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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-

erslips. Coverslips contained three paraffin 'dots' on one side (same side on which the neurons are plated) to suspend it over the glial cell layer without contact [2]. Cells were allowed to adhere to the coverslips for 1–4 h in a humidified 37 °C incubator with 7% CO_2. Coverslips were rinsed with PBS and transferred to 24-well plates containing the glial cells, neuron side down. Cytosine-β-D-arabinoside (5 μM) was added to the co-cultures ∼24 h later to inhibit division of non-neuronal cells. Co-cultures were maintained by changing three-fourth of the media at 3–4 days in culture.

2.4. Toxicity experimental design

Aβ42 was added to co-culture media at 6–8 days *in vitro* (DIV), media at this point would have been 'conditioned' for a minimum of 3 days. Individual well volumes were adjusted to accommodate the treatment samples so that the final volumes were equivalent. Aβ42 oligomers and fibