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Anti-Aβ single-chain antibody delivery via adeno-associated virus for treatment of Alzheimer's disease

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Immunization of mouse models of Alzheimer disease (AD) with amyloid-peptide (Aβ) reduces Aβ deposits and attenuates their memory and learning deficits. Recent clinical trials were halted due to meningoencephalitis, presumably induced by T cell mediated and/or Fc-mediated immune responses. Because injection of anti-Aβ F(ab')$_2$ antibodies also induces clearance of amyloid plaques in AD mouse models, we have tested a novel gene therapy modality where an adeno-associated virus (AAV) encoding anti-Aβ single-chain antibody (scFv) is injected into the corticohippocampal regions of AD mouse models. One year after injection, expression of scFv was readily detectable in the neurons of the hippocampus without discernible neurotoxicity. AD mouse models subjected to AAV injection had much less amyloid deposits at the injection sites than the mouse models subjected to PBS injection. Because the scFv lacks the Fc portion of the immunoglobulin molecule, this modality may be a feasible solution for AD without eliciting inflammation.

Keywords: Gene therapy; Vaccine; Amyloid protein; Amyloid plaque; Transgenic mouse; Immune therapy

Introduction

Alzheimer's disease (AD) is the most common cause of dementia after the age of 60. The pathological hallmarks of AD include deposition of amyloid β-peptide (Aβ) in neuritic plaques and cerebral blood vessels, neurofibrillary tangles, and loss of neurons. Aβ is derived from its precursor, APP, by proteolytic processing. Generation of Aβ requires cleavage of APP by β- and γ-secretase at the N-terminal and C-terminal end of Aβ, respectively. The amyloid cascade hypothesis claims that accumulation of Aβ in the brain is an initial and essential event in the pathogenesis of AD (Hardy and Selkoe, 2002). This hypothesis is supported by increasing lines of evidence: (1) mutations in 3 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) (Scheuner et al., 1996); (2) Aβ1–42 is considered to be highly amyloidogenic and oligomeric forms of Aβ are neurotoxic (Lambert et al., 2001; Walsh et al., 2002); (3) patients with Down's syndrome (carrying three APP genes) develop AD pathology in their fourth or fifth decade of life; and (4) overexpression of the mutant forms of APP in transgenic mice leads to AD-like pathologies including amyloid plaques in the brain. While the causes of sporadic AD are not clear, based on the similarities in the pathological and clinical manifestations between familial and sporadic AD, it is widely considered that Aβ also plays a pathogenetic role in sporadic AD.

The efficacy of Aβ vaccination on AD treatment was first demonstrated in a transgenic mouse model of AD, where immunization of AD mouse models with synthetic Aβ by repeated needle injection prevented or reduced Aβ deposits and improved their memory and learning deficits (Janus et al., 2000; Morgan et al., 2000; Schenk et al., 1999). These groundbreaking results prompted initiation of phase I/II clinical trials to evaluate the safety and tolerability of aggregated Aβ (AN1792) vaccination. AD patients subjected to this treatment showed less decline among the patients with positive antiserum titers against Aβ, compared with control patients in Disability Assessment for Dementia Scores, and no safety concerns arose during phase I clinical trial (Bayer et al., 2005). The phase IIa clinical trial, however, was halted due to meningoencephalitis found in 6% of AD patients subjected to the vaccine trial (Orgogozo et al., 2003). Clinical studies and autopsy results indicated aseptic meningoencephalitis, presumably induced

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by T-cell-mediated and/or Fc-mediated immune responses (Ferrer et al., 2004; Nicoll et al., 2003; Orgogozo et al., 2003). In other experiments, peripheral administration of antibodies against Aβ induced clearance of preexisting amyloid plaques in an AD mouse model despite modest serum levels of the antibodies (Bard et al., 2000), indicating that an active T-cell-mediated immune response was unnecessary. Based upon these observations, intravenous injection of humanized monoclonal antibodies against Aβ (passive immunization) has been proposed as a possible therapeutic means for AD and such a clinical trial has been started (Bacskai et al., 2001; DeMattos et al., 2001; Nitsch, 2004; Sigurdsson et al., 2002; Weksler, 2004). This modality, however, suffers from repeated administrations of antibodies, leading to a large financial and physical burden to AD patients. Because topical injection of Fc-removed antibodies against Aβ led to clearance of amyloid deposits in the AD mouse models (Bacskai et al., 2002), single-chain antibodies (scFvs) against Aβ, which lack Fc, may be effective also. In this study, we attempt to demonstrate the feasibility of a new gene therapy modality for AD, where scFv against Aβ is delivered by adeno-associated virus (AAV) encapsidated in serotype 2 capsids to the brain. This approach may eliminate repeated administrations of antibodies or, as in the case for the clinical trial of gene therapy for hemophilia B (Kay et al., 2000; Manno et al., 2003), may reduce the number of antibody injections.

