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Induction of a Th2 immune response by co-administration of recombinant adenovirus vectors encoding amyloid β-protein and GM-CSF

Hong-Duck Kim[a], Yunpeng Cao[a,d], Fan-Kun Kong[c], Kent R. Van Kampen[c], Terry L. Lewis[a], Zhendong Ma[a], De-chu C. Tang[b,c], Ken-Ichiro Fukuchi[a,*]

[a] *Department of Genetics, Schools of Medicine and Dentistry, University of Alabama at Birmingham, KHGB 640B, 720 20th Street South, Birmingham, AL 35294-0024, USA*
[b] *Department of Dermatology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA*
[c] *Vaxin Inc., Birmingham, AL 35294, USA*
[d] *Department of Neurology, First Affiliated Hospital, China Medical University, Shenyang 110001, PR China*

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Abstract

Lines of experimental evidence indicate that induction of humoral immune responses in transgenic mouse models of Alzheimer disease (AD) by repeated injection of synthetic amyloid β-protein (Aβ) is effective in prevention and clearance of deposits of Aβ aggregates in the brain of the mice. We have tested a *non-injection* modality whereby replication-defective adenovirus vectors encoding Aβ or the 99-amino acid carboxyl terminal fragment of Aβ precursor were intranasally administered to mice to elicit immune responses against Aβ. When mice were immunized only with the adenovirus vectors, immune responses against Aβ were negligible. By co-immunization with an adenovirus vector encoding granulocyte-macrophage colony stimulating factor (GM-CSF), the adenovirus vector encoding Aβ effectively elicited an immune response against Aβ. Immunoglobulin isotyping demonstrated a predominant IgG1 and IgG2b response, suggesting a Th2 anti-inflammatory type. Thus, adjuvantation is essential for induction of an immune response against Aβ by adenovirus-mediated nasal vaccination.

Keywords: Alzheimer disease; Vaccine; Granulocyte-macrophage colony stimulating factor

1. Introduction

Alzheimer disease (AD) is the most common neurodegenerative disease in the elderly and characterized by amyloid plaques, neurofibrillary tangles, and neuronal loss in the brain. To date, no satisfactory treatments are available for AD. The main constituent of amyloid plaques is amyloid β-protein (Aβ). Increasing evidence supports the notion that Aβ and amyloid β-protein precursor (APP) play important roles in the pathogenesis of AD. Mutations in three different genes (APP, presenilin 1 and 2) implicated in the etiology of familial AD have been shown to increase Aβ production, particularly, a longer form of Aβ consisting of 42 amino acids (Aβ1-42). Aβ1-42 is considered to be highly amyloidogenic and oligomeric forms of Aβ are neurotoxic [1,2]. Overexpression of the mutant forms of APP in transgenic mice led to AD-like pathologies including amyloid plaques in brain. Immunization of these AD model mice with synthetic Aβ by repeated needle injection prevented or reduced Aβ deposits [3] and ameliorated their memory and learning deficits [4,5]. Clinical trial of Aβ immunization, however, was halted due to brain inflammation presumably induced by toxic Aβ and/or T-cell-mediated immune response [6,7]. Thus, safer immunization modalities have to be exploited for AD treatment.

Genetic immunization is an approach for eliciting immune responses against specific proteins by expressing genes encoding the proteins in animal's own cells and can be a safer modality for AD treatment. Genetic immunization may simplify the vaccination protocol because the difficult steps of

* Corresponding author. Tel.: +1 205 975 8715; fax: +1 205 975 5519.
E-mail address: fukuchi@uab.edu (K.-I. Fukuchi).

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protein purification and combination with adjuvant, both routinely required for vaccine development, are eliminated. It has been demonstrated that genetic vaccines may be more effective than contemporary clinically licensed vaccines in animal models [8]. Indeed, we previously demonstrated that nasal administration of adenovirus vector encoding the tetanus toxin C fragment induces a protective immune response against tetanus in mice [9]. In the present study, we have tested a *non-injection* vaccination modality whereby defective adenovirus vectors encoding Aβ or the 99-amino acid carboxyl terminal fragment (C99) of Aβ precursor (APP) are intranasally inoculated into mice to elicit an immune response against Aβ. Endogenous expression of the antigens may play important roles not only in the relatively greater efficacy of genetic vaccines but also in induction of a different type of immune response compared with conventional vaccines. Because cytokines play a critical regulatory role in the development of these immune responses induced by genetic immunization and granulocyte-macrophage colony stimulating factor (GM-CSF) is one of the most potent cytokines that augment such immune responses [10,11], we have tested the potential of GM-CSF as a genetic adjuvant in induction of an immune response against Aβ. We have performed histopathological and immunohistochemical analyses of the immunized mice to determine the safety of the modality, also.

