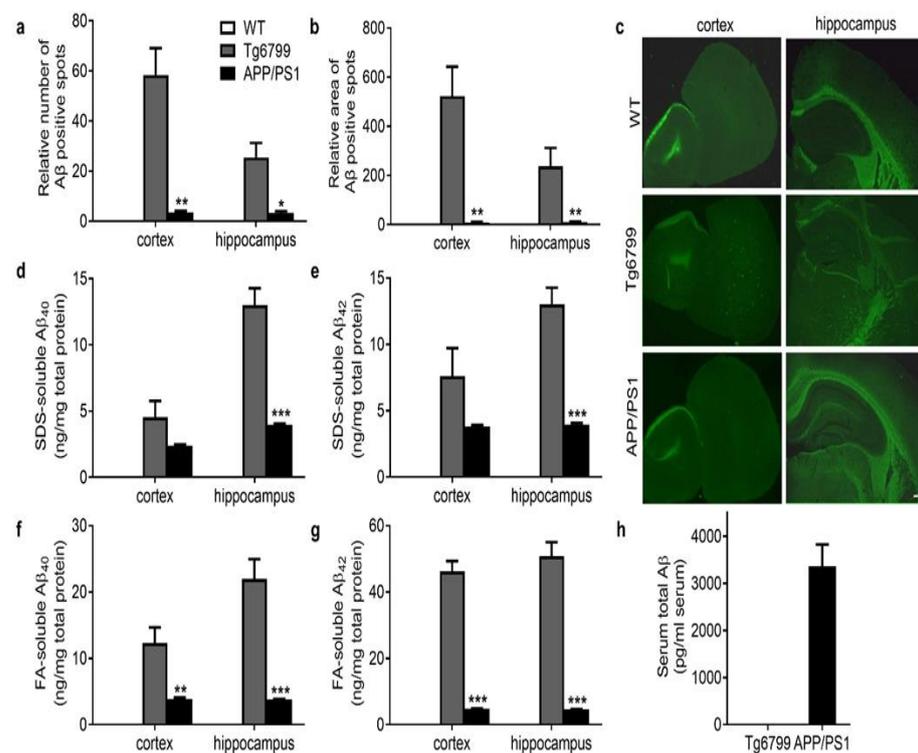


Figure 2. Serum and brain A β levels in Tg6799 and APP/PS1 transgenic mice. (a,b) The relative number and area of A β plaques in cortex and hippocampus of WT, Tg6799, and APP/PS1 mice were quantified. (c) Representative images of senile plaques with thioflavin-S staining in cortex and hippocampus sections. (d–g) Brain A β levels, including (d,e) SDS-soluble A β 42 and A β 40 and (f,g) FA-soluble A β 42 and A β 40 in the cortex and hippocampus of WT, Tg6799, and APP/PS1 mice by ELISA. $n = 3–5$. Data are mean \pm SEM, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ (Student's t -test). (h) Serum A β levels in Tg6799 and APP/PS1 transgenic mice were measured by using the human total A β ELISA kits. $n = 6$. Scale bars, 200 μ m.

3.3. Intraperitoneal Administration of A β 42 Peptides Ameliorated the Clinical Symptoms of EAE in Tg6799 Mice

To test whether the serum A β level is one of the key factors interfering with EAE severity, systemic administration of A β was carried out before EAE onset in Tg6799 mice. Either 50 μ g of synthesized A β 42 peptides or the PBS vehicle control was administered twice daily via intraperitoneal injection from day 5 post-EAE induction until the end of the study. Strikingly, A β 42 peptide administration was able to not only significantly reduce the peak severity and cumulative clinical score of EAE but also delay the onset of the disease in Tg6799 mice (Figure 3a–c).

