Int J Clin Exp Med 2015;8(4):5340-5350
www.ijcem.com /ISSN:1940-5901/IJCEM0006585

Original Article

Amyloid-β immunization enhances neurogenesis and cognitive ability in neonatal mice

Hongguang Chen[1*], Min Wang[2*], Aihong Jiao[1], Guotai Tang[1], Wei Zhu[1], Peng Zou[1], Tuo Li[1], Guangqiang Cui[1], Peiyou Gong[1]

[1]Department of Neurosurgery, Yantai Yuhuangding Hospital, 20 Yuhuangding East Road, Yantai 264000, Shandong Province, PR China; [2]Department of Neurology, Yantaishan Hospital, Yantai 264000, Shandong Province, PR China. [*]Equal contributors.

Received February 1, 2015; Accepted April 2, 2015; Epub April 15, 2015; Published April 30, 2015

Abstract: Whether Aβ actually has a physiological as well as a pathological role is not known. In order to investigate the effect of endogenous Aβ, wild type C57BL/6 mice were immunized with human or mouse derived Aβ1-42. The anti-Aβ antibody concentrations were increased in both treated groups. Compared to the human Aβ1-42 treated group, level of serum Aβ significantly decreased in mouse Aβ1-42 treated group. Western blot results revealed that these two derived Aβ1-42 had no cross-reaction. The new dentate granule survival cells increased in Aβ1-42 immunization groups, indicated by more BrdU+/NeuN+ and BrdU+/DCX+ cells as compared to PBS-treated group, accompanied by behavioral performance improving in a hippocampus-dependent learning task. Immunohistochemical analysis showed that BrdU+/Iba1+ cells also increased, however new born astrocytes (BrdU+/GFAP+) were unaffected in all treated mice. Interestingly, according the results of ELISA analysis both vaccines up-regulated IL-4 and IFN-γ levels in the brains and sera, but the TNF-α level did not changed. Of note, human Aβ1-42 immunization in neonatal mice enhanced neurogenesis and cognitive ability, might via Aβ immune response rather than cleaning endogenous Aβ.

Keywords: Amyloid-β, immunization, neurogenesis, cognitive function, neonatal mice

Introduction

Amyloid-β peptides (Aβ) is generated through sequential cleavage of the transmembrane region of APP by β- and γ-secretases and the length range from 38 to 43 amino acids [1]. Currently, the physiological function of endogenous Aβ is still unclear and may even has neuroprotective roles [2]. It was suggested that Aβ as the product reacting to the damage of brain. Some investigators proposed that Aβ acting as an anti-infectious agent played a key role in central nervous system [3, 4]. Whether Aβ could be against further infections or maintains neurogenesis is unknown. Overall, the role of Aβ goes beyond being the pathological causative agent of Alzheimer's disease. It is generally believed that various forms of Aβ peptides, either alone or in combination with other immune factors, serve as activators of the innate immune response in the brain and the periphery system [5].

The amino acids sequence of APP is particular conservative. Only 17 amino acids of APP are different between rodents and human. The N-terminal domain product of APP only has three different amino acids. Furthermore, this particular area is considered to be Aβ race different character [6]. Different B cell epitopes could induce different immune reactions. Human or rodent Aβ could induce different anti-Aβ antibodies. In this study, neonatal mice were immunized with human or mouse derived Aβ1-42 to explore the potential differences of immunological reaction. Lots of evidence confirmed that amyloid-β peptides could destroy neurons and impair cognitive functions, spatial learning abilities then lead to memory loss. The traditional viewpoint was that fibrillar forms of Aβ in the amyloid plaques, not monomeric or dimmer tended towards neurotoxicity and neurodegeneration. There was a stronger correlation between cortical levels of soluble Aβ species and synaptic loss which indicated that soluble Aβ oligomers could impair synaptic plasticity by inhibiting hippocampal long-term potentiation (LTP) [7, 8]. Plaque burden in brains of AD patients was not the important indicator to cog-

nitive ability improvement. Vaccination with Aβ1-42 in PDAPP mouse resulted in striking mitigation of AD-like pathology [9, 10]. Following reports showed immunotherapy could reduce cerebral Aβ levels and improve cognitive abilities in APP-tg mouse [11, 12]. Early protective immunization before the occurrence of substantial neuropathology, neuronal loss and cognitive deficits might be more beneficial and safer for potential AD patients. Interestingly, our results revealed that the neonatal mice after Aβ immunization improved neurogenesis and cognitive ability comparing to the control group, but the endogenous Aβ clearance was not obvious.

The neonatal time is the key period of cognitive function formation and development of neurogenesis. Neonatal rats with or without hydrocephalus had low levels of Aβ and transporters comparing to adult rats with hydrocephalus [13]. In this study, the effects of human and mouse derived Aβ immunization on neurogenesis and cognitive ability were explored in neonatal C57BL/6 mice. Both derived Aβ immunization could improve neurogenesis and cognitive ability in neonatal mice, without clearing endogenous Aβ.

An MII alternative activation of macrophages by IL-4 has been reported to impair pathogens phagocytosis, potentiate microbial-induced signaling and promote pro-inflammatory cytokine secretion [14]. Some researchers supported the idea that increase of TNF in the brain was the result of viral infections [15]. Age-associated alterations of sex hormone levels have also been suggested to induce an increase of pro-inflammatory cytokines such as TNF-α in some AD patients' brain regions [16]. TNF-α production is initiated and remained for an extended period of time in brain [17]. Quantification of microglia, serum and brain IL-4, IFN-γ but not TNF-α showed significant increasing after Aβ immunization.

Materials and methods

Animals and immunization protocol

All the neonatal wild-type C57BL/6 mice were obtained from the Animal Centre of Sun Yat-Sen University. The mice were housed according to the National Institutes of Health Care and Treatment of Laboratory Animals. The study protocol was approved by the Ethics Committee of Sun Yat-Sen University. Thirty-six mice were randomly assigned to three groups (n = 12 for each group, six males/six females) and vaccination was begun at 1 week of age. Two treatment groups were treated with human Aβ1-42 peptide vaccine or mouse Aβ1-42 peptide vaccine which were both purchased from Sigma-Aldrich (St. Louis, MO, USA). Each mouse was vaccinated with 50 μg protein that was already emulsified with Freund's complete adjuvant (1:1 v/v) in the first injection. The mice were vaccinated by subcutaneous injection with the same antigen (50 μg per injection) that was emulsified with Freund's incomplete adjuvant (1:1 v/v) at 2-week intervals after the first immunization. The control mice immunized with PBS were also treated according to the above procedure. Immunization was performed three times.

Bromodeoxyuridine labeling

One week after the final vaccination, all mice were injected twice daily for five days with BrdU (Sigma-Aldrich; 50 mg/kg, i.p., dissolved in potassium-phosphate-buffered saline, KPBS) for labeling of mitotic cells [18]. Mice were sacrificed under deep anesthesia five weeks after the BrdU injections. To ensure proper fixation and immunostaining of brain tissues, mice were exsanguinated by transcardial perfusion with normal saline. Then brains were removed and bisected in midsagittal plane. The right hemisphere was snap frozen for biochemical analysis [19], whereas the left hemisphere was fixed in 4% paraformaldehyde for immunohistochemical analysis.

Antibody titer measurements

One week after each vaccination, all blood samples were regularly drawn and utilized for hematological and biochemical examinations. Sera were stored at -80°C. Antibody titers were measured using a method to dissociate Aβ from the anti-Aβ antibodies in the serum. This method was previously described [20]. Briefly, serum was diluted 1:100 using a dissociation buffer at pH 3.5 and incubated at room temperature for 20 min. The sera were then centrifuged at 8000× g for 20 min at room temperature using a Microcon centrifugal device. The sample reservoir was then placed inverted into a second tube and centrifuged at 1000× g for 3 min. The collected solution containing the anti-

body dissociated from the Aβ peptide was adjusted to pH 7.0. These samples were then analyzed by enzyme-linked immunosorbent assay to determine the antibody titer. Ninety-six-well plates were coated with Aβ1-42 peptide overnight, blocked and then incubated for1h at 37°C with two fold serial dilutions of serum samples. Anti-mouse peroxidase (Sigma-Aldrich) was added and the ELISA was developed using 3, 3', 5, 5'-tetramethylbenzidine (Sigma-Aldrich). The experiment protocols followed the manufacturer's directions. Plates were read spectrophotometrically at 450 nm within 30 minutes, with P/N > 2:1 as the positive reaction.

Aβ ELISA

The soluble serum Aβ1-42 levels were determined. ELISAs specific for human or mouse Aβ1-42 were performed as previously described [21].

Morris water maze

Morris Water Maze was use for assessing spatial of learning and memory [22]. Mice participated in Morris water maze test in the 5th week. The apparatus of water maze and the rules of the operations were in accordance with Zou et al. [23]. The water maze consisted of a pool that was 100 cm in diameter and 60 cm in height and filled with water with the temperature at (22±1)°C. It was painted white and made opaque with nontoxic white tempera paint powder and was placed in a room surrounded by distinct extramaze cues. The pool was divided into four quadrants. A movable black circular platform (10 cm in diameter) was located in the center of quadrant and submerged 2 cm below the water surface. The water in the tank was stirred in between animal trials to disrupt odor trails. The room was furnished with several extramaze cues immobile throughout the entire experiment process. Task in the MWM comprised of two times-spatial reference memory tests and two times-reversal learning tests. The animal's movement was recorded and analyzed using a computerized video-tracking system (Ethovision@8.0, Noldus Information Technology, Wageningen, Netherlands). In the spatial reference memory test, from the fourth to sixth day, mice were trained to find the hidden platform with the extra maze cues to examine acquisition of spatial reference memory. The platform was located in the southwest quadrant from the fourth to sixth day. Mice were trained twice a day for three consecutive days and each training consisted of four trials. In each trial, mice were placed into the pool, facing the wall at each of the four quadrant edges, pseudo randomly chosen across trials. If the mice found the platform within 60 s, it was allowed to stay in the platform for 10 s. If not, the experimenter guided the mouse to the platform and allowed it to rest for 10 s. The mice were then returned to a holding cage for 60 s before the next trial. Two parameters were recorded: escape latency, swimming time to locate the hidden platform; path length, the distance of the swim path from the start location to the hidden platform. In the reversal learning test, mice were assessed reversal learning ability. The platform was moved to the quadrant opposite that in which it had been placed during the prior reference memory task. Mice were tested once and each test consisted of four trials. The method used and parameters recorded were the same as reference memory task.

Immunofluorescence

The left hemispheres were post-fixed overnight, and then were put in 20% sucrose in 0.1 M phosphate buffer for 24 h. Systemically random coronal sections (40 μm thick) were cut on a sliding microtome, with an interval of 320 μm between consecutive sections, and stored in cryoprotective solution. Sections were washed with PBS and incubated with 2 N HCl for 30 min at 37°C. Sections were blocked for 1 h with a permeabilization/blocking solution containing 10% fetal calf serum, 2% bovine serum albumin, 1% glycine, and 0.05% Triton X-100 (Sigma-Aldrich). Specimens were then stained with the primary antibodies rat anti-BrdU (1:400; Oxford Biotechnology), or with specified combinations of the following primary antibodies: rat anti-BrdU (1:200; Oxford Biotechnology)/goat anti-DCX (1:100; Santa Cruz Biotechnology), rat anti-BrdU (1:400; Oxford Biotechnology)/ mouse anti-NeuN (1:400; sigma). We used secondary antibodies Cy-3-conjugated donkey anti-rat, Alexa488-conjugated donkey anti-goat, Alexa488-conjugated goat anti-mouse, (1:200; Jackson ImmunoResearch).

Microscopic analyses and stereology

All measurements were performed by an observer blind to animal identifications. For qu-

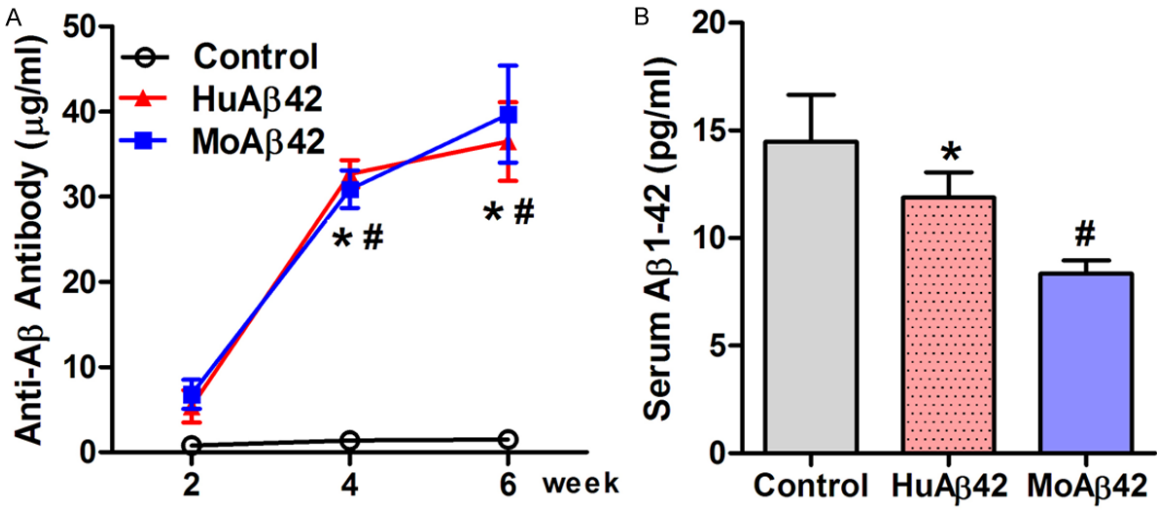

Figure 1. Immune response against mouse and human Aβ in neonatal mice and the quantity of Aβ42 in serum after Aβ1-42 immunization. A. Quantification of anti-Aβ antibodies in mouse serum by anti-Aβ ELISA. Data are presented as mean ± SD (n = 12) of Aβ antibodies (μg/ml). One-way ANOVA followed by post hoc comparison revealed significant differences in anti-Aβ titers of human Aβ1-42-immunized group when comparing week 4 and week 6 (*P < 0.05). The same trend was observed in mouse Aβ1-42-immunized group (*P < 0.01). There was no significant difference between the two treated groups. B. For Aβ analysis, blood samples were individually collected from human or mouse Aβ1-42 or PBS immunized mice at the end of 6th week and were measured separately by Aβ ELISA. Data were presented as mean ± SD (pg/ml). The level of mouse Aβ1-42-treated group was decreased compared to the control group (*P < 0.01).

antification of proliferation and neurogenesis in the dentate gyrus, we used the protocol previously described [24]. Proliferation was assessed by bilateral counting of BrdU+ cells in the SGZ of the dentate gyrus (defined as a zone of the hilus, the width of two cell bodies, along the base of the granular layer). Neurogenesis in the dentate gyrus was evaluated by counting the cells that were double labeled with BrdU and DCX or with BrdU and NeuN. With the same method, we counted the number of labeled cells in six coronal sections (370 μm apart) per mouse brain that were stained and mounted on coded slides. Absolute numbers and ratios of double-labeled cells versus BrdU-positive cells are given. All images were taken in sequential scanning mode using a confocal laser scanning microscope (ZEISS) under a 40× objective and further processed in Adobe Photoshop 7.0. Only general contrast adaptations were made and figures were not otherwise manipulated. Exemplary and representative images are presented.

Measurements of mouse cytokines

All samples were normalized to 400 μg/ml before analysis. Levels of the pro-inflammatory cytokines interleukin-4 (IL-4) and tumor necrosis factor-α (TNF-α) as well as level of interferon γ (IFN-γ) in hippocampal supernatants and serum were measured using ELISA kits per manufacturer's instructions (Invitrogen, Basel, Switzerland). Cytokines concentrations were normalized to total hippocampal protein concentration.

Statistical analysis

All experiments were repeated for three times and the data were described as mean ± SD for each group. The difference between groups was analyzed with one-way ANOVA and LSD-t was used for comparison of two groups with SPSS18.0. P < 0.05 was considered statistically significant.

Results

Aβ antibody production in WT mice immunized with human or mouse Aβ1-42 combined with Freund's adjuvant

To determine the presence of circulating Aβ antibodies, antibody ELISA analysis was use to evaluate the success of Aβ1-42 vaccination. After the second vaccination, anti-Aβ1-42 of mouse Aβ1-42 treated group was (7.46±2.69)