2. Materials and methods

2.1. Construction of expression vectors

In order to optimize expression of Aβ, four expression vectors (Table 1) were constructed. These vectors differ in presence and absence of a Kozak consensus sequence [12] and in DNA sequences of codon usage [13]. In choosing more frequently used codons for Aβamino acid sequences, putative hairpin formation were avoided using GENETYX software for DNA analysis (Software Development Co. Ltd., Tokyo, Japan). All the cDNA constructs were placed under the control of the CMV enhancer/β-actin promoter in pCAGGS [14] or pCAGGSnc [15]. (i) pCA-Aβ for expression of methionine + Aβ1-42: to obtain the initiation ATG codon + Aβ1-42 sequence + stop TAG codon, PCR was performed using: ALA12 primer 5′-CCG CTC GAG GTC GCG ATG GAT GCA GAA TTC CGA CAT GAC TCA-3′, and ALA11 primer 5′-CCG CTC GAG GGG GGT CTA CGC TAT GAC AAC ACC GCC CAC CAT-3′, and pCA-SβC as a template [16], which contain the native cDNA sequence of C99. A 162-bp PCR product was digested with *Xho*I and cloned into the *Xho*I cloning site of pCAGGS [14] to generate pCA-Aβ that contains the native translation start site of APP. (ii) pCA-SAβ for expression of the signal sequence of APP + Aβ1-42: the 162-bp PCR product above was digested with *Eco*RI and *Xho*I restriction enzymes and cloned into the *Eco*RI and *Xho*I sites of pKS$^+$-SβC [16] from which the *Eco*RI/*Xho*I fragment was previously removed, to create pKS$^+$-SAβ. The *Not*I/*Xho*I fragment containing the signal sequence + Aβ1-42 was isolated from pKS$^+$-SAβ and the *Not*I site was ligated onto the *Not*I site of pCAGGSnc [15] that was previously digested with restriction enzymes, *Not*I and *Cla*I. The other ends were ligated after blunt end formation by Klenow fill-in reaction to create pCA-SAβ that has the native translation start site of APP. (iii) pCA-fSAβ for expression of the signal sequence of APP + Aβ1-42 using Kozak sequence and more frequently used codons for human and mouse: using four overlapping oligonucleotides, a double stranded DNA for an *Eco*RI site + Kozak consensus sequence + signal sequence of APP + Aβ1-42 sequence + stop TAA codon + *Eco*RI site was synthesized. Two oligonucleotides, 5′-TTG AAT TCG CCA CCA TGC TGC CCG GCC TGG CCC TGC TGC TGC TGG CCG CCT GGA CCG CCA G-3′ and 5′-GTG GTG CAC CTC GTA GCC GCT GTC GTG TCT GAA CTC GGC GTC GGC TCT GGC GGT CCA GGC G-3′, with 15 overlapping nucleotides (underlined) were annealed and the double stranded DNA was synthesized using Taq DNA polymerase. The other set of oligonucleotides, 5′-GAG GTG CAC CAC CAG AAG CTG GTG TTC TTC GCC GAG GAC GTG GGC AGC AAC AAG GGC GCC ATC AT-3′ and 5′-TTG AAT TCT TAG GCG ATC ACC ACG CCG CCC ACC ATC AGG CCG ATG ATG GCG CCC TTG-3′, were annealed and the double stranded DNA was similarly produced. After *Alw*44I

Table 1
Expression vectors and their DNA constructs for expression of Aβ1-42

Expression vectors	Translation start sites and coding sequences
pCA-Aβ	5′-*Xho*I-Native[a]-Met[b]-Aβ1-42[c]-Stop[d]-*Xho*I-3′
pCA-SAβ	5′-*Bam*HI-Native[a]-Signal[e]-Met[b]-Aβ1-42[c]-Stop[d]-*Xho*I-3′
pCA-fSAβ	5′-*Eco*RI-Kozak[f]-f[g]Signal[e]-f[g] Aβ1-42[c]-Stop[d]-*Eco*RI-3′
pCA-fKSAβ	5′-*Eco*RI-Kozak[f]-f[g]KSignal[h]-f[g] Aβ1-42[c]-Stop[d]-*Eco*RI-3′

[a] Native translation start signal of APP, GTCGCG.
[b] Methionine codon, ATG.
[c] cDNA for the Aβ peptide consisting of 42 amino acids, DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA.
[d] A stop codon.
[e] cDNA for the signal peptide of APP, MLPGLALLLLAAWTARA.
[f] A Kozak consensus sequence, GCCACC.
[g] More frequently used codons in humans and mouse were used for cDNA.
[h] cDNA for the signal peptide of immunoglobulin Kappa light chain, MSVPTQVLGLLLLWLTDARC.