Amyloid Deposition and Inflammation in APPswe/PS1dE9 Mouse Model of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is characterized by amyloid plaques and neurofibrillary tangles associated with chronic inflammation. APPswe/PS1dE9 is an AD mouse model bearing mutant transgenes of amyloid precursor protein and presenilin-1. Amyloid deposition is present in this mouse model at early stage of life. However, the progression of inflammation and its relationship with amyloid deposition have not been characterized. Here we showed that amyloid plaques were present at 4 months of age and increased with age. CD11b-positive microglia clusters appeared in hippocampus and neocortex at 4 months of age and increased with age. Clustered glial fibrillary acidic protein (GFAP)-positive astrocytes were observed in hippocampus and cortex after 6 months of age and increased with age. Double staining with CD11b/GFAP antibody and thioflavin S showed clustered microglia and astrocytes were in close association with amyloid plaques. Expression of TNF-α was detected at 8 months of age, while IL-1β, IL-6 and MCP-1 at 10 months. These cytokines increased with age. Double immunostaining of cell specific marker and cytokine indicated TNF-α, IL-1β, IL-6 and MCP-1 were expressed by activated microglia and a small part of activated astrocytes. MCP-1 was also expressed by neurons, which support recent finding that MCP-1 expression was increased in neurons of AD patient. These results demonstrate amyloid plaques and its associated inflammatory response developed at early stage of life and progressively increased with age, both activated glia and neurons are involved in chronic inflammation in AD. APPswe/PS1dE9 model provides a mean for studying the mechanisms and novel therapeutics for AD.

Keywords: Alzheimer's disease, APPswe/PS1dE9, transgenic mouse, amyloid deposition, inflammation, microglia, astrocyte, neuron.

INTRODUCTION

Alzheimer's disease (AD) is a progressive, irreversible brain disorder pathologically characterized by senile plaques, neurofibrillary tangles and neuronal cell loss [1, 2]. Senile plaques composed mainly of the 39-43 amino acid peptide amyloid β (Aβ) derived from proteolytic processing of amyloid precursor protein (APP). Neurofibrillary tangles contain hyperphosphorylated forms of the microtubule-associated protein tau. The senile plaques are often associated with activated microglial cells and surrounded by reactive astrocytes. Considerable evidence from studies with postmorterm AD brain tissues, along with those on glial cell cultures and Aβ-developing transgenic mouse models have provided evidence that glial cell activation is a crucial event mediating inflammatory responses in AD [3, 4].

While most AD cases are sporadic (90%), the early onset familial AD can be caused by mutations in APP, presenilin-1 (PS1), or presenilin-2 (PS2). The limited knowledge of the development of AD and the lack of effective therapeutic approaches have led to the production of a wide range of transgenic animal models. Transgenic mice expressing mutated human genes associated with familial forms of AD offered good models to study the etiology, progression and therapeutic modulation for AD [5]. These models display several pathological characteristics of AD, such as amyloid plaques which can be detected at 6-9 months of age and are accompanied by reactive gliosis [6]. These changes are paralleled by significant cognitive deficits [7-10]. Double transgenic mouse models, such as APP/PS1 and APP/PS2, have been reported to have accelerated development of pathology and cognitive deficits relative to single transgenics [11, 12], facilitating studies that would be difficult in very old animals.

A recently developed and readily available AD model is the APPswe/PS1dE9 model expressing both APPswe and PS1dE9 transgenes**.** This model was generated by co-injection of APPswe (mouse/human chimeric APP695 harboring the Swedish mutation (KM594/5N)) and PS1dE9 (exon 9-deleted PS1) constructs into pronuclei [13]. Mice expressing APPswe alone only develop visible amyloid deposits after 24 months of age [14], whereas it has been reported that co-expression of PS1dE9 induces amyloid deposition by 4-6 months of age [15, 16]. A recent study showed that glial fibrillary acidic protein (GFAP) level was increased in hippocampus of APPswe/PS1dE9 mouse at 14 months of age, and GFAP-positive astrocytes were concentrated around

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amyloid plaques [17]. However, the progression of inflammation (microglia and astrocyte activation, proinflammatory cytokine and chemokine expression) and its relationship with amyloid deposition have not been characterized in this AD model. In this study, we have undertaken a systematic description of brain amyloid deposition and inflammation in APPswe/PS1dE9 mouse model. Male mice were studied in order to avoid the possible influence of gender on amyloid plaque formation and inflammation in AD [18, 19].

MATERIALS AND METHODS

Animals

APPswe/PS1dE9 double-transgenic mice were obtained from Jackson Laboratory. The male transgenic mice aged 2-12 months and age-matched non-transgenic littermates were used in the present study. All animal experiments were in accordance with the National Institutes of Heath Guide for the Care and Use of Laboratory Animals and were approved by the Biological Research Ethics Committee, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Reagents

Primary antibodies used in immunohistochemistry are summarized in Table **1**. Thioflavin S (Thio-S) and other reagents were from Sigma, unless otherwise indicated.

Brain Tissue Preparation

Mice were transcardially perfused with 0.9% NaCl after deep anesthesia with pentobarbital (100 mg/kg, i.p.). The brain was quickly removed, frozen in liquid nitrogen, and stored at -70 ^{o}C. Series of coronal sections (14 μM) were cut through the brain using a sliding, freezing microtome, mounted on slides and dried in air at room temperature. Tissue sections were stored at -70 ^{o}C or used immediately.

Immunohistochemistry

Immunostaining was performed according to previous methods with minor modifications [20]. Briefly, tissue sections were fixed in 4% paraformaldehyde in PBS containing 0.5% glucose, washed with PBS-S (PBS containing 0.3% Triton X-100 and 0.1% glucose), immersed in 1% H_2O_2 in PBS-S for 30 minutes, quickly rinsed with PBS-S and blocked in 5% serum (from same host of the secondary antibodies) for 10 minutes. After incubation with the primary antibody (Table **1**) or corresponding control antibody overnight at 4 ^{o}C, slides were washed and incubated with HRP-conjugated secondary antibody (1:100, Dako, Carpinteria, CA, USA) for 1 h at room temperature in the dark. The sections were rinsed three times with PBS-S and incubated with DAB substrate (Dako, Carpinteria, CA, USA) for 3~5 minutes.

Double-labeled fluorescent immunohistochemistry was used to examine the expression of cell specific marker (CD11b, GFAP, or NeuN) and cytokine (TNF-α, IL-1β, IL-6, or MCP-1). The procedures for detecting cell specific marker were same as above except that the secondary antibody was fluorescence-conjugated (1:500). Then the slides were washed and incubated with primary antibody against cytokine (Table **1**) or corresponding control antibody for 1 h at room temperature, washed and applied fluorescence-conjugated secondary antibody for 30 min at room temperature. After washing with PBS-S three times, sections were coversliped by fluorescent mounting medium (Dako, Carpinteria, CA, USA) and stored in dark at 4 ^{o}C. Double-labeled immunostaining was evaluated with a fluorescence microscope (Olympus BX 61, Tokyo, JP). The secondary antibodies used were goat anti-rabbit Alex546, goat anti-rat Alex350, goat anti-mouse Alex546, donkey anti-goat Alex488 and goat anti-rabbit FITC (Molecular Probe, Eugene, OR, USA).

Thio-S Staining

Mouse brain sections were stained in 1% Thio-S for 5 minutes followed by differentiation in 70% ethanol for 5 minutes, rinsing in water and dehydration in 70%, 95%, and 100% ethanol for 5 minutes. The sections were mounted in neutral balsam. Signal was visualized using a specific filter set with excitation of 405-445 nm.

Table 1. Antibodies Used for Immunohistochemistry

Antibody	Host and Formulation	Specificity	Source	Concentration
$A\beta_{1-16}$	mouse monoclonal (6E10)	human	Signet	1:500
CD11b	rat monoclonal (M1/70)	human, mouse	BD Pharmingen	1:25
GFAP	rat monoclonal (2.2B10)	bovine, human, rodent	Zymed	1:100
TNF-α	rat monoclonal (MP6-XT22)	mouse	Serotec	1:100
TNF-α	goat polyclonal	mouse	R&D	1:30
IL-1β	rabbit antiserum (AAM13G)	rabbit antiserum	Serotec	1:100
IL-6	rat monoclonal (MP5-20F3)	mouse	Serotec	1:100
IL-6	rabbit polyclonal	mouse	Pierce	1:200
MCP-1	rabbit antiserum (AAM43)	mouse	Serotec	1:100
NeuN	mouse monoclonal (A60)	avian, mouse, rat, primate, etc.	Chemicon	1:500

Image Analyses

Images were acquired using Olympus BX 61 microscope connected to an Olympus DP70 digital microscope camera. The density of amyloid plaques was determined by counting the total number of plaques present in frontal cortex and hippocampus of the section after Thio-S staining or 6E10 immunostaining using Image-Pro Plus imaging software (version 5.0, Media Cybernetics, Bethesda, MD, USA) which allows identification of objects based on thresholding of the optical density to identify Aβ deposits [21]. The number of plaques, the average plaque size and the plaque burden expressed as percentage of analyzed area, were calculated for each age group under study.

Statistical Analysis

To assess the evolution of amyloid plaques, we used one-way ANOVA followed by a Bonferroni t-test. The data of glia activation and cytokine expression were analyzed using one-way ANOVA followed by a Tukey test.

RESULTS

Progressive Accumulation of Cerebral Amyloid Plaques with Age

Amyloid plaques were examined by Thio-S staining and 6E10 immunohistochemistry in 2-12 months old male APPswe/PS1dE9 mice. Sections stained with Thio-S or immunostained with 6E10 showed a similar profile for plaque number, size and burden evolution at the different time points examined (Figs. **1H** and **I**). Consistent with the report by Garcia-alloza *et al*. [15], an overall progressive increase in plaque number and plaque burden was observed between 4 and 12 month old age (Figs. **1 A-F**, **H** and **I**). 6E10 immunostaining showed that at the early stage the majority of plaques were tiny but compact, and both compact and diffuse plaques increased with age (data not shown). As 6E10 antibody stained both compact and diffuse plaques, it detected bigger plaque burden than Thio-S did, especially at age of 10 and 12 month (Figs. **1G**, **H** and **I**). No labeling was observed in non-transgenic, age-matched littermates (data not shown).