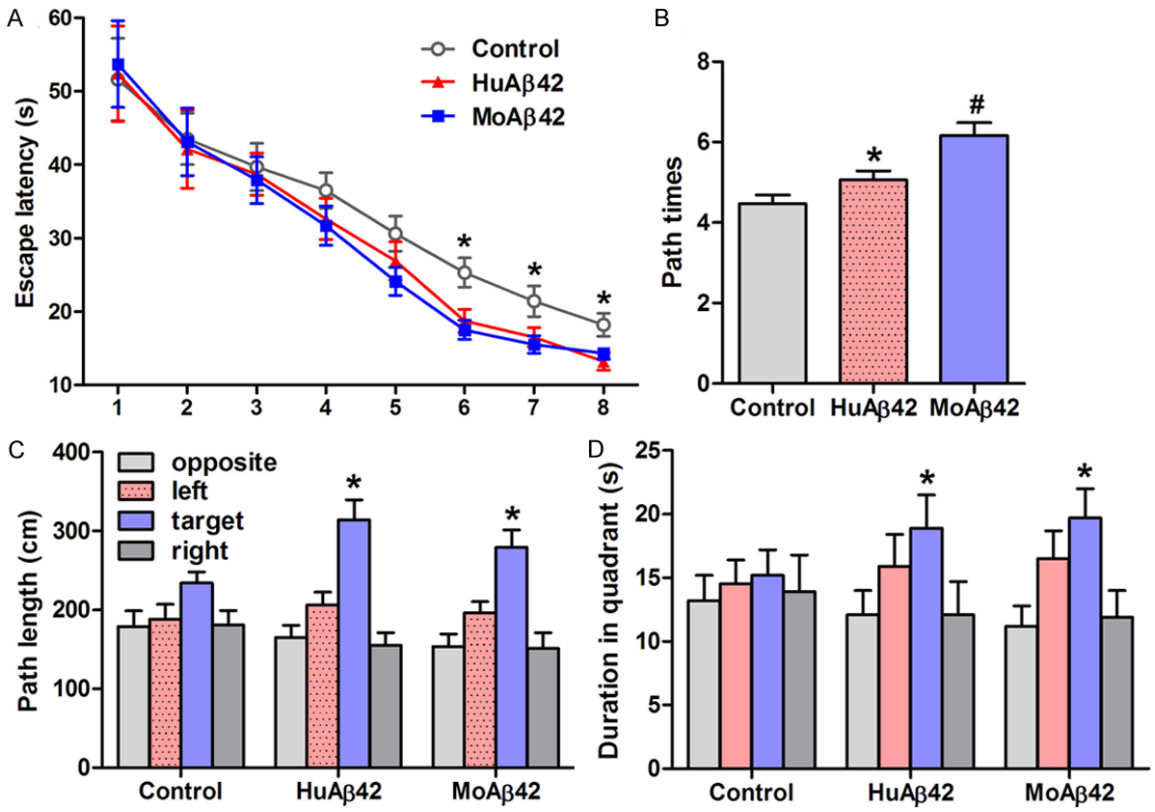

Figure 2. Cognitive performance of mice submitted to mouse or human Aβ1-42 injection, evaluated by the Morris water maze test. A. Performance in the reference memory protocol, based on escape latency. Each point represents the mean ± SD. There was no significantly difference in the first two days ($P > 0.05$). Significant differences were detected by comparing Aβ1-42 injection groups and control group from day 3 (n = 12, repeated measures analysis of variance, $*P < 0.05$); B. Number of crossings over the platform position. Values are mean ± SD. Significantly different from treated groups (n = 12, two-way ANOVA, followed by Tukey's test, $*P < 0.05$). C. The path length of each group in each quadrant in the probe trial (n = 12, Student's t test, $*P < 0.05$). D. Time spent of each group in each quadrant in the probe trial. Values are mean ± SD (n = 12, Student's t test, $*P < 0.05$).

μg/ml and human Aβ1-42 treated group was (6.94±2.94) μg/ml. Two weeks later, antibodies of human Aβ1-42 group was (28.55±4.78) μg/ml and mouse Aβ1-42 group was (30.48±4.19) μg/ml. At 6th week, the anti-Aβ1-42 antibody quantity of mouse Aβ1-42 group was (43.61±4.67) μg/ml, while human Aβ1-42 group was (39.12±4.69) μg/ml (**Figure 1A**). They were all higher than the control group ($*P < 0.01$). Meanwhile, to ensure whether Aβ vaccine clean up mouse endogenous Aβ as anti-Aβ1-42 antibodies were detected in the Aβ groups. The Aβ quantity in serum of mouse Aβ group (9.22±1.76) pg/ml was much less than the control group (14.92±3.88) pg/ml and human Aβ group (14.21±2.37) pg/ml, however there is no significant difference between human Aβ group and the control group (**Figure 1B**).

Behavioral effects

To evaluate the effect of Aβ1-42 immunization to C57BL/6 mice, we used the MWM to analyze the spatial reference learning and memory. The data acquired were analyzed with general linear model (GLM) and ANOVA. In learning trials, the escape latencies of mice in Groups decreased by day with significant difference, which also had statistical significance among the groups with different treatment. From **Figure 2A**, the best performance happened in Aβ1-42 treated groups compared with the control group. **Figure 2B** represented the path length, duration in zone and the time spent passing the platform in probe trial, respectively. Mice that had learned the location of the platform were expected to spend more time with longer path length in the target quadrant

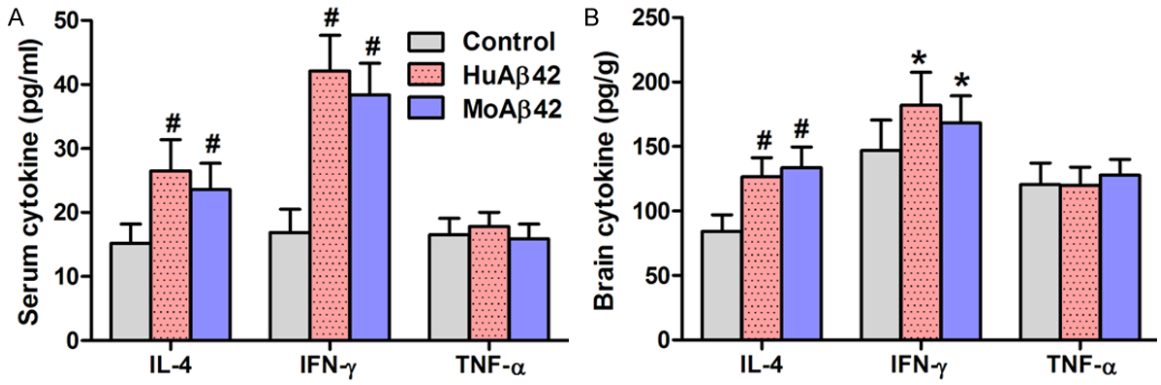

Figure 3. The quantity of cytokines in mice immunized with human Aβ1-42, mouse Aβ1-42 and control mice. A. The levels of IL-4, IFN-γ and TNF-α in serum of three groups. There was a significant difference in cytokine levels of IL-4 and IFN-γ between Aβ1-42 challenge groups and control group (*$P < 0.05$). There was no significant difference in cytokine levels TNF-α of the three groups. B. The levels of IL-4, IFN-γ and TNF-α in mouse brains of three groups. IL-4 and IFN-γ increased in brains of Aβ1-42 treated groups (*$P < 0.05$), while there was no change in TNF-α level comparing to the control group. Values are mean ± SD.

than in any other quadrants and more time passing the platform (**Figure 2C**, **2D**). The trial data implied that spatial reference memory of human Aβ1-42 group (*$P < 0.05$) and mouse Aβ1-42 group (*$P < 0.05$) was better improved compared with the control.

