Journal of Neuroimmunology 274 (2014) 62–70

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Active immunotherapy facilitates Aβ plaque removal following through microglial activation without obvious T cells infiltrating the CNS

Sha Sha [a,1], Xiao-Na Xing [b], Yun-Peng Cao [c,*]

[a] Department of Geriatrics, The First Affiliated Hospital of China Medical University, Shenyang 110001, Liao Ning Province, China
[b] Department of Neurology, Provincial People's Hospital, Shenyang 110001, Liao Ning Province, China
[c] Department of Neurology, the First Affiliated Hospital of China Medical University, China

ARTICLE INFO

Article history:
Received 11 March 2014
Received in revised form 13 June 2014
Accepted 17 June 2014

Keywords:
Alzheimer's disease
Microglial
Amyloid β-protein
T cell infiltration

ABSTRACT

Immunization of AD mouse models with Aβ reduced Aβ deposits and improved memory and learning deficits, but some clinical trials of immunization with Aβ were halted due to brain inflammation which was presumably induced by a T cell-mediated autoimmune response. We have developed a "possibly safer" vaccine. Our results demonstrate that pcDNA3.1 vector encoding ten repeats of Aβ3-10 fragments elicited high titers of antibodies which reacted well with not only monomeric but also oligomeric and fibrillar forms of Aβ42 peptide. Induced antibodies strongly reacted with amyloid plaques in the brain, demonstrating functional activity of the antibodies. Immunohistochemical and immunofluorescence showed there was significantly less plaque deposition accomplied with less microglia activation as detected both in the frontal cortex and hippocampus. These data suggested that microglial activation is necessary for efficient removal of compact amyloid deposits with immunotherapy. No obvious inflammation T cell and Prussian blue positive cell was found indicated that inflammation T cell infiltration and microhemmorage can be avoided or at least reduced to the minimum level.

1. Introduction

Alzheimer's disease (AD) is characterized pathologically by amyloid plaques, neurofibrillary tangles, neuron loss and an inflammatory response which involves the activation and proliferation of microglia (Akiyama et al., 2000; Hardy and Selkoe, 2002).Accordingly, strategies for AD therapy are aimed at reducing the level of Aβ in the brain, or preventing the assembly of this peptide into pathological form. There are a strong body of evidences that immune therapy in mouse models of AD is able to remove Aβ which ultimately improves the cognitive decline (Janus et al., 2000; Hock et al., 2003).Active immunotherapy significantly elicited high titers of antibodies which decreased the cerebral level of the peptide by promoting microglial clearance and redistributing the peptide from the brain to the systemic circulation (Bard et al., 2000; Janus et al., 2000; Morgan et al., 2000; Hock et al., 2003). These data largely justified the first clinical trial in AD patients, but the phase II clinical trial was halted because of acute meningoencephalitis, an inflammatory reaction in the CNS, presumably related to the induction of Th1-type responses (Orgogozo et al., 2003). T cell infiltrates were present in the leptomeninges, perivascular spaces, and brain parenchyma, suggesting an autoimmune T cell reaction to Aβ42 (Gilman et al., 2005).Collectively, further refinement of vaccines for AD is needed in order to eliminate, or at least attenuate the potential adverse events. Strategies of targeting Aβ B cell epitope while avoiding Aβ-specific T cell epitope may be safer and avoid adverse sequelae triggered by fibrillar full-length Aβ42 (Orgogozo et al., 2003). Passive immunization was also effective in reducing Aβ burden and attenuating cognitive deterioration of the animals (Wilcock et al., 2004; Lee et al., 2006). However, this strategy is not likely to be useful for protective vaccination due to the substantial cost, invasive nature of the treatment, and the recurrent clinical visits necessary for effective delivery of the immunotherapy (Movsesyan et al., 2008a,b). Gene vaccine provided an attractive alternative to synthetic peptide and adjuvant approach for inducing long-term, high levels of anti-Aβ antibodies as well as suppress cellular immunity. A unique property of DNA-based vaccination is the ability to induce prolonged, endogenous antigen synthesis and processing within the immunized host own cells. There are growing consensuses among some researchers based on both analysis of pre-clinical studies that early preventive immunization prior to substantial neuropathology, neuronal loss, and cognitive deficits have become firmly established may be more effective and safer for future patients receiving immunotherapy (Cribbs, 2010).