Early Glial Response Accompanies Amyloid Plaque Deposition

We examined the activation of microglia and astrocytes by immuostaining with anti-CD11b and anti-GFAP antibody, respectively. Control antibody corresponding to antibody against CD11b or GFAP detected no signal in both APPswe/PS1dE9 mice and non-transgenic, age-matched littermates (data not shown). Rare CD11b-positive microglia was detected in non-transgenic, age-matched littermates (data not shown). In APPswe/PS1dE9 mice, clustered microglia appeared in hippocampus and neocortex at 4 months of age and increased with age (Figs. **2B-F, J**). The increase of CD11b-positive staining was parallel with the increase of amyloid plaques (Figs. **2J** and Fig. **1**). Double staining with CD11b and Thio-S confirmed that amyloid plaques were in close association with activated microglial cells (Figs. **2G-I**).

Scattered GFAP-positive astrocytes were observed in hippocampus in both APPswe/PS1dE9 mice and non-transgenic mice at age of 2-6 months (Figs. 3**A-C** and data not shown). This non-specific activation was not different between these two groups of mice. Clustered astrocytes were observed in hippocampus and cortex after 6 months of age, and continued to progress with age (Figs. **3D-F, J**). Double staining for GFAP and Thio-S demonstrated that most amyloid plaques were surrounded by a large amount of astrocytes. A small part of amyloid plaques were surrounded by a few astrocytes. Some scattered activated astrocytes were not associated with amyloid plaques (Figs. **3G-I**).

Proinflammatory Cytokine Expression

In order to reveal the expression profile of proinflammatory cytokines TNF-α, IL-1β and IL-6, as well as chemokine MCP-1, coronal brain sections from mice age of 2, 4, 6, 8, 10, and 12 months were subjected to immunohistochemistry assay. The control antibody corresponding to antibody against TNF-α, IL-1β, IL-6, or MCP-1 detected no signal in both APPswe/PS1dE9 mice and non-transgenic, age-matched littermates (data not shown). No staining of TNF-α, IL-1β, IL-6 and MCP-1 were observed in age-matched non-transgenic littermates (data not shown). In APPswe/PS1dE9 mice, TNF-α expression presented at 8 months of age, while IL-1β, IL-6 and MCP-1 were detected at 10 months of age. These cytokines increased at 12 months in both cortex and hippocampus. Double-staining of cytokine and amyloid plaques showed that TNF-α, IL-1β, IL-6 and MCP-1 immunoreactive cells assembled around ThioS-positive amyloid plaques, or isolated (Fig. **4A**). We then checked the type of cells expressing these cytokines or chemokine in brain tissue of 12 month old mice by double staining of cell-specific marker and cytokine. Figs. (**4B** and **4C**) demonstrated that activated microglia expressed TNF-α, IL-1β, IL-6 and MCP-1, while only a small part of activated astrocytes expressed these cytokines. MCP-1 was also expressed by part of neurons throughout brain sections (Fig. **4D** and data not shown).

DISCUSSION

AD is a progressive neurodegenerative disorder, of which the pathogenesis is thought to involve increased Aβ deposition and abnormal inflammatory responses. APPswe/PS1dE9 mice, a transgenic mouse model generated with mutant transgenes for APP and PS1, showed Aβ deposition by 4 months with a progressive increase in plaque number up to 12 months [15]. Our study confirmed an early appearance of amyloid plaques in APPswe/PS1dE9 mice at 4 months of age which increased with age. Thio-S and anti-Aβ antibody 6E10 staining showed a similar profile for plaque number, size and burden evolution (Fig. **1**). 6E10 immunostaining showed that at the early stage the majority of plaques were tiny but compact, and both compact and diffuse plaques increased with age. Similarly, both diffuse and compact plaques were detected as early as 9-10 weeks of age in TgCRND8 mice harboring the human APP gene with the Indiana and Swedish mutations [22]. However, amyloid plaques appeared later in life in other transgenic models. For example, Congo red-positive plaques started accumulating at around 6 months of age in APP23 mice expressing human APP with the Swedish double mutation (670/671 KM→NL) [23, 24]. Both diffuse and compact plaques developed between 9 and 12 months of age in Tg2576 mice carrying a transgene coding for the 695 amino-acid isoform of human

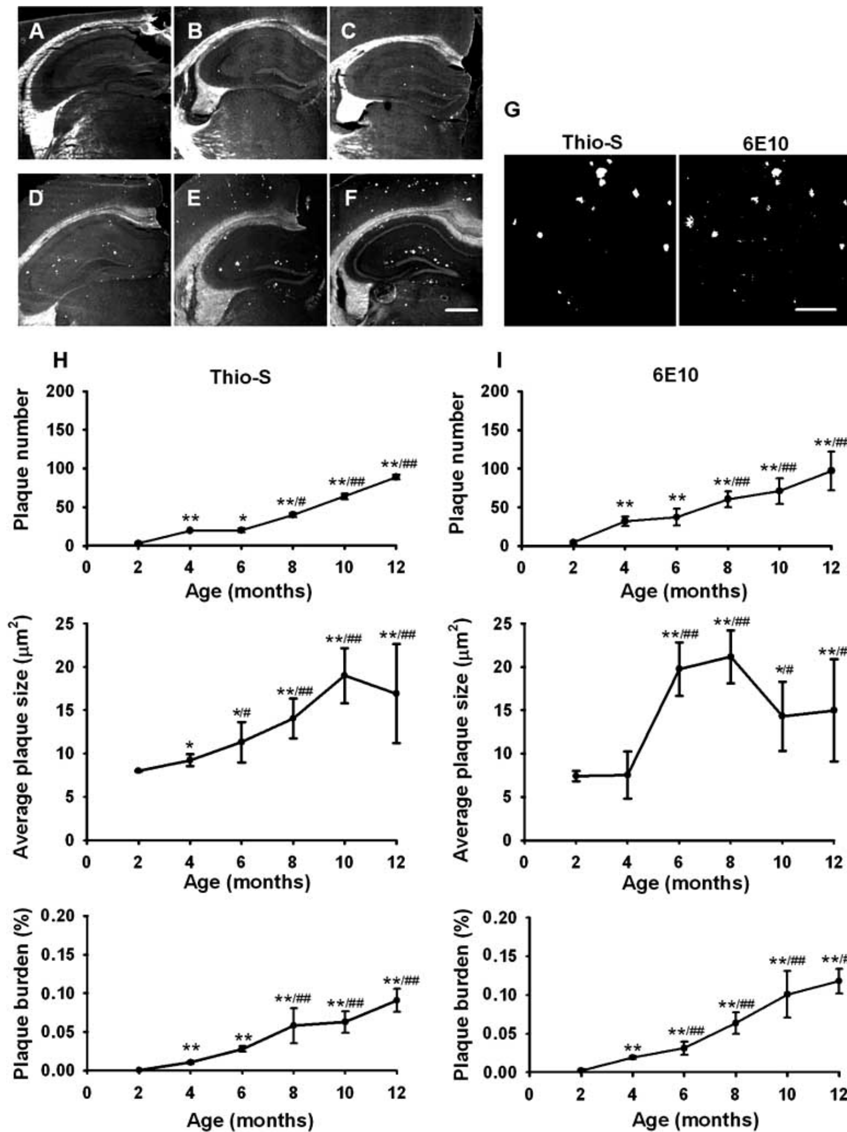

Fig. (1). Amyloid deposition with age in APPswe/PS1dE9 mice. Coronal sections from mice at age of 2, 4, 6, 8, 10 and 12 month were stained with anti-Aβ_{1-16} (6E10) antibody or Thio-S. Representative illustrations of senile plaques stained with Thio-S at age of 2 **(A)**, 4 **(B)**, 6 **(C)**, 8 **(D)**, 10 **(E)** and 12 **(F)** month were shown. Scale bar =100 µm. **(G)** Illustrative examples of brain sections from 12 month old mice stained with 6E10 antibody followed by Thio-S staining. Scale bar =10 µm. **(H)** and **(I)** showed the quantification of amyloid plaques in cerebral cortex and hippocampus after Thio-S staining and 6E10 immunohistochemistry, respectively. Data were representative of 3-9 sections from 3-5 animals. One-way ANOVA followed by a Bonferroni t-test. *$p<0.05$, **$p<0.001$ vs. 2 months old group. $^{\#}p<0.05$, $^{\#\#}p<0.001$ vs. 4 months old group.

APP derived from a large Swedish family [9, 25]. The difference of the time-course of Aβ deposition among different animal models may be caused by the difference in transgenes and mouse genetic backgrounds.

There are more and more evidence supporting the important role of inflammation in the pathogenesis of AD. Activated microglia and astrocytes are found in AD patients and transgenic mouse models, and they are located in close proximity to senile plaques [3, 4]. Activated microglia and astrocytes increased with age and amyloid deposition in brain tissue of AD mouse models [26, 27]. In APPswe/PS1dE9 mouse model, we detected clustered microglia in hippocampus and neocortex at 4 months of age which increased with age. Clustered microglia were associated with amyloid plaques (Figs. **2G-I**). The fact that activated microglia appeared as soon as amyloid deposits were visible suggest that microglial activation is an early event in response to Aβ deposits. We also observed astrocyte activation during disease process in APPswe/PS1dE9 mice. The presence of astrocytes was scattered in young mice as in non-transgenic littermates. Consistently, this phenomenon was also observed in wild type mice and TgCRND8 mice harboring human APP gene with the Indiana and Swedish mutations [22]. Minkeviciene *et al.* reported that GFAP level was increased in hippocampus of APPswe/PS1dE9 mouse at 14 months of age, and GFAP-positive astrocytes were concentrated around amyloid plaques [17]. Our present study showed that clusters of activated astrocytes appeared in the hippocampus at around 6