Aβ vaccine up-regulated IL-4, IFN-γ

To explore whether the underlying Aβ1-42 specific T cells mediated the improvement of neurogenesis in the dentate gyrus and cognitive function, the quantity of some pro-inflammatory cytokines in brain and serum, such as IL-4, IFN-γ and TNF-α were measured. The quantity of IL-4 was significantly increased both in serum (**Figure 3A**) and brain (**Figure 3B**), while the two Aβ1-42 groups comparing to the control group (*$P < 0.05$). After Aβ1-42 vaccination, IL-4 detected in the serum of the human Aβ1-42 group was (24.70±6.19) pg/ml and (116.49±23.42) pg/g in the brain; meanwhile, IL-4 of mouse Aβ1-42 group was (24.15±5.68) pg/ml in serum and (199.39±14.88) pg/g in brain. Meanwhile, human Aβ1-42 group was (44.55±6.39) pg/ml in serum and (200.64±19.30) pg/ml in brain, they were much higher than the control group (*$P < 0.05$). The amount of IFN-γ in mouse Aβ42 group was (41.61±5.30) pg/ml in serum and (199.39±14.88) pg/g in brain, significantly higher than the control (*$P < 0.05$). The difference between the mouse Aβ1-42 group and human Aβ1-42 group had no statistical difference. The level of serum and brain TNF-α was quantified as well. However, there is no statistical significant difference among these three groups ($P > 0.05$).

Immunization with Aβ1-42 induce more newly formed neurons and microglia

Since the better performance of the two Aβ1-42 groups in ethological test, to analyze the detailed morphology of the new granule cells, we labeled them using the antibodies to double staining of BrdU with DCX (**Figure 4A**) and BrdU with NeuN (**Figure 4B**). Neonatal neurons (BrdU+/DCX+) in mouse Aβ1-42 group and human Aβ1-42 group were significantly increased than the control group, so as to the newly formed maturate neurons (BrdU+/NeuN+) (*$P < 0.05$, **Figure 4E**). Double staining of BrdU with Iba1 (**Figure 4C**) and BrdU with GFAP (**Figure 4D**) represented the newborn microglia and astrocyte. Stereology analysis showed that BrdU+/ Iba1+ cells also increased in Aβ1-42 treated groups (*$P < 0.05$, **Figure 4F**), nevertheless, there was nearly no difference of astrocyte (BrdU+/GFAP+) between the three groups ($P > 0.05$, **Figure 4F**).

Discussion

Although much attention has been focused on Aβ itself in this respect, it may not actually be as an attractive target as current doubts concerning its causative role is substantiated [25, 26]. Aβ is the main component of senile plaques and generating excessive or clearance decrease would result in the formation of neurofi-