In our current study, we chose pcDNA3.1 as a vector, used ten tandem repeats of B cell-activating epitope Aβ3-10 as an antigen to enhance immunogenicity and then added melatonin (MT), which is

* Corresponding author at: Department of Neurology, the First Affiliated Hospital of China Medical University, No.155 Nanjing North Street, Heping District, Shenyang Liaoning, 110001, China. Tel.: +86 24 83282516.
E-mail addresses: yanbingxingya@163.com (S. Sha), ypengcao@yahoo.com (Y.-P. Cao).
[1] Department of Geriatrics, the First Affiliated Hospital of China Medical University, No.155 Nanjing North Street, Heping District, Shenyang Shenyang 110001, Liao Ning province, China. Tel.: +86 24 83282031.

http://dx.doi.org/10.1016/j.jneuroim.2014.06.008
0165-5728/

secreted by the pineal gland, as a novel adjuvant to enhance the humoral immune response. The vaccine was then injected intramuscularly into 3-month-old Tg-APPswe/PSEN1dE9 (Tg) mice using in vivo EP. Our previous study has demonstrated that our novel vaccine can induce high levels of anti-Aβ antibodies and subsequently results in a more robust Th2-polarized humoral immune response as well as decreased Aβ accumulation and deposition in the brain with a resultant decrease in plaque-associated astrocytosis and improved memory and cognition. The present study was designed to test the hypothesis that this vaccine should induce a robust humoral immune response, have prophylactic effects that can prevent the deposition of Aβ through microglial activation without T cell infiltration and microhemorrage.

2. Materials and methods

2.1. Plasmid construction and preparation

According to the cDNA sequence of Aβ3-10 in GenBank, the DNA fragment was synthesized:5'-TT-EcoRI-cozak-ATG(initiation bcodon)-(Aβ3-10)10-TAG(stop codon)-NotI-XhoI-GG-3′, and 10 × Aβ3-10 was cloned into a mammalian expression vector (pcDNA3.1) using EcoRI/XhoI restriction sites. The recombinant plasmid was verified by NotI/EcoRI digestion and gel electrophoresis. Correct plasmid sequence was confirmed using nucleotide sequence analysis (Shanghai GeneCore Bio Technologies, Shanghai, China).The recombinant plasmid was amplified in DH5 alpha E.coli competent cells. The plasmids were purified in large quantities using E.Z.N.A TM Fastfilter Endo-Free Plasmid Maxi Kit (OMEGA, USA).

2.2. Immunization of mice by in vivo electroporation

Twenty-four three-month-old APPswe/PSEN1dE9 mice were obtained from the Animal School of China Medical University. APPswe/PSEN1dE9 mice develop beta-amyloid deposits in the brain by 6 to 7 months of age. All mice were maintained in a 12-h light/dark cycle with food and water ad libitum. All animal experiments were done in compliance with NIH and institutional animal care and use committee guidelines at UCR and USF. Procedures had approval from the University of China Medical University ethics committee.

The mice were randomly assigned three groups and injected with the p(Aβ3-10)10-MT vaccine (n = 8), an Aβ42 peptide (n = 8), or pcDNA3.1 (n = 8) respectively. The immunization protocol was mentioned as (Sha et al., 2012). Aβ42 (50 μg per injection) was emulsified 1:1 (v/v) with complete Freund's adjuvant (Sigma, USA) for the first immunization, followed by a boost in incomplete Freund's adjuvant at three-week intervals (total of 10 times). The protocol for the preparation of the synthetic Aβ42 peptide (AnaSpec, San Jose, CA) and Freund's adjuvant was adapted from Schenk et al. (Schenk et al., 1999). The p(Aβ3-10)10-MT and pcDNA3.1 group were injected with their plasmid(100 μg in 100 μl PBS) intramuscularly into the left hind leg for a total of 10 immunizations at three-week intervals. After the mice were anaesthetized, a pair of electrode needles (26-gauge) were inserted into the muscle 5 mm apart to cover the DNA injection sites and electrical pulses were delivered using an electric pulse generator (ECM830, BTX Harvard Apparatus company, USA). Six pulses of 75 V each were delivered at a rate of one pulse every 200 ms (Otten et al., 2004).

