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Early Aβ-HBc virus-like particles immunization had better effects on preventing the deficit of learning and memory abilities and reducing cerebral Aβ load in PDAPP mice

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ABSTRACT

For nearly two decades, immunization against the β-amyloid peptide (Aβ) has been investigated as a potential treatment for Alzheimer's disease (AD). Despite some disappointing results in clinic trials, greater significance has been attached by some researchers to exploring the immune effects on pathological and cognitive changes in AD or producing new vaccines of AD. In the previous study, we have made a virus-like particles (Aβ-HBc VLPs) as Aβ vaccine candidate. Aβ-HBc VLPs could ameliorate the learning and memory abilities and reduce cerebral Aβ deposit in the old PDAPP mice. In the present study, to observe the preventive effect and the proper time of immunization, 3, 6 and 9-month old PDAPP mice were immunized with Aβ-HBc VLPs for 3 months. All mice generated high titer of anti-Aβ antibody after Aβ-HBc VLPs immunizations. When the mice were 15-month old, Morris Water Maze was used to test their learning and memory abilities. The escape latencies of Aβ-HBc VLPs immunized mice were shorter than that of control mice. These immunized mice entered platform region frequently and spent more time on the platform region and quadrant. 3 m and 6 m Aβ-HBc VLPs immunized groups performed better than the 9 m group. In immunohistochemistry tests, all the Aβ-HBc VLPs immunized mice had less amyloid deposit in cortex and hippocampus. ELISA results showed that soluble Aβ was reduced in the brain homogenates of the Aβ-HBc VLPs immunized mice, and 3- and 6-month groups had less soluble Aβ than the 9-month group. In conclusion, our study showed that Aβ-HBc VLPs immunization could elicit a strong immune response in adult APP mice, and early immunization had better effects on preventing learning and memory deficits, lowering Aβ burden in PDAPP mice.

1. Introduction

Alzheimer disease (AD) is a complex neurodegenerative disease characterized by progressive impairment of memory. Due to increasing of the morbidity and without valid therapy, AD becomes the most common form of dementia and the third major cause of disability and death for the elderly, only after cardiovascular and cerebrovascular diseases and malignant tumors [1]. However, only five drugs are approved by the FDA to treat AD, and these drugs just alleviate some of symptoms of AD without slowing the progression of the disease. Thus, developing drugs for more effective AD treatment is urgently needed.

An imbalance between production and clearance of amyloid β peptide (Aβ) causes Aβ overload in the brain. Overloaded Aβ accumulates and aggregates to form amyloid plaques, the neuropathological hallmark of AD. So, preventing the Aβ over-producing, and/or increasing its clearance are strategies for treating AD. It has been proved that anti-Aβ immunotherapy is effective to remove Aβ from the brains of animal models and AD patients [2–4]. In our previous studies, the gene of 2Aβ15 (linked by -AAGAAG-) was inserted in the truncated *HBc* gene (coding for amino acids 1–71 and 88–144 of HBcAg) to construct the recombinant gene c-2Aβ15-c that was expressed in *Escherichia coli*. The purified fusion protein could form chimerical virus-like particles (Aβ-HBc VLPs). These VLPs effectively induced B cell response without adverse effects in BALB/c mice. The main isotypes of antibody were IgG1 and IgG2b [5]. Aβ-HBc VLPs also ameliorated the learning and memory and reduced cerebral Aβ deposit in the

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old PDAPP mice [6]. However, it remains unclear whether the Aβ-HBc VLPs immunization could prevent the deficit of the learning and memory abilities and is capable of preventing Aβ deposition in AD transgenic mice before the symptoms appearance or at the very beginning of the disease stage. And it is also not clear when the proper time to treat the disease is. So in the present study, we immunized young and adult PDAPP mice (with no or mild behavioral and pathological changes) with Aβ-HBc VLPs to investigate the prophylactic effects.

2. Materials and methods

2.1. Animals and immunization

PDAPP transgenic mice were purchased from Institute of Laboratory Animal Science, Chinese Academy of Medical Science (Beijing, China). Mice were housed and fed under specific pathogen-free (SPF) conditions with food and water ad libitum. All treatments were in accordance with Guide for the Care and Use of Laboratory Animals of Xi'an Jiaotong University.

PDAPP mice with 3 different age stages were used in the present study: 3-month old (without behavioral and pathological changes), 6-month old (behavioral changes begin to appear) and 9-month old (both typical behavioral and pathological changes appear). All mice were divided into 6 groups: 3-month Aβ-HBc VLPs immunization group (Aβ-HBc VLPs, 3 m) and 3-month phosphate buffered saline (PBS) group (PBS, 3 m), 6-month Aβ-HBc VLPs immunization group (Aβ-HBc VLPs, 6 m) and 6-month PBS group (PBS, 6 m), 9-month Aβ-HBc VLPs immunization group (Aβ-HBc VLPs, 9 m) and 9-month PBS group (PBS, 9 m). 10 mice in each group (n = 10; 5 males and 5 females).