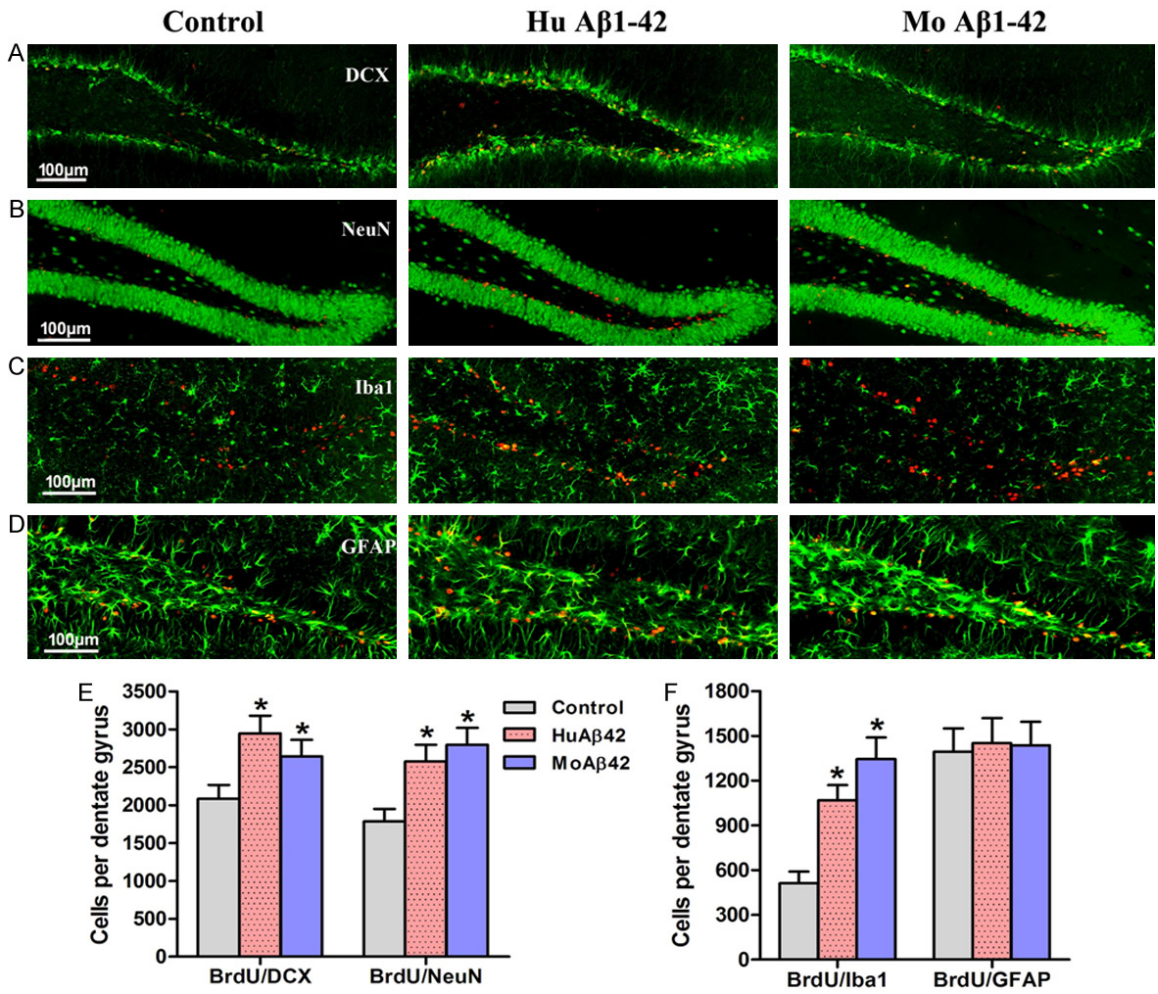

Figure 4. The improvement of neurogenesis in mouse and human Aβ1-42 vaccinated groups. A. Representative confocal micrographs of the dentate gyrus of the control, human Aβ1-42 and mouse Aβ1-42 treated group mice that were double stained for BrdU (red) and DCX (green). B. Representative confocal micrographs of the dentate gyrus of the three groups that were double stained for BrdU (red) and NeuN (green). C. Representative confocal micrographs of the three groups that were double stained for BrdU (red) and Iba1 (green) for the changes of microglia in dentate gyrus after Aβ1-42 immunization. D. Representative confocal micrographs of the dentate gyrus of the three groups that were double stained for BrdU (red) and GFAP (green) for the changes of astrocyte in dentate gyrus after Aβ1-42 immunization. E. Quantification analysis of BrdU+/DCX+ cells and BrdU+/NeuN+ cells in treated groups comparing to the control group, respectively (n = 12, $*P < 0.05$). F. Quantification analysis of BrdU+/Iba1+ cells and BrdU+/GFAP + cells in treated groups comparing to the control group, respectively (n = 12, $*P < 0.05$). Data were presented as mean ± SD. Scale bar = 100 μm.

brillary tangles, which is a key factor of AD [27]. Aβ cascade hypothesis is the most widely recognized. The traditional view was that the neurotoxicity of Aβ was mostly amyloid fibrils, but more and more studies found that while compared to fibrillar Aβ, soluble Aβ was more neurotoxicity. The level of Aβ oligomers inducing microglia-mediated neurotoxicity was 5-50 nmol/L, and fibrillar Aβ neurotoxicity concentration is above 15 mmol/L [28]. The mechanisms of Aβ neurotoxicity were mainly neuron insult, synaptic dysfunction and reduction of synaptic plasticity, then impairing cognitive function [29]. Aβ1-42 vaccination in AD transgenic mice could not only induce anti-Aβ1-42 antibodies to prevent and reduce the formation of amyloid plaques, but also significantly reduce the cognitive impairment or improve cognitive ability [30].

Three amino acid sequences of Aβ are different between rodents and human. All three different sites locate in the N-terminal, and this particular region is B cell epitopes. The different B cell

epitopes of rodent and human Aβ would induce different anti-Aβ antibodies. In our research, both mouse and human Aβ1-42 immunized in newborn mice, induced amounts of anti-Aβ antibodies, improved neurogenesis and development of cognitive function, while compared with the control group. There is no significant difference between the two Aβ1-42 groups. After immunization, the quantity of serum Aβ was reduced in mouse Aβ1-42-treated group, however, there is no significant difference between human Aβ1-42 group and the control group.

It was showed that Aβ triggers an inflammatory response by activating microglial cells and astrocytes [31]. Numerous studies demonstrated increasing pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) in patient CSF and serum and, moreover, suggested that their levels correlated with the transformation of MCI to AD despite a lack of changes in Aβ [32, 33]. Further mechanistic studies revealed that the improvement of neurogenesis and development of cognitive function after Aβ immunization could not attribute to anti-Aβ antibody mediated clearance of endogenous mouse Aβ. Since endogenous Aβ clearance was not in favor of improvement neurogenesis and cognitive ability, there must have another way. In our research, more neonatal microglia was found in SGZ of mice after Aβ1-42 vaccination which indicated immune response taking part in the improvement. Some central nervous system (CNS) self-proteins, such as myelin basic protein (MBP), can be recognized by CNS-specific T cells, through interacting with CNS dendritic cells [34]. The controlled autoimmune T cells activity in CNS not only plays a crucial role in neuroprotection for injured brain, but also have to do with neurogenesis [19, 35]. Aβ is also the CNS protein and could activate the CNS autoimmune T cells and inducing meningoencephalitis. The controlled autoimmune T cells may benefit to neurogenesis and the development of cognitive function in newborn mice. Most recent researches showed TNF-α, mostly a excretion of reactive microglia, is anti-neurogenic [36]. Phenotype of microglia is determined by the local environment. IFN-γ and IL-4,secreted by T cell, could induce a protective phenotype in microglia, while some other microglia, such as exposed to LPS, would result in a cytotoxic microglial phenotype, with up-regulation of TNF-α [37]. In the present study, we evaluated that mice inoculated with mouse Aβ1-42 and human Aβ1-42, higher amount of serum and brain IFN-γ, IL-4 was found comparing to the control group, but there was no difference between the two Aβ1-42 groups. TNF-α was lower than the control group in both mice Aβ1-42 and human Aβ1-42 treated group, but the difference was not statistically significant, which indicated that neonatal mice inoculating with Aβ42 would change the status of immune system, then affect the CNS neurogenesis. After immunization, IFN-γ and IL-4 were up-regulated in serum and brain with more activated microglia in CNS. Meantime, the levels of TNF-α in serum and brain were not change or lower, suggested the neonatal microglia was neuroprotective [38]. Immunized with Aβ1-42 in neonatal mice involved in IL-4 and IFN-γ inducing activation of microglia, which would secret some cytokines to promote the neurogenesis, and improve the cognitive ability, meanwhile, TNF-α decreased or unchanged meant that neurogenesis inhibition was not appear. Thus, Aβ1-42 immunotherapy in neonatal mice would contribute to the improvement of neurogenesis and cognitive function through evoking CNS Aβ-specific T cells, which secreted some cytokines, such as IFN-γ, IL-4, activating microglia to erect some neuroprotective factors. However, to confirm the Aβ-specific T cells contribute to neurogenesis and cognitive ability in neonatal mice remains to be further explored.

