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Aβ42 neurotoxicity in primary co-cultures: Effect of apoE isoform and Aβ conformation

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Abstract

Autosomal dominant mutations that increase amyloid-β(1–42) (Aβ42) cause familial Alzheimer's disease (AD), and the most common genetic risk factor for AD is the presence of the ε4 allele of apolipoprotein E (apoE). Previously, we characterized stable preparations of Aβ42 oligomers and fibrils and reported that oligomers induced a 10-fold greater increase in neurotoxicity than fibrils in Neuro-2A cells. To determine the effects of apoE genotype on Aβ42 oligomer- and fibril-induced neurotoxicity *in vitro*, we co-cultured wild type (WT) neurons with glia from WT, apoE-knockout (apoE-KO), and human apoE2-, E3-, and E4-targeted replacement (TR) mice. Dose-dependent neurotoxicity was induced by oligomeric Aβ42 with a ranking order of apoE4-TR > KO = apoE2-TR = apoE3-TR > WT. Neurotoxicity induced by staurosporine or glutamate were not affected by apoE genotype, indicating specificity for oligomeric Aβ42-induced neurotoxicity. These *in vitro* data demonstrate a gain of negative function for apoE4, synergistic with oligomeric Aβ42, in mediating neurotoxicity.

Keywords: ApoE; Alzheimer disease; Amyloid-β; Neurotoxicity; Co-cultures; ApoE transgenic mice

1. Introduction

Autosomal dominant mutations in the amyloid precursor protein (APP) and presenilin genes, which result in an overall increase in production of the peptide amyloid-β(1–42) (Aβ42), cause the familial form of Alzheimer's disease (AD) [73]. Although amyloid deposits are a defining pathological hallmark of AD, plaque density in both AD patients and transgenic mice exhibits an imperfect correlation with neurodegenerative pathophysiology and cognitive symptoms [1,8,14,23,31,56]. Therefore, recent research has focused on soluble oligomeric assemblies of Aβ42 as the proximate cause of neuronal injury, synaptic loss and the eventual dementia associated with AD [33]. Aβ42 oligomers have now been incorporated as an early, causal factor in the pathogenesis of AD in revisions of the "amyloid hypothesis" [18,20,72]. However, the relative contributions of fibrillar and oligomeric Aβ42 to the disease process remain unresolved. To directly assess the conformation-dependent differences among Aβ42 assemblies *in vitro*, we have developed protocols for the preparation of homogenous unaggregated, oligomeric, and fibrillar assemblies of Aβ42 [75], and demonstrated that *in vitro*, oligomeric Aβ42 is ∼10-fold more toxic than the fibrils in a neuroblastoma cell line, Neuro-2A cells [9]. Oligomeric Aβ42 also caused a significant increase in the inflammatory response when compared to fibrils in cultured primary rat glial cells [86]. In addition, oligomeric Aβ42 inhibited long-term potentiation (LTP) at the medial perforant path in the dentate gyrus of hippocampal slices while equivalent doses of unaggregated peptide had no effect [84],

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further demonstrating an Aβ42 conformation-dependent mechanism.

The ε4 allele of apolipoprotein E (apoE), a lipid transport protein in the plasma, is the only established genetic risk factor for AD. Three major apoE isoforms exist in humans that differ at two residues: apoE2 (Cys^{112}, Cys^{158}), apoE3 (Cys^{112}, Arg^{158}), and apoE4 (Arg^{112}, Arg^{158}). Inheritance of one or two copies of the ε4 allele is associated with a dose-dependent risk for AD, as well as an earlier onset of the disease [7,69]. ApoE2, on the other hand, offers cognitive protection from aging, as well as AD [7,67]. The conformation of apoE has been shown to vary based on the source of the protein, i.e. synthetic, recombinant, glial-secreted, CSF, or plasma. Like Aβ42, the conformation of apoE results in functional heterogeneity, particularly with regard to the affinity of apoE for specific apoE receptors [6,15,28,29,41,70], members of the low-density lipoprotein (LDL) receptor (LDLR) gene family [34]. Because of this conformational specificity, cultured glial cells isolated from human apoE-targeted replacement (TR) mice were the source of apoE-containing particles for the *in vitro* experiments described herein. Human apoE-TR mice are perhaps the most biologically relevant transgenic mouse model for human apoE [78,79]. ApoE-knock out (apoE-KO) mice [60,62] have been used to assess the role of apoE in CNS function and are the background for a number of transgenic mouse lines where heterologous promoters drive the expression of human apoE [3,27,66,74,80,81]. However, in the apoE-TR mice, only the coding domain of human apoE replaces the coding domain of mouse apoE. This is particularly important as apoE is part of a 48 kb multi-gene complex and this extensive DNA sequence is critical for the expression of apoE in the brain, and includes, for example, two regulatory sequences 3.3 and 15 kb downstream of the apoE gene that are required for the expression of apoE by astrocytes [19]. Thus, in apoE-TR mice, human apoE is expressed in a conformation and at physiological levels in a temporal and spatial pattern comparable to endogenous mouse apoE [78,88].