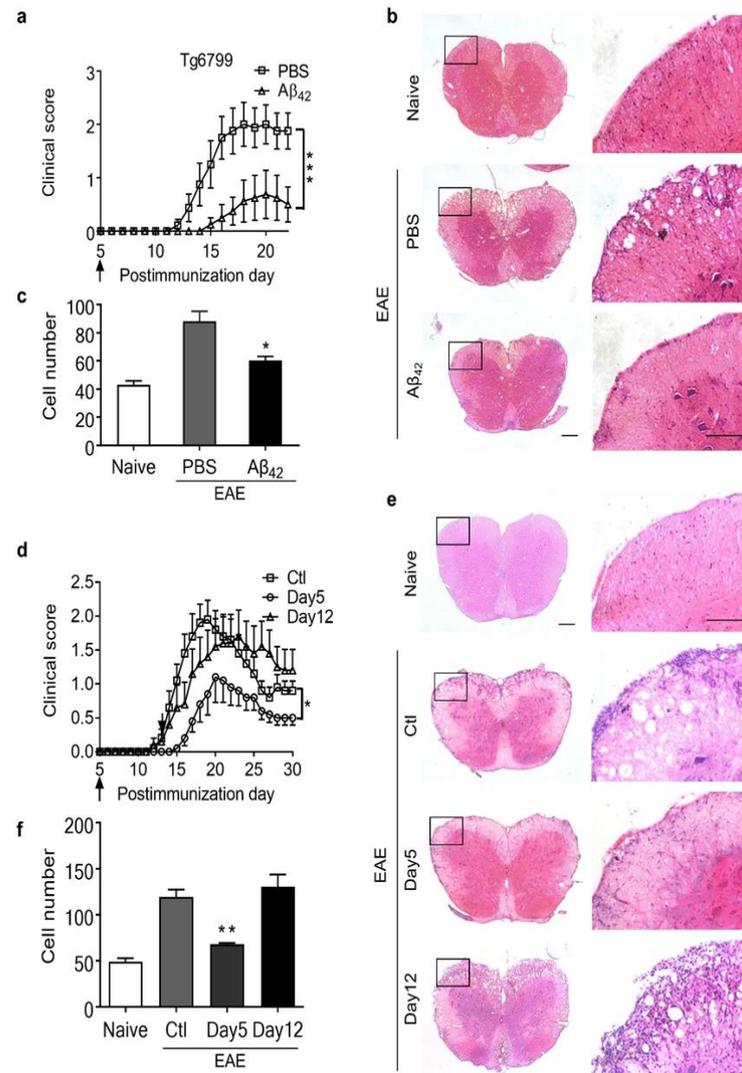


Figure 3. Reduction in EAE clinical symptoms by intraperitoneal injection of A β 42 peptides. **(a)** Clinical scores of EAE mice treated with A β 42 peptides or PBS in Tg6799 mice. EAE was induced in 10-week Tg6799 mice by immunization with MOG35–55 peptide, A β 42 peptides, or PBS, which were administered daily via intraperitoneal injection from day 5 postimmunization. Data are mean \pm SEM, *** $p < 0.001$ (PBS group, $n = 9$; A β 42 group, $n = 9$). **(b,c)** H&E staining of spinal cord sections and quantification of infiltrating cells. * $p < 0.05$ (Student's t -test). **(d)** Clinical scores (mean \pm SEM) of MOG-immunized mice treated with A β 42 peptides or PBS in 10-week C57BL/6J mice. A β 42 peptides or PBS were administered daily via intraperitoneal injection from day 5 or day 12 postimmunization. * $p < 0.05$ ($n = 10$). **(e,f)** H&E staining of spinal cord sections and quantification of infiltrating cells. Data are mean \pm SEM, ** $p < 0.01$ (Student's t -test). The boxed areas in the left columns are presented enlarged on the right. Scale bars, 200 μ m.

To determine whether the protective effect of A β treatment on EAE was restricted to the AD models, we treated C57BL/6J mice with A β 42 or PBS twice daily from day 5 or day 12 postimmunization. We found that peripheral injection of A β 42 from day 5 postimmunization could eliminate EAE clinical symptoms, as well as infiltrating cells, in normal mice (Figure 3d–f). More interestingly, the protective effect of A β against EAE was dependent on the time point of administration. When administered after the onset of the disease (day 12 postimmunization), A β 42 was unable to reduce the severity of EAE in C57BL/6J mice (Figure 3d), which indicates a preventative effect rather than a therapeutic benefit of this peptide in these mice.