Mice in Aβ-HBc VLPs immunization groups were subcutaneously injected with Aβ-HBc VLPs (5 μg/mouse [5,6]) in the absence of adjuvant at 2 weeks intervals for the first 3 immunizations (1 month) and then monthly immunization (2 months, 2 times immunization) (Fig. 1). The control mice were immunized with PBS in the same schedule.

2.2. Serological tests

Animals were bled before the first immunization and every one week after each immunization for the serological tests. Antibody titers in the sera were detected by indirect enzyme-linked immunosorbent assay (ELISA) as described previously [5].

2.3. Behavioral tests

At 15 months of age, Morris Water Maze (MWM) tests were used to measure the learning and memory abilities as described before [6]. In brief, the tests were divided into 2 trials: 5-day spatial navigation trial and one day probe trial. In the spatial navigation trial, an escape platform was submerged 1 cm beneath the water surface. Each trial lasted a maximum of 60 s. The time to reach platform from the start location was recorded as latency. If a mouse failed to find the platform within 60 s, it was manually guided to the platform and allowed to stay on the platform for 10 s, the latency was recorded as 60 s.

The platform was withdrawn 24 h after the last spatial navigation trial. All mice were given the probe trial to assess the memory. In probe trials, platform crossings and time spent in target quadrant were measured. Performance in all tasks was recorded and analyzed by a computer-based video tracking system and image analyzing software (Chengdu TME Technology company, Chengdu, China).

2.4. Immunohistochemistry tests

After the Morris Water Maze tests, all animals were anesthetized with 10% chloral hydrate followed by perfusing with saline intracardially. For each mouse, brain was removed and cut into two symmetrical halves. Left half of the brain was fixed in 4% paraformaldehyde (PFA, PH 7.4) for 48 h and subsequently incubated in 30% sucrose till sunk. Coronal sections of 30 μm thickness were collected and stored in PBS at 4 °C until use.

For immunohistochemistry staining, one complete series of sections containing the dorsal hippocampus (8 sections of each mouse brain) with 150 μm distance between the sections were used for each mice. Staining was performed under standard free-floating labeling procedures. In brief, after blocking the endogenous peroxidase and nonspecific background, sections were incubated with primary antibodies ($A\beta_{1-42}$, 1:1000, cell signaling technology) overnight at 4 °C, followed by biotinylated secondary antibodies (anti-rabbit, and anti-goat), detection with an ABC peroxidase kit (Boster biotechnology company, China), and visualization with a

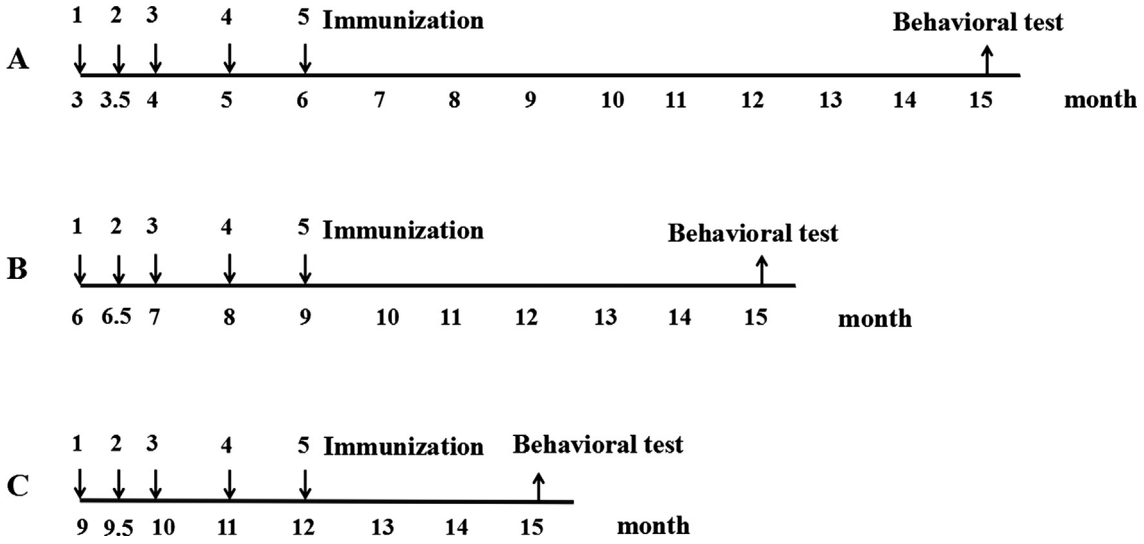

Fig. 1. Immunization protocols. PDAPP mice were immunized five times as indicated. Blood was collected and humoral immune responses were analyzed. The behavioral tests were performed when the mice were 15 months old. After that, they were sacrificed and analyzed for neuropathological changes in the brain.

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