To determine the effect of apoE isoform on Aβ42 oligomeric- and fibrillar-induced neurotoxicity *in vitro*, we co-cultured wild type (WT) neurons with glia isolated from WT, apoE-KO, and human apoE2-, E3-, and E4-TR mice. Our results demonstrate that oligomeric Aβ42 induced significant neurotoxicity in co-cultures with WT, KO, apoE2-, E3-, and E4-TR glia, an effect that was dose-dependent. Compared to comparable doses of oligomeric Aβ42, fibrillar Aβ42 did not induce significant neurotoxicity. Oligomer-induced neurotoxicity was significantly higher when cultured with apoE4-TR glia compared to apoE-KO, E2- or E3-TR glia. WT co-cultures exhibited the least neurotoxicity. Additionally, apoE isoform did not affect staurosporine or glutamate neurotoxicity, suggesting that the effect of apoE isoform is specific to oligomeric Aβ42-induced toxicity. This study provides direct evidence for a gain of negative function for apoE4, synergistic with oligomeric Aβ42 in mediating neurotoxicity. Overall, these findings provide an additional functional link between conformational states of Aβ42 and apoE isoforms in mediating neuronal loss, and possibly the pathology of AD.

2. Materials and methods

2.1. Peptide

Aβ42 peptide was purchased from rPeptide, Inc. (Athens, GA) as lyophilized powder. Peptide was prepared as previously described [75] to generate Aβ42 oligomers and fibrils. Briefly, peptide is initially solubilized in HFIP, aliquoted, and stored at −20 °C as an HFIP film. Aliquoted peptide is resuspended with anhydrous DMSO to 5 mM and diluted with phenol red-free F12 media (oligomers) or 10 mM HCl (fibrils) to a concentration of 100 μM. Peptide for the oligomer preparation was incubated at 4 °C and for the fibril preparation at 37 °C, both for 24 h prior to use.

2.2. Animals

Timed pregnant WT C57Bl/6 mice were purchased from Jackson Labs. Timed pregnant mice of the genotypes apoE-KO, apoE2-TR, apoE3-TR, and apoE4-TR were obtained from our breeding colonies maintained at Taconic laboratories. The apoE-TR mice have been backcrossed to C57Bl/6 greater than eight times to establish a strain background consistent with the WT and apoE-KO mice and are maintained in a homozygous background [79].

2.3. Neuron:glia primary co-cultures

Glial cultures were prepared from the cortices of 1–2-day-old neonatal WT, apoE-KO, E2-, E3-, or E4-TR mice, as previously described [24,40,43]. Cells were maintained in α-minimum essential medium (α-MEM, Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Invitrogen), 2 mM glutamine, and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). Confluent 'secondary' cultures were used to seed 24-well plates at 5×10^4 cells/well. The following day, glia were rinsed twice with PBS to remove serum-containing media, and neurobasal media containing B27 supplements was added to the cultures (NB/B27, Invitrogen). This change in media was done at least 24 h prior to addition of the neurons. These tertiary glial cultures have ∼95–97% astrocytes and ∼2–5% microglial cells [26].

Neuron cultures were prepared as previously described [32] with the following modifications. Cortices were dissected from E14–E16 WT mouse embryos, incubated with 0.25% trypsin for 10 min at 37 °C, and then triturated with a fire polished Pasteur pipette. FBS was added (10%) to the dissociated cells to stop trypsinization. Cells were then pelleted, resuspended in NB/B27, and counted. Cells were plated (5×10^4) onto poly-L-lysine-coated 10 mm round glass cov-