3.4. A β 42 Regulated Th17 Development by Reducing IL-6 Production by DCs

Th1 and Th17 cells are known to be the main pathogenic CD4+ T effector cells in EAE [22]. To gain further insight into the mechanism of A β 42 protective effects in EAE, we analyzed the subpopulation of CD4+ T cells in the spleen of A β 42-treated or untreated mice. Compared to PBS-treated EAE mice, the percentage of Th17 cells in the CD4+ cells was significantly lower in A β 42-treated mice, whereas no significant change in the Th1 percentage was observed (Figure 4a,b). Next, we tested whether A β 42 directly affects T cell differentiation using an in vitro T cell differentiation assay. Naïve CD4+ cells were isolated from the spleen of C57BL/6J mice and induced to differentiate into Th1, Th17, or Tregs under the corresponding polarization conditions. As shown in Figure 4d–f, administration of A β 42 did not affect Th1, Th17, or iTReg differentiation in vitro.

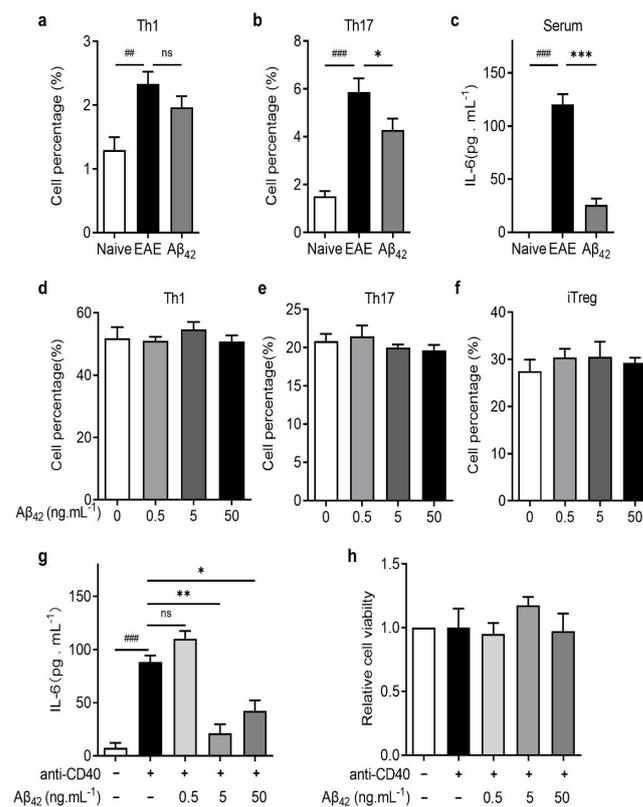


Figure 4. A β 42 reduced IL-6 production and inhibited Th17 development in vivo. (a,b) Th1 and Th17 cells were analyzed in vivo. (c) The levels of IL-6 in serum of naïve mice, EAE mice, and A β -treated EAE mice were measured in vivo. (d–f) Naïve CD4+ T cells isolated from C57BL/6J mice were induced to differentiate into Th1, Th17, and iTReg cells with the addition of different concentrations of A β 42 (0.5, 5, or 50 ng/mL) in vitro. (g,h) The IL-6 level and the relative viability of dendritic cells were measured by ELISA in vitro. Data are presented as mean \pm SEM, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ### $p < 0.001$, ## $p < 0.01$, ns: not significant ($n = 3$ –5, Student's t -test).

IL-6 is a critical pro-inflammatory cytokine for Th17 development [23]. Indeed, the serum concentration of IL-6 was significantly increased in PBS-treated EAE mice but decreased dramatically after A β 42 treatment (Figure 4c). DCs are the main antigen-presenting cells (APCs) in the periphery, which produce potent pro-inflammatory molecules upon maturation [24]. We then tested whether A β 42 can affect IL-6 production by DC cells. In the *in vitro* IL-6 production assay, DCs isolated from the lymph nodes of naive mice were stimulated with anti-CD40 alone or in combination with A β 42 at various concentrations. The anti-CD40 treatment enhanced IL-6 production by DCs, and A β 42 at 5 ng/mL and 50 ng/mL significantly decreased the anti-CD40-stimulated IL-6 production to nearly 75% and 50% (Figure 4g), without affecting the relative viability of DCs (Figure 4h). These data suggest that A β 42-mediated reduction in the Th17 subpopulation *in vivo* does not arise from a direct effect on T cell differentiation but is an indirect effect via modulation of upstream cytokine production by APCs.