Materials and methods

Isolation of human scFvs against Aβ

A synthetic peptide containing amino acid residues 1–42 of Aβ was purchased from U.S. Peptide (Rancho Cucamonga, CA). scFvs that specifically react with the synthetic peptide were obtained through screening the ETH-2 human antibody phage library (Eidgenössische Technische Hochschule, Zurich, Swiss). The synthetic peptide was coated onto immunotubes at a concentration of 20 μg/ml in PBS overnight at 4°C. Unbound antigen was washed away, and the tubes were blocked by incubation with 2% dried milk in PBS for 2 h at room temperature. A library of approximately 10^{12} phages, encompassing 100–1000 copies of 10^{9} distinct clones, was incubated with the immobilized antigen. After 2 h at room temperature, unbound phages were washed away and the remaining phages were eluted by exposure to 100 mM triethylamine. Logarithmically growing *E. coli* (suppressor strain TG1) was infected with a portion of the eluted phages, and the titers of eluted phages were determined by serial dilution. The remaining phages were grown in bacterial culture overnight, packaged and expressed through co-infection with helper phage, and precipitated from the bacterial supernatant. The precipitated phages were then used for subsequent rounds of antigen panning. Phages were isolated from single ampicillin-resistant colonies of infected TG1 cells using helper phage, and binding specificity for antigen was determined by enzyme-linked immunosorbent assay (ELISA). Ampicillin-resistant colonies were used to inoculate culture broth (200 μl/colony) in microtiter plates, and the expression of soluble scFv fragments was induced by addition of 1 mM isopropyl-D-thiogalactopyranoside to the cultures. Bacteria were pelleted, and the supernatants containing monoclonal phage populations were screened for binding to antigen by ELISA. Binding specificity was determined by comparing signals obtained from plates coated with the relevant antigen versus those obtained with the negative control antigen. Phages with high ELISA titer were isolated and used to infect the *E. coli* HB2151 (non-suppressor). Soluble scFv fragments induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside to the cultures were used to further screen scFv phage clones for binding to the synthetic Aβ1–42 peptide by ELISA using anti-FLAG M2 monoclonal antibody (scFv contains Flag sequences as a marker) (Sigma, St. Louis, MO) as the detecting reagent. An scFv clone, scFv59, that demonstrated the highest ELISA titer for Aβ immunoreactivity was selected for cloning of scFv cDNA. As a control, scFv-Gag was isolated by screening the ETH-2 library for HIV-1 Gag immunoreactivity.

Construction of an expression vector coding for scFvs

The cytomegalovirus promoter (CMV) in pAAV-MCS (Stratagene, La Jolla, CA) was replaced with CMV-enhancer/β-actin promoter (Niwa et al., 1991) to generate pAAV-CA. The woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) was cloned by PCR using 5′-GGA AGC TTA ATC AAC CTC TGG ATT ACA A-3′ and 5′-CTC TCG AGC AGG CGG GGA GGC GGC CCA A-3′ as primers and pWHV8 as a template (ATCC, Manassas, VA) and inserted into the HindIII and XhoI sites of pAAV-CA to generate pAAV-CAWPRE. The Kozak and signal sequences of the immunoglobulin heavy chain (VH) gene were prepared by annealing and ligating 4 overlapping oligonucleotides listed in Table 1. The ligated oligonucleotides contained EcoRI and BsrGI restriction enzyme sites (bold letters in the table) at the 5′ and 3′ ends of the sequences, respectively. cDNA for scFv was isolated by PCR using 2 primers (5′-TGG **TGT ACA** GTG TGA GGT GCA GCT GTT GGA GTC TGG G-3′and 5′-CC**A AGC TT**C TAA TGG TGA TGG TGA TGG TGC TTG TCG TC-3′) with NcoI and HindIII restriction enzyme sites (in bold letters). After adding the signal sequences of the VH gene to the 5′end of the PCR product in frame, the signal and scFv cDNA were inserted into the EcoRI and HindIII sites of pAAV-CAWPRE to generate pAAV-CAscFv59 (Fig. 1). pAAV-CAscFv-Gag was similarly prepared by cloning cDNA for scFv-Gag into pAAV-CAWPRE. In the vectors, produced scFvs contained the D3 SD2-FLAG-His tag as a marker at the C-terminal ends. The DNA fragments prepared by PCR were verified by DNA sequencing.

Expression and secretion of scFv from cultured cells

COS (an African green monkey kidney cell line) cells were cultured in Dulbecco's modified Eagle's medium (DMEM)

Table 1
Oligonucleotides for the Kozak and signal sequences of the immunoglobulin heavy chain (VH) gene

DNA sequences of oligonucleotides
5′-CG**G AAT TC**[1]*G CCA CC*[2]A TG*G* AGT TTG GGC TGA GCT-3′
5′-*GGC TTT T*[3]TC TTG TGG CTA TTT TAA AAG GTG G**TG TAC A**[4]GT G-3′
5′-CAC **TGT ACA**[4] CCA CCT TTT AAA ATA GCC ACA AGA-3′
5′-*AAA AGC C*[3]AG CTC AGC CCA AAC T*CC* AT*G GTG GC***G AAT TC**[1]C G-3′

1. EcoRI site is in bold.
2. Kozak sequence is in italics.
3. Overlapping sequences are underlined.
4. BsrGI site is in bold.