2.3. Serum and tissue collection

Blood samples were drawn from each mouse via the orbital vein before immunization and one week after each immunization. After incubation at room temperature for 3hs, the blood was centrifuged for 10 min at 10,000 rpm. The mice were sacrificed two weeks after the final immunization, and the cerebrum was sagittally divided, and the left hemisphere was weighed and stored at −70 °C used for chemistry. The right hemisphere was fixed in 75% ethanol-formaldehyde for 48hs and embedded in paraffin following dehydration

2.4. ELISA analysis of anti-Aβ antibodies in serum and immunoreactivity of antisera to monomeric and oligomeric Aβ

The method for an ELISA(Enzyme-linked immunosorbent assay) to monitor the humoral immune response was adapted from publications by (Cribbs et al., 2003; Ghochikyan et al., 2006). In brief, mouse serum was used to detect Aβ peptide with a 96-microwell plate coated with glutathione S-transferase–Aβ proteins. Antibody titers were determined using a standard curve generated with serial dilutions of 6E10 antibody(Covance).Bound antibody was detected using horseradish peroxidase(HRP)-conjugated goat anti-mouse IgG (Zymed, CA).The reaction was developed with 3,3′,5,5′-tetramethylbenzidine(TMB) substrate. The optical density(OD)at 450 nm was read using a microplate reader(Elx800,BioTek Instruments, USA).HRP-conjugated anti-mouse IgG1, IgG2a, and IgG2b (Zymed, CA) were used to determine the isotypes of immunoglobulin. For western blot analysis, oligomeric Aβ was prepared as described by Dahlgren et al. (Dahlgren et al., 2002). The peptide was dissolved in 1 mM hexafluoroisopropanol (Sigma) and then removed under vacuum in a Speed Vac (Savant, Holbrook, NY). The residual peptide was resuspended in dimethyl sulfoxide (Sigma) to a concentration of 5 mM. By adding phenol red free Hams F-12 medium (Mediatech, Herndon, VA) to the resuspended peptide, the concentration was made to 100lM and the peptide was kept at 4 °C for 24 h. The samples were diluted in NuPage sample buffer (Invitrogen, Carlsbad, CA) and separated by 16.5% Tris–Tricine SDS–PAGE. Western blotting was performed using induced anti-sera and an enhanced chemiluminescence system (Amersham, Arlington Heights, IL) as described previously (Fukuchi et al., 1998).

2.5. Immunoreactivity of antisera to amyloid plaques in the brain:

Brain sections were used for testing the binding ability of anti-Aβ antibodies from sera of vaccinated mice. A 15-month-old-transgenic mouse (Mo/Hu APPswe PS1dE9) was deeply anesthetized and perfused transcardially with 4% paraformaldehyde. The brain was removed and postfixed in the perfusate for 16 h. Coronal sections (5 μm thick) were prepared for immunohistochemistry of amyloid plaques using different dilutions of sera from vaccinated mice with p(Aβ3-10)10-MT, empty vector, Aβ42 peptide respectively. After three washes with PBS, the corresponding biotin-labeled secondary antibodies (rabbit anti-mouse IgG, 1:500, Sigma, USA) and horseradish peroxidase-labeled streptomycin avidin working solution were added. Peroxidase activity was detected with DAB, and the sections were counterstained with hematoxylin.

2.6. ELISA for brain Aβ

For ELISA of brain Aβ, frozen cerebral hemisphere was homogenized in 10 volumes of ice-cold guanidine buffer (5.0 M guanidine HCl/50 mM Tris Cl, pH 8.0) and mixed for 3 to 4 h at room temperature. The homogenates were further diluted 1:10 with ice-cold casein buffer before centrifugation (16,000 ×g for 20 min at 4 °C). Final dilutions were made in 0.5 M guanidine and 0.1% bovine serum albumin and assessed for Aβ using commercially available ELISA kits (Invitrogen, Camarillo, CA).The absorbance of the plates was read at 450 nm with a spectrophotometer.

2.7. Immunohistochemistry staining and confocal laser scanning microscopy

Immunoreactivity was used to assess plaque load using the 6E10 anti-Aβ antibody, while Iba1 was used to assess microgliosis immunoreactivity and anti-CD3,anti-CD4 were used to analysis infiltration of T cells. We performed fluorescence immunolabeling to determine the relationship between Aβ burden and microgliosis activity. For double