4. Discussion

Alzheimer's disease and multiple sclerosis are relatively common neural degenerative diseases that cause CNS neuronal damage and activate inflammation. Due to the extended potential life span of humans and advancements in therapeutic strategies, people with MS are living longer than ever and will likely face the same age-related diseases, such as AD, as other seniors. In this study, we investigated EAE progression in two transgenic AD models, both with abundant A β plaque deposits in the brain. Interestingly, by inducing EAE in two AD mouse models, we found that MS clinical symptoms could still be established under an AD pathological background, which agrees with previous case reports of the coexistence of AD and MS in the same person [14,15].

More surprisingly, the clinical severity of induced EAE was distinct in the two AD models, which correlated with the serum A β 42 level in these mice but not with brain A β plaques. Both AD mice with a high serum level of A β 42 (APP/PS1) and mice with undetectable serum A β 42 (Tg6799) but receiving A β 42 peripherally showed alleviated EAE symptoms. These results not only showed us how MS-like disease might progress under an AD background but also reflected the two sides of A β . A β is mostly considered one of the supervillains of AD; however, it is highly conserved throughout evolution and may have crucial physiological roles [25]. The bad reputation of A β stems from its propensity to form aggregates called amyloid plaques in the brain that are regarded as characteristic pathological features of AD [26]. Current therapeutic efforts have been aimed at reducing the detrimental insoluble A β 42 peptide, but little attention has been devoted to its soluble precursor, which is proposed to have various physiological functions, such as regulating learning and memory, angiogenesis, and neurogenesis; repairing leaks in the blood–brain barrier; promoting recovery from injury; acting as an antimicrobial peptide and tumor suppressor; and modulating mitochondrial function and even neuroprotection [25]. Recent publications have shown that amyloid-positive individuals with normal cognition have higher soluble A β 42 levels in the brain than those with mild cognitive impairment or dementia, and higher soluble A β 42 levels in the human brain were associated with better cognitive performance and greater hippocampal volume [27,28]. Consistent with these reports, our findings in the current study showed that under an AD pathological background, the serum soluble A β level rather than insoluble senile plaques of A β in the brain had an important role in ameliorating EAE, supporting that the soluble, non-aggregating forms of A β 42 have some positive effects.

It was reported that blood A β levels were increased before dementia onset in patients with familial AD [29], and in some patients, the blood A β 42 level presented an initial increase followed by a decrease before and during the early stages of AD [27]. However, these patterns of blood A β in AD patients have not been found in other clinical analyses [30–32]. Nevertheless, many studies have been working on using the variation in A β 42 in the blood as an early diagnostic biomarker of AD prior to the appearance of clinical symptoms [33,34]. Consistent with our study, others reported that the serum A β 42 level reached 900 pg/mL

in 3-month-old APP/PS1 mice prior to the appearance of learning and memory deficits [35]. At present, the factor that leads to the especially high level of serum A β in APP/PS1 mice is unclear. Amyloid- β peptide is a substrate of the human 20S proteasome, and the decline in proteasome function associated with aging would contribute to increased A β levels [36–38]. Substantial evidence also points to ubiquitin–proteasome system (UPS) malfunction as an important factor playing a key role in A β amyloid growth and AD pathogenesis [39,40]. Therefore, proteasomal dysfunction could be one of the additional factors promoting A β accumulation, which needs to be further investigated in the future.