MWM is one of the most frequently used tools in behavioral neuroscience to investigate cognitive ability, underlying hippocampus mechanisms. The escape latency is an estimate of spatial learning and memory capacity, while the probe trail is use to evaluate retentive faulty. Our study confirmed that both human and mouse Aβ1-42 groups performed equally well than the control group, suggesting that cognitive ability was improved after Aβ1-42 vaccine since neonatal period. Aβ-specific T cellular immune response rather than anti-Aβ1-42 antibody may contribute to neurogenesis and development of cognitive function.

Previous studies have identified that neurogenesis continues throughout the whole life, including the adult, especially in immediate postnatal period [39]. Hippocampus, in which new neurons generate actively, may have a function in cognition [40, 41]. DCX is expressed in imma-

ture neurons and continued for 2-3 weeks as neural precursor cells mature into neurons [42]. At the meanwhile, NeuN is expressed in these mature neurons and GFAP+ was used to activity mark the astrocyte. In this research, newly formed immature neurons were labeled both BrdU and DCX, while double staining for BrdU and NeuN was stand for the mature neurons. Through the analysis of stereology, we next verified more newly formed immature and mature neurons were found in DG, which was suggesting neurogenesis was promoted, other than astrocyte, after mouse or human Aβ1-42 vaccine in neonatal mice.

Although there is no direct evidence that neurogenesis is required for emotional regulation and hippocampus-dependent spatial learning ability [43], there is still an explicit association between neurogenesis and certain hippocampal activities, especially the memory formation. The neonatal mice immunized with human or mouse Aβ1-42 had better hippocampus dependent memory and spatial learning ability, likewise, the morphological features of the two Aβ1-42 groups showed more neurogenesis. Conclusively, whatever Aβ1-42 vaccination in neonatal mice absolutely had an effect on neurogenesis and cognitive ability.

Taken together, these data provide evidence of Aβ1-42 vaccination could promotes recognition memory and neurogenesis of the neonatal wild type mice, which are possibly not associated with clearance of endogenous Aβ. Moreover, IFN-γ and IL-4 were observed up-regulating in serum and brain. These findings contribute to the understanding the Aβ potential factors for neurogenesis.

Acknowledgements

This research was supported by the project Yantai science and technology development plan (No. 2013WS214).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Guangqiang Cui and Peiyou Gong, Department of Neurosurgery, Yantai Yuhuangding Hospital, 20 Yuhuangding East Road, Yantai 264000, Shandong Province, PR China. E-mail: cuiguangqiang001@163.com (GQC); 13954562823@163.com (PYG)

References

[1] Chow VW, Mattson MP, Wong PC and Gleichmann M. An overview of APP processing enzymes and products. Neuromolecular Med 2010; 12: 1-12.

[2] Porayette P, Gallego MJ, Kaltcheva MM, Bowen RL, Vadakkadath Meethal S and Atwood CS. Differential processing of amyloid-beta precursor protein directs human embryonic stem cell proliferation and differentiation into neuronal precursor cells. J Biol Chem 2009; 284: 23806-23817.

[3] Lukiw WJ, Cui JG, Yuan LY, Bhattacharjee PS, Corkern M, Clement C, Kammerman EM, Ball MJ, Zhao Y, Sullivan PM and Hill JM. Acyclovir or Abeta42 peptides attenuate HSV-1-induced miRNA-146a levels in human primary brain cells. Neuroreport 2010; 21: 922-927.

[4] Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE and Moir RD. The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One 2010; 5: e9505.

[5] Zaghi J, Goldenson B, Inayathullah M, Lossinsky AS, Masoumi A, Avagyan H, Mahanian M, Bernas M, Weinand M, Rosenthal MJ, Espinosa-Jeffrey A, De Vellis J, Teplow DB and Fiala M. Alzheimer disease macrophages shuttle amyloid-beta from neurons to vessels, contributing to amyloid angiopathy. Acta Neuropathol 2009; 117: 111-124.

[6] Jankowsky JL, Younkin LH, Gonzales V, Fadale DJ, Slunt HH, Lester HA, Younkin SG and Borchelt DR. Rodent Abeta Modulates the Solubility and Distribution of Amyloid Deposits in Transgenic Mice. J Biol Chem 2007; 282: 22707-22720.

[7] Balducci C, Beeg M, Stravalaci M, Bastone A, Sclip A, Biasini E, Tapella L, Colombo L, Manzoni C, Borsello T, Chiesa R, Gobbi M, Salmona M and Forloni G. Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein. Proc Natl Acad Sci U S A 2010; 107: 2295-2300.

[8] Wicklund L, Leao RN, Stromberg AM, Mousavi M, Hovatta O, Nordberg A and Marutle A. Beta-amyloid 1-42 oligomers impair function of human embryonic stem cell-derived forebrain cholinergic neurons. PLoS One 2010; 5: e15600.

[9] Wilcock DM and Colton CA. Anti-amyloid-beta immunotherapy in Alzheimer's disease: relevance of transgenic mouse studies to clinical trials. J Alzheimers Dis 2008; 15: 555-569.

[10] Town T. Alternative Abeta immunotherapy approaches for Alzheimer's disease. CNS Neurol Disord Drug Targets 2009; 8: 114-127.

[11] Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M and Arendash GW. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000; 408: 982-985.

[12] Maier M, Seabrook TJ, Lazo ND, Jiang L, Das P, Janus C and Lemere CA. Short amyloid-beta (Abeta) immunogens reduce cerebral Abeta load and learning deficits in an Alzheimer's disease mouse model in the absence of an Abeta-specific cellular immune response. J Neurosci 2006; 26: 4717-4728.

[13] Deren KE, Forsyth J, Abdullah O, Hsu EW, Klinge PM, Silverberg GD, Johanson CE and McAllister JP 2nd. Low levels of amyloid-beta and its transporters in neonatal rats with and without hydrocephalus. Cerebrospinal Fluid Res 2009; 6: 4.

[14] Varin A, Mukhopadhyay S, Herbein G and Gordon S. Alternative activation of macrophages by IL-4 impairs phagocytosis of pathogens but potentiates microbial-induced signalling and cytokine secretion. Blood 2010; 115: 353-362.