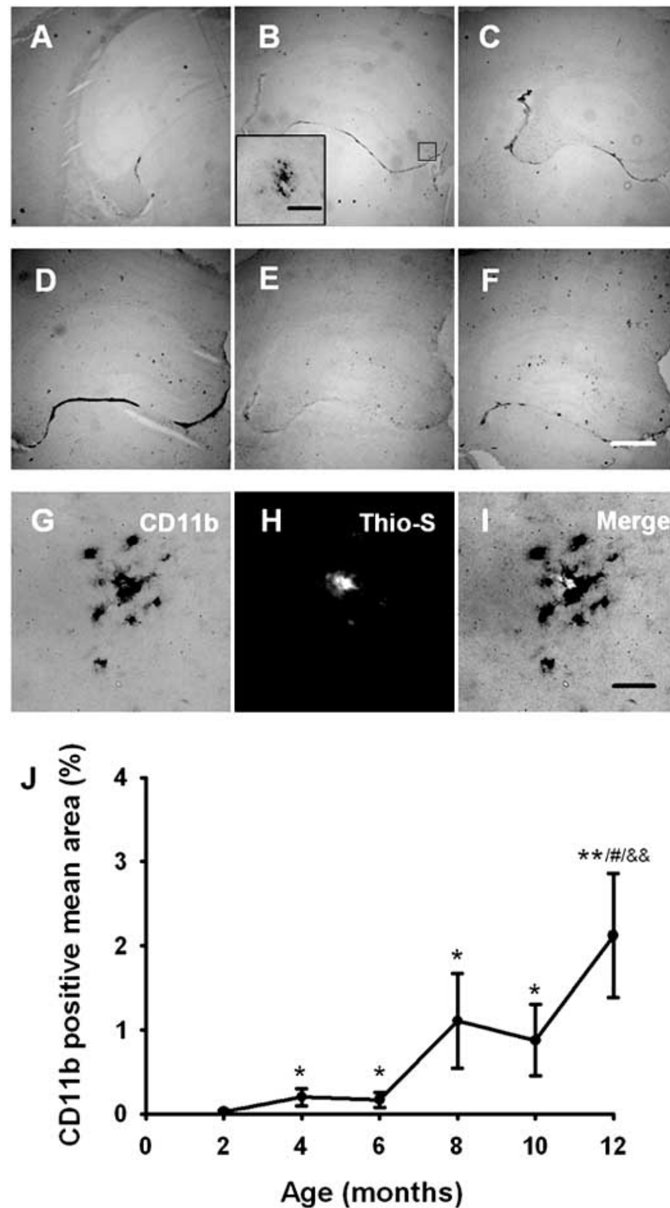

Fig. (2). Time-course of microglia activation in frontal cortex and hippocampus of APPswe/PS1dE9 mice. Brain sections from mice at age of 2 **(A)**, 4 **(B)**, 6 **(C)**, 8 **(D)**, 10 **(E)** and 12 **(F)** months were stained with anti-CD11b antibody. Representative illustrations of sections from 3 animals were shown. Equivalent regions were selected for each animal. **(G-I)** double labeling of amyloid plaque (Thio-S; green fluorescence) and microglia (CD11b; dark product) in the hippocampus of mice at 8 month of age. Scale bar =100 μm in A-F, 25 μm in A insert and B insert, 10μm in G-I. **(J)** CD11b positive areas of cerebral cortex, molecular layer and fimbria hippocampus of 3 sections from 3 animals were assessed using Image-Pro Plus (version 5.0) (Media Cybernetics, Bethesda, MD, USA) analysis software. Data are presented as mean ± SEM. One-way ANOVA followed by a Tukey test. *$p<0.05$, **$p<0.001$ vs. 2 months old group. $^{\#}p<0.05$ vs. 4 months old group. $^{\&\&}p<0.001$ vs. 6 months old group.

months of age and appeared in the cerebral cortex thereafter. Such a shift in the distribution occurred following the initiation of Aβ deposition and microglial activation. Double staining the brain tissues of APPswe/PS1dE9 mice demonstrated that a large number of GFAP-positive astrocytes surrounded amyloid plaques (Fig. **3**). Activated microglia and astrocytes have been reported to be able to degrade Aβ [28-31], thus the activation of microglia and astrocytes at early stage of AD in APPswe/PS1dE9 mice may be beneficial for clearance of Aβ.