In the current study, we found that serum A β in AD mice conferred protection against EAE by regulating the balance between Th1, Th17, and iTreg, which are CD4+ T effector cells that have a pivotal role in the pathogenesis of MS and EAE [41]. We found that the administration of A β 42 can impair Th17 cell development, probably through a reduction in IL-6, an important cytokine secreted from DCs that governs the differentiation of Th17 cells in the development of EAE [42–44]. Therefore, at least in EAE, serum A β plays a beneficial role against the disease. A previous report also showed that peripheral administration of A β could reverse paralysis and reduce inflammation in an EAE model [13]. Others also reviewed the literature and reported that A β might regulate learning and memory, angiogenesis, and neurogenesis; repair leaks in the blood–brain barrier; promote recovery from injury; and act as an antimicrobial peptide and tumor suppressor in certain contexts [25]. How the serum A β level in AD correlates with AD diagnosis and prognosis remains elusive and is worthy of further investigation. Comprehensive consideration of both the physiological and pathological roles of A β under certain circumstances might be essential for the design of safe and effective therapeutic strategies for certain diseases.

5. Conclusions

We revealed that AD and EAE could coexist in the same mouse, and the clinical symptoms of EAE were ameliorated in APP/PS1 AD mice but not in 5xFAD transgenic (Tg6799) AD mice. The A β residing in the peripheral circulation plays an anti-inflammatory role in preventing EAE progression in AD mice. We highlight a potentially beneficial role for A β , and the findings may be of benefit to clinical diagnosis or therapy of both neurodegenerative diseases.

Author Contributions: Data curation, C.S., J.C., J.G., P.Z., J.L. (Junjiang Lian), B.Z. and Q.H.; formal analysis, C.S., J.C., J.G., P.Z., J.L. (Junjiang Lian), B.Z. and Q.H.; investigation, C.S. and S.W.; project administration, X.X. and R.Z.; supervision, R.Z.; visualization, J.C.; writing—original draft, J.C., C.S. and R.Z.; writing—review and editing, J.C., R.Z., J.L. (Jie Lv) and C.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Technology of China, grant number 2017YFA0104002.

Institutional Review Board Statement: The study was approved by Tongji University Animal Care Committee (Approval Code: TJAB04121102, Approval Date: 7 March 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sospedra, M.; Martin, R. Immunology of multiple sclerosis. *Annu. Rev. Immunol.* **2005**, *23*, 683–747. [[CrossRef](#)]
2. Compston, A.; Coles, A. Multiple sclerosis. *Lancet* **2008**, *372*, 1502–1517. [[CrossRef](#)]
3. Constantinescu, C.S.; Hilliard, B.; Fujioka, T.; Bhopale, M.K.; Calida, D.; Rostami, A.M. Pathogenesis of neuroimmunologic diseases. Experimental models. *Immunol. Res.* **1998**, *17*, 217–227. [[CrossRef](#)]
4. Steinman, L.; Zamvil, S.S. Virtues and pitfalls of EAE for the development of therapies for multiple sclerosis. *Trends Immunol.* **2005**, *26*, 565–571. [[CrossRef](#)]

5. Hardy, J.; Selkoe, D.J. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **2002**, *297*, 353–356. [[CrossRef](#)] [[PubMed](#)]
6. Scheltens, P.; Blennow, K.; Breteler, M.M.; de Strooper, B.; Frisoni, G.B.; Salloway, S.; Van der Flier, W.M. Alzheimer's disease. *Lancet* **2016**, *388*, 505–517. [[CrossRef](#)]
7. Selkoe, D.J. The molecular pathology of Alzheimer's disease. *Neuron* **1991**, *6*, 487–498. [[CrossRef](#)]
8. Lichtenthaler, S.F.; Wang, R.; Grimm, H.; Uljon, S.N.; Masters, C.L.; Beyreuther, K. Mechanism of the cleavage specificity of Alzheimer's disease gamma-secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3053–3058. [[CrossRef](#)]
9. Selkoe, D.J. The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol.* **1998**, *8*, 447–453. [[CrossRef](#)]
10. Yang, L.B.; Lindholm, K.; Yan, R.; Citron, M.; Xia, W.; Yang, X.L.; Beach, T.; Sue, L.; Wong, P.; Price, D.; et al. Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. *Nat. Med.* **2003**, *9*, 3–4. [[CrossRef](#)]
11. Brothers, H.M.; Gosztyla, M.L.; Robinson, S.R. The Physiological Roles of Amyloid- β Peptide Hint at New Ways to Treat Alzheimer's Disease. *Front. Aging Neurosci.* **2018**, *10*, 118. [[CrossRef](#)] [[PubMed](#)]
12. Kumar, D.K.; Choi, S.H.; Washicosky, K.J.; Eimer, W.A.; Tucker, S.; Ghofrani, J.; Lefkowitz, A.; McColl, G.; Goldstein, L.E.; Tanzi, R.E.; et al. Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. *Sci. Transl. Med.* **2016**, *8*, 340ra72. [[CrossRef](#)]
13. Grant, J.L.; Ghosn, E.E.; Axtell, R.C.; Herges, K.; Kuipers, H.F.; Woodling, N.S.; Andreasson, K.; Herzenberg, L.A.; Herzenberg, L.A.; Steinman, L. Reversal of paralysis and reduced inflammation from peripheral administration of beta-amyloid in TH₁ and TH₁₇ versions of experimental autoimmune encephalomyelitis. *Sci. Transl. Med.* **2012**, *4*, 145ra105. [[CrossRef](#)] [[PubMed](#)]
14. Avitan, I.; Halperin, Y.; Saha, T.; Bloch, N.; Atrahimovich, D.; Polis, B.; Samson, A.O.; Braitbard, O. Towards a Consensus on Alzheimer's Disease Comorbidity? *J. Clin. Med.* **2021**, *10*, 4360. [[CrossRef](#)] [[PubMed](#)]
15. Luczynski, P.; Laule, C.; Hsiung, G.-Y.R.; Moore, G.R.W.; Tremlett, H. Coexistence of Multiple Sclerosis and Alzheimer's disease: A review. *Mult. Scler. Relat. Disord.* **2019**, *27*, 232–238. [[CrossRef](#)]
16. Lv, J.; Zhuang, W.; Zhang, Y.; Xie, L.; Xiang, Z.; Zhao, Q.; Jiang, X.; Shen, J.; Du, C. 9,10-Anhydrodehydroartemisinin Attenuates Experimental Autoimmune Encephalomyelitis by Inhibiting Th₁ and Th₁₇ Cell Differentiation. *Inflammation* **2021**, *44*, 1793–1802. [[CrossRef](#)]
17. Wang, C.; Yang, J.; Xie, L.; Saimaier, K.; Zhuang, W.; Han, M.; Liu, G.; Lv, J.; Shi, G.; Li, N.; et al. Methyl Butyrate Alleviates Experimental Autoimmune Encephalomyelitis and Regulates the Balance of Effector T Cells and Regulatory T Cells. *Inflammation* **2021**. [[CrossRef](#)]
18. Xie, L.; Saimaier, K.; Wang, C.; Yang, J.; Han, M.; Lv, J.; Zhuang, W.; Liu, G.; Du, C. Methyl acetate arrests Th₁ in peripheral immune system and alleviates CNS inflammation in EAE. *Int. Immunopharmacol.* **2021**, *101*, 108291. [[CrossRef](#)]
19. Wei, W.; Du, C.; Lv, J.; Zhao, G.; Li, Z.; Wu, Z.; Hasko, G.; Xie, X. Blocking A2B adenosine receptor alleviates pathogenesis of experimental autoimmune encephalomyelitis via inhibition of IL-6 production and Th17 differentiation. *J. Immunol.* **2013**, *190*, 138–146. [[CrossRef](#)] [[PubMed](#)]
20. Dong, H.; Locci, A.; Keszycki, R.M.; Orellana, H.D.; Rodriguez, G.; Fisher, D.W. Characterization and comparison of memory and affective behavior in common transgenic mouse models of Alzheimer's disease. *Alzheimer's Dement.* **2020**, *16*, e045211. [[CrossRef](#)]
21. Oakley, H.; Cole, S.L.; Logan, S.; Maus, E.; Shao, P.; Craft, J.; Guillozet-Bongaarts, A.; Ohno, M.; Disterhoft, J.; Van Eldik, L.; et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. *J. Neurosci.* **2006**, *26*, 10129–10140. [[CrossRef](#)]
22. Domingues, H.S.; Mues, M.; Lassmann, H.; Wekerle, H.; Krishnamoorthy, G. Functional and pathogenic differences of Th₁ and Th₁₇ cells in experimental autoimmune encephalomyelitis. *PLoS ONE* **2010**, *5*, e15531. [[CrossRef](#)]
23. Burgler, S.; Ouaked, N.; Bassin, C.; Basinski, T.M.; Mantel, P.-Y.; Siegmund, K.; Meyer, N.; Akdis, C.A.; Schmidt-Weber, C.B. Differentiation and functional analysis of human T_H17 cells. *J. Allergy Clin. Immunol.* **2009**, *123*, 588–595. [[CrossRef](#)] [[PubMed](#)]
24. Comabella, M.; Montalban, X.; Münz, C.; Lünemann, J.D. Targeting dendritic cells to treat multiple sclerosis. *Nat. Rev. Neurol.* **2010**, *6*, 499–507. [[CrossRef](#)]
25. Kent, S.A.; Spires-Jones, T.L.; Durrant, C.S. The physiological roles of tau and A β : Implications for Alzheimer's disease pathology and therapeutics. *Acta Neuropathol.* **2020**, *140*, 417–447. [[CrossRef](#)] [[PubMed](#)]
26. Benilova, I.; Karran, E.; De Strooper, B. The toxic A β oligomer and Alzheimer's disease: An emperor in need of clothes. *Nat. Neurosci.* **2012**, *15*, 349–357. [[CrossRef](#)] [[PubMed](#)]
27. Espay, A.J.; Sturchio, A.; Schneider, L.S.; Ezzat, K. Soluble Amyloid- β Consumption in Alzheimer's Disease. *J. Alzheimer's Dis.* **2021**, *82*, 1403–1415. [[CrossRef](#)]
28. Sturchio, A.; Dwivedi, A.K.; Young, C.B.; Malm, T.; Marsili, L.; Sharma, J.S.; Mahajan, A.; Hill, E.J.; Andaloussi, S.E.; Poston, K.L.; et al. High cerebrospinal amyloid- β 42 is associated with normal cognition in individuals with brain amyloidosis. *EClinicalMedicine* **2021**, *38*, 100988. [[CrossRef](#)] [[PubMed](#)]
29. Scheuner, D.; Eckman, C.; Jensen, M.; Song, X.; Citron, M.; Suzuki, N.; Bird, T.D.; Hardy, J.; Hutton, M.; Kukull, W.; et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med.* **1996**, *2*, 864–870. [[CrossRef](#)] [[PubMed](#)]

30. Gao, H.; Liu, M.; Zhao, Z.; Yang, C.; Zhu, L.; Cai, Y.; Yang, Y.; Hu, Z. Diagnosis of Mild Cognitive Impairment and Alzheimer's Disease by the Plasma and Serum Amyloid-beta 42 Assay through Highly Sensitive Peptoid Nanosheet Sensor. *ACS Appl. Mater. Interfaces* **2020**, *12*, 9693–9700. [[CrossRef](#)] [[PubMed](#)]
31. Lopez, O.L.; Kuller, L.H.; Mehta, P.D.; Becker, J.T.; Gach, H.M.; Sweet, R.A.; Chang, Y.F.; Tracy, R.; DeKosky, S.T. Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study. *Neurology* **2008**, *70*, 1664–1671. [[CrossRef](#)]
32. Rissman, R.A.; Trojanowski, J.Q.; Shaw, L.M.; Aisen, P.S. Longitudinal plasma amyloid beta as a biomarker of Alzheimer's disease. *J. Neural Transm.* **2012**, *119*, 843–850. [[CrossRef](#)] [[PubMed](#)]
33. Nakamura, A.; Kaneko, N.; Villemagne, V.L.; Kato, T.; Doecke, J.; Doré, V.; Fowler, C.; Li, Q.-X.; Martins, R.; Rowe, C.; et al. High performance plasma amyloid- β biomarkers for Alzheimer's disease. *Nature* **2018**, *554*, 249–254. [[CrossRef](#)]
34. Yang, Y.-H.; Situmeang, R.F.; Ong, P.A. Can blood amyloid levels be used as a biomarker for Alzheimer's disease? *Brain Sci. Adv.* **2021**, *7*, 17–25. [[CrossRef](#)]
35. He, J.; Qiao, J.-P.; Zhu, S.; Xue, M.; Chen, W.; Wang, X.; Tempier, A.; Huang, Q.; Kong, J.; Li, X.-M. Serum β -amyloid peptide levels spike in the early stage of Alzheimer-like plaque pathology in an APP/PS1 double transgenic mouse model. *Curr. Alzheimer Res.* **2013**, *10*, 979–986. [[CrossRef](#)] [[PubMed](#)]
36. Zhao, X.; Yang, J. Amyloid- β peptide is a substrate of the human 20S proteasome. *ACS Chem. Neurosci.* **2010**, *1*, 655–660. [[CrossRef](#)] [[PubMed](#)]
37. Flood, F.; Murphy, S.; Cowburn, R.F.; Lannfelt, L.; Walker, B.; Johnston, J.A. Proteasome-mediated effects on amyloid precursor protein processing at the gamma-secretase site. *Biochem. J.* **2005**, *385*, 545–550. [[CrossRef](#)] [[PubMed](#)]
38. Bellia, F.; Lanza, V.; Garcia-Viñuales, S.; Ahmed, I.M.M.; Pietropaolo, A.; Iacobucci, C.; Malgieri, G.; D'Abrosca, G.; Fattorusso, R.; Nicoletti, V.G.; et al. Ubiquitin binds the amyloid β peptide and interferes with its clearance pathways. *Chem. Sci.* **2019**, *10*, 2732–2742. [[CrossRef](#)] [[PubMed](#)]
39. Tundo, G.R.; Sbardella, D.; Santoro, A.M.; Coletta, A.; Oddone, F.; Grasso, G.; Milardi, D.; Lacal, P.M.; Marini, S.; Purrello, R.; et al. The proteasome as a druggable target with multiple therapeutic potentialities: Cutting and non-cutting edges. *Pharmacol. Ther.* **2020**, *213*, 107579. [[CrossRef](#)]
40. Santoro, A.M.; Lanza, V.; Bellia, F.; Sbardella, D.; Tundo, G.R.; Cannizzo, A.; Grasso, G.; Arizzi, M.; Nicoletti, V.G.; Alcaro, S.; et al. Pyrazolones Activate the Proteasome by Gating Mechanisms and Protect Neuronal Cells from β -Amyloid Toxicity. *ChemMedChem* **2020**, *15*, 302–316. [[CrossRef](#)] [[PubMed](#)]
41. Zozulya, A.L.; Wiendl, H. The role of regulatory T cells in multiple sclerosis. *Nat. Clin. Pract. Neurol.* **2008**, *4*, 384–398. [[CrossRef](#)]
42. Heink, S.; Yogev, N.; Garbers, C.; Herwerth, M.; Aly, L.; Gasperi, C.; Husterer, V.; Croxford, A.L.; Moller-Hackbarth, K.; Bartsch, H.S.; et al. Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic TH17 cells. *Nat. Immunol.* **2017**, *18*, 74–85. [[CrossRef](#)] [[PubMed](#)]
43. Okuda, Y.; Sakoda, S.; Fujimura, H.; Saeki, Y.; Kishimoto, T.; Yanagihara, T. IL-6 plays a crucial role in the induction phase of myelin oligodendrocyte glycoprotein 35–55 induced experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **1999**, *101*, 188–196. [[CrossRef](#)]
44. Quintana, F.J. Old dog, new tricks: IL-6 cluster signaling promotes pathogenic TH₁₇ cell differentiation. *Nat. Immunol.* **2016**, *18*, 8–10. [[CrossRef](#)]