[15] Carter CJ. Interactions between the products of the Herpes simplex genome and Alzheimer's disease susceptibility genes: relevance to pathological-signalling cascades. Neurochem Int 2008; 52: 920-934.

[16] Clark IA and Atwood CS. Is TNF a link between aging-related reproductive endocrine dyscrasia and Alzheimer's disease? J Alzheimers Dis 2011; 27: 691-699.

[17] Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, Knapp DJ and Crews FT. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 2007; 55: 453-462.

[18] Dolbeare F. Bromodeoxyuridine: a diagnostic tool in biology and medicine, Part II: Oncology, chemotherapy and carcinogenesis. Histochem J 1995; 27: 923-964.

[19] Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J and Schwartz M. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci 2006; 9: 268-275.

[20] Qu B-X, Xiang Q, Li L, Johnston SA, Hynan LS and Rosenberg RN. Aβ42 gene vaccine prevents Aβ42 deposition in brain of double transgenic mice. J Neurol Sci 2007; 260: 204-213.

[21] Petrushina I, Ghochikyan A, Mktrichyan M, Mamikonyan G, Movsesyan N, Davtyan H, Patel A, Head E, Cribbs DH and Agadjanyan MG. Alzheimer's disease peptide epitope vaccine reduces insoluble but not soluble/oligomeric Abeta species in amyloid precursor protein transgenic mice. J Neurosci 2007; 27: 12721-12731.

[22] Vorhees CV and Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols 2006; 1: 848-858.

[23] Zou J, Yao Z, Zhang G, Wang H, Xu J, Yew DT, Forster EL. Vaccination of Alzheimer's model mice with adenovirus vector containing quadrivalent foldable Aβ1-15 reduces Aβ burden and behavioral impairment without Aβ-specific T cell response. J Neurol Sci 2008; 272: 87-98.

[24] Iacono D, O'Brien R, Resnick SM, Zonderman AB, Pletnikova O, Rudow G, An Y, West MJ, Crain B and Troncoso JC. Neuronal hypertrophy in asymptomatic Alzheimer disease. J Neuropathol Exp Neurol 2008; 67: 578-589.

[25] Selkoe DJ. Preventing Alzheimer's disease. Science 2012; 337: 1488-1492.

[26] Naylor MD, Karlawish JH, Arnold SE, Khachaturian AS, Khachaturian ZS, Lee VM, Baumgart M, Banerjee S, Beck C, Blennow K, Brookmeyer R, Brunden KR, Buckwalter KC, Comer M, Covinsky K, Feinberg LF, Frisoni G, Green C, Guimaraes RM, Gwyther LP, Hefti FF, Hutton M, Kawas C, Kent DM, Kuller L, Langa KM, Mahley RW, Maslow K, Masters CL, Meier DE, Neumann PJ, Paul SM, Petersen RC, Sager MA, Sano M, Schenk D, Soares H, Sperling RA, Stahl SM, van Deerlin V, Stern Y, Weir D, Wolk DA and Trojanowski JQ. Advancing Alzheimer's disease diagnosis, treatment, and care: recommendations from the Ware Invitational Summit. Alzheimers Dement 2012; 8: 445-452.

[27] Hardy J and Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297: 353-356.

[28] Maezawa I, Zimin PI, Wulff H and Jin LW. Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity. J Biol Chem 2011; 286: 3693-3706.

[29] Malchiodi-Albedi F, Paradisi S, Matteucci A, Frank C and Diociaiuti M. Amyloid Oligomer Neurotoxicity, Calcium Dysregulation, and Lipid Rafts. International Journal of Alzheimer's Disease 2011; 2011: 1-17.

[30] Lizard G, Rouaud O, Demarquoy J, Cherkaoui-Malki M and Iuliano L. Potential roles of peroxisomes in Alzheimer's disease and in dementia of the Alzheimer's type. J Alzheimers Dis 2012; 29: 241-254.

[31] Solito E and Sastre M. Microglia function in Alzheimer's disease. Front Pharmacol 2012; 3: 14.

[32] Laurin D, David Curb J, Masaki KH, White LR and Launer LJ. Midlife C-reactive protein and

risk of cognitive decline: a 31-year follow-up. Neurobiol Aging 2009; 30: 1724-1727.
[33] Schuitemaker A, Dik MG, Veerhuis R, Scheltens P, Schoonenboom NS, Hack CE, Blankenstein MA and Jonker C. Inflammatory markers in AD and MCI patients with different biomarker profiles. Neurobiol Aging 2009; 30: 1885-1889.
[34] Ziv Y and Schwartz M. Immune-based regulation of adult neurogenesis: Implications for learning and memory. Brain, Behavior, and Immunity 2008; 22: 167-176.
[35] D'Hooge R and De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 2001; 36: 60-90.
[36] Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S, Martino G and Schwartz M. Microglia activated by IL-4 or IFN-γ differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cellular Neurosci 2006; 31: 149-160.
[37] Butovsky O, Talpalar AE, Ben-Yaakov K and Schwartz M. Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. Mol Cell Neurosci 2005; 29: 381-393.
[38] Cribbs DH, Ghochikyan A, Vasilevko V, Tran M, Petrushina I, Sadzikava N, Babikyan D, Kesslak P, Kieber-Emmons T, Cotman CW and Agadjanyan MG. Adjuvant-dependent modulation of Th1 and Th2 responses to immunization with beta-amyloid. Int Immunol 2003; 15: 505-514.
[39] Smart JL, Dobbing J, Adlard BP, Lynch A and Sands J. Vulnerability of developing brain: relative effects of growth restriction during the fetal and suckling periods on behavior and brain composition of adult rats. J Nutr 1973; 103: 1327-1338.
[40] McKay R. Stem cells in the central nervous system. Science 1997; 276: 66-71.
[41] van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD and Gage FH. Functional neurogenesis in the adult hippocampus. Nature 2002; 415: 1030-1034.
[42] Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L and Kuhn HG. Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 2003; 467: 1-10.
[43] Deng W, Aimone JB and Gage FH. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 2010; 11: 339-350.