Aβ peptides are potent activators of glia cells. Once activated, microglia and astrocytes release a variety of cytokines, chemokines and free radical oxygen species [32], which can contribute to neuronal dysfunction and death. In addition, some specified glia-derived cytokines may also increase Aβ generation [33]. TNF-α has been reported to possess of both neurotoxic activity [34] and neuroprotective properties [3] in the brain of AD. IL-1β is thought to promote microglia and astrocytes activation and recruitment to the site of deposition and plays a key role in the pathogenesis of AD inflammation by start up a cytokine cycle [35]. IL-6

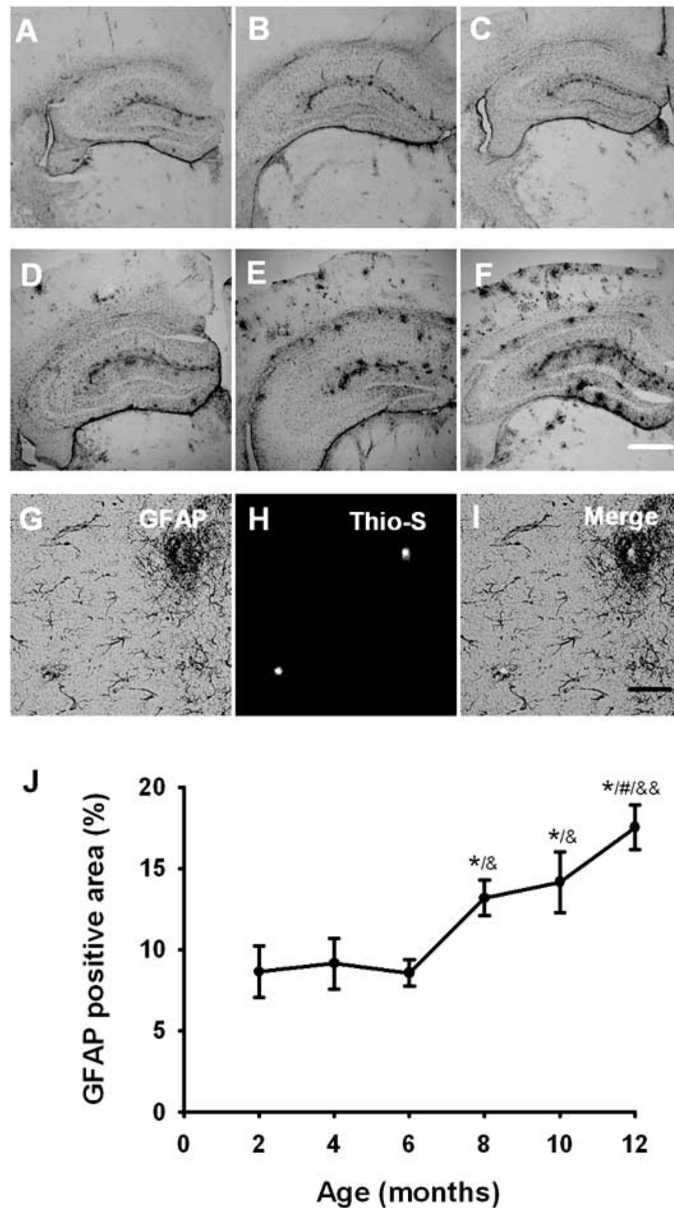

Fig. (3). Astrocyte activation with age in frontal cortex and hippocampus of APPswe/PS1dE9 mice. Brain sections were stained with anti-GFAP antibody at age of 2 (**A**), 4 (**B**), 6 (**C**), 8 (**D**), 10 (**E**) and 12 (**F**) months. Representative illustrations of 3 sections from 3 animals were shown. Equivalent regions were selected for each animal. **(G-I)** double labeling of amyloid plaque (Thio-S; green fluorescence) and astrocyte (GFAP; dark product) in the frontal cortex of mice at 8 month of age. Scale bar =100 µm in A-F and 10 µm in G-I. (**J**) GFAP positive areas of cerebral cortex, molecular layer and CA1 from 3 sections of 3 animals were assessed using Image-Pro Plus (version 5.0) (Media Cybernetics, Bethesda, MD, USA) analysis software. Data are presented as mean ± SEM. One-way ANOVA followed by a Tukey test, *p<0.05 vs. 2 months old group. $^{\#}$p<0.05 vs. 4 months old group. $^{\&}$p<0.05, $^{\&\&}$p<0.001 vs. 6 months old group.

can promote astrogliosis [36], microgliosis [37] and the production of acute phase proteins [38]. Moreover, IL-6 may also be involved in the memory processes [39]. MCP-1 is a member of chemokine family that may amplify subsequent tissue reactions through regulation of microglia migration and recruitment of astrocytes to the area of neuroinflammation [40, 41]. Although the expression and function of pro-inflammatory cytokines have been widely explored *in vitro*, and increased expression of TNF-α, IL-1β, and IL-6 has been identified in amyloid plaques and/or glial cells in brain tissues of AD patients [3, 42-44], only a limited number of studies have addressed the progression of neuroinflammation in transgenic AD mouse models with variable results. In APP[V717I] mice overexpressing the London mutant of APP, IL-1β and IL-6 could be detected as early as 3 months of age [45]. In Tg2576 transgenic mice, the expression pattern of cytokines (IL-1α/β, IL-6, IL-10, IL-12, IL-18, TNF-α, IFN-γ) and chemokines (MCP-1, macrophage migration inhibitory factor) were not consistent as reported by different researchers [46-49]. The controversial results reported in the same strain of APP transgenic mice may be caused by different methodology. Our results showed that in APP-swe/PS1dE9 mice, TNF-α positive cells appeared at 8 months of age, while the expression of IL-1β, IL-6 and

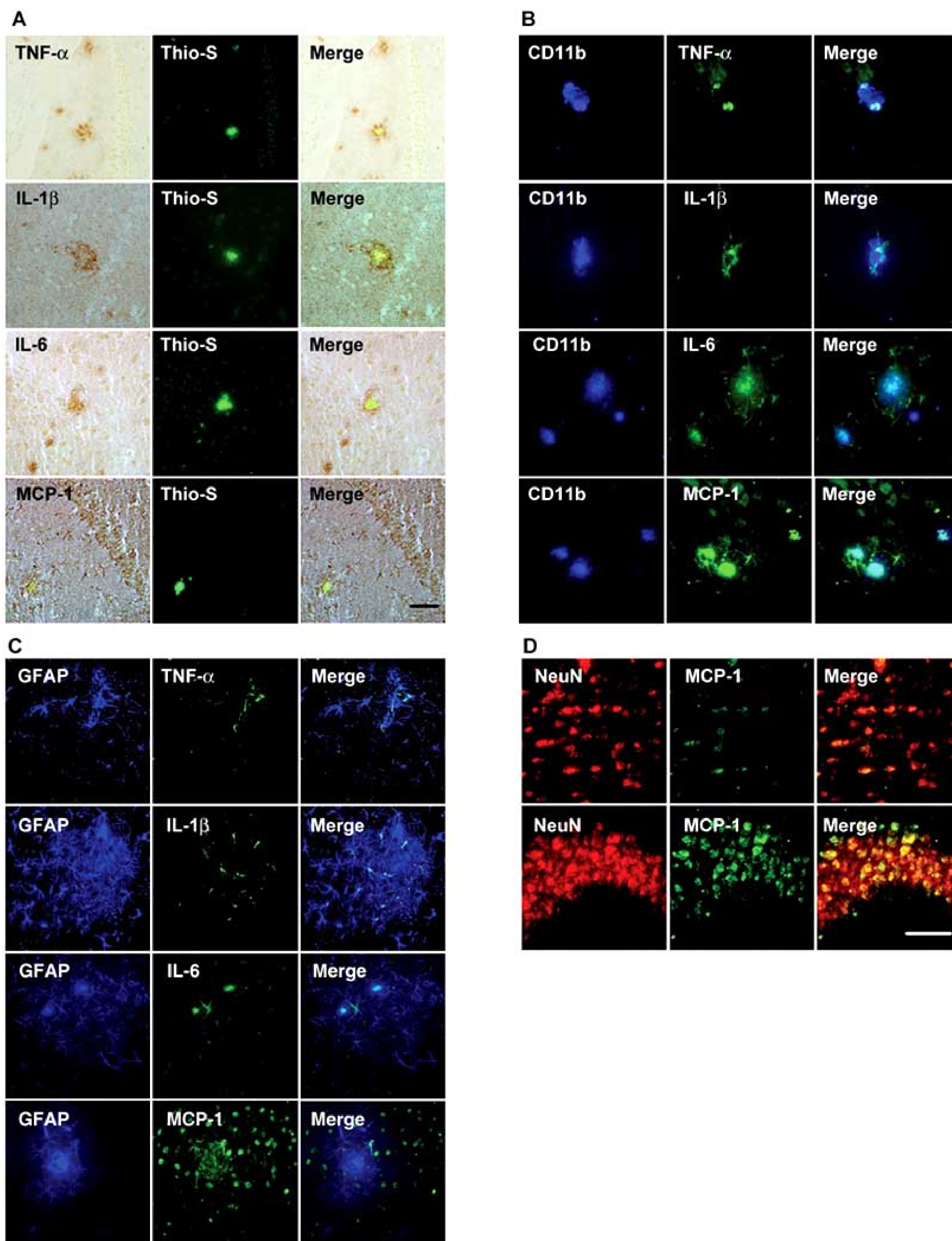

Fig. (4). Expression of inflammatory cytokines in APPswe/PS1dE9 mice. **(A)** Brain sections from mice were double stained with HRP-conjugated anti-cytokine antibody (brown product. TNF-α for 8 month old mice; IL-1β, IL-6, and MCP-1 for 10 month old mice) and thioflavin-S (Thio-S, green fluorescence). Representative illustrations of cerebral cortex (TNF-α, IL-1β, IL-6) and CA1 region of hippocampus (MCP-1) from 3-5 animals were shown. Scale bar =10 µm. **(B-D)** Brain sections from 12 month old mice were double stained with fluorescent labeled anti-cell specific marker (CD11b, GFAP, or NeuN) antibody and anti-cytokine (TNF-α, IL-1β, IL-6, or MCP-1) antibody. Representative illustrations of cerebral cortex (B, C and the first panel of **D)** and CA3 region of hippocampus (D, the second panel) from 3 animals were shown. Scale bar = 40 µm.

MCP-1 presented at 10 months of age. The expression of TNF-α, IL-1β, and IL-6 in APPswe/PS1dE9 mice was associated with amyloid plaques or scattered, and expressed by activated microglia and a small part of activated astrocytes (Fig. **4**). The expression pattern of these proinflammatory cytokines in APPswe/PS1dE9 mice are consistent with those observed in brain tissues of AD patients.

Elevated MCP-1 expression has been detected in amyloid plaques, microglia and astrocytes in the brain tissues of AD patients [50, 51]. In this study, we also detected MCP-1 expression in microglia and astrocytes (Figs. **4B** and **C**). In addition, we found MCP-1 was expressed by part of neurons in the brain tissues of APPswe/PS1dE9 mice (Fig. **4D**). To our knowledge, this is the first report for expression of MCP-1 by neurons in transgenic AD mouse models. Our observation strongly supports recent findings in AD patients that MCP-1 expression is increased in neurons in addition to astrocytes [51]. Increased levels of MCP-1 are found in the sera and cerebrospinal fluid of AD patients, as well as in patients with mild cognitive impairment who later develop AD [52, 53]. APP transgenic mice that overexpress MCP-1 have increased plaque formation in association with increased inflammatory response compared to APP transgenic counterparts [54]. These results suggest that MCP-1 contribute to monocyte infiltration and neuroinflammation in AD. Our present studies demonstrated that activated microglia and astrocytes produced both proinflammatory cytokines and chemokine MCP-1, neuron was an additional source of MCP-1 besides these cells. Therefore, both activated glia and neurons are involved in chronic inflammation in AD. Recent studies showed that different cognitive deficits are present in APPswe/PS1dE9 mice at early and late stages of the disease [55-57], which may result from the progression of amyloid deposition and inflammation in different stages of AD.

Although the major stimulus for the inflammatory responses at different stages of AD is currently unknown, a variety of molecules have been demonstrated to be involved. Aβ has been reported to directly induce glial cell activation and upregulation of TNF-α, IL-1β, IL-6 and MCP-1 *in vitro* and *in vivo* [58, 59]. Proinflammatory cytokines and chemokines stimulated by Aβ may further contribute to the activation of glial cells [35-37, 40, 41]. In addition, activated complement components are found to locate in close proximity the amyloid plaques in AD patients and transgenic mouse models [60, 61]. It has been reported that amyloid deposition, or fibril formation, is capable of causing the activation of both the classical and alternative complements *in vitro*. Bioactive peptides produced through complement activation can mediate a variety of pro-inflammatory responses. For example, C5a can stimulate an immune response that results in the activation and influx of microglia cells [62-65].

Studies with various transgenic mouse models, including APPswe/PS1dE9 mice, have demonstrated that chronic inflammation played important role in the pathogenesis of AD. For example, overexpression of MCP-1 in APP transgenic mice Tg2576 show accelerated diffuse beta-amyloid deposition [54]; sustained hippocampal IL-1β overexpression in APPswe/PS1dE9 mice resulted in plaque amelioration and altered chronic neuroinflammation [66]; chronic neuronal TNF-α expression promoted inflammation and finally resulted in neuronal cell death in a triple transgenic AD mouse model [67]; TNF-α receptor (TNFR1) deletion reduces Aß pathology, microglia activation, BACE1 activity, neuron loss, and memory deficits compared to transgenic APP23 mice expressing normal levels of TNFR1[68]; C1q deficient mouse model of AD displayed less neuropathology than do the C1q sufficient mouse [69]. Transgenic AD mouse models have also been used to explore the therapeutic potential for targeting inflammatory molecules in AD. Inhibition of soluble TNF signaling in triple transgenic mice (APP_{Swe}, tau_{P301L}, and $PS1_{M146V}$) by either hippocampal infusion of dominant-negative-TNF or intracerebroventricular injection of lentivirus encoding dominant-negative-TNF reduced inflammation-induced accumulation of C-terminal APP fragments in the hippocampus, cortex, and amygdale [70]. Recently studies showed that early intracranial administration of vaccinia virus complement control protein (VCP), a protein which could inhibit amyloid protein mediated up-regulation of complement system *in vitro*, improved cognitive deficits present in APPswe/PS1dE9 mice at early and late stages of the disease [56, 57].

In summary, we demonstrated that in APPswe/PS1dE9 mouse model of AD, amyloid plaques and its associated inflammatory response develop at early stage of the life and progressively increase with age. Characterization of the relationship between amyloid deposition and inflammation may provide useful information for using this mouse model to investigate the pathogenesis of AD, assess potential biomarkers, as well as develop of new therapeutic interventions that would also be effective in human.

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ABBREVIATIONS

AD	=	Alzheimer's disease
Aβ	=	Amyloid β peptide
APP	=	Amyloid precursor protein
GFAP	=	Glial fibrillary acidic protein
IL	=	Interleukin
MCP-1	=	Monocyte chemotactic peptide-1
PS1	=	Presenilin-1
PS2	=	Presenilin-2
Thio-S	=	Thioflavin S
TNF-α	=	Tumor necrosis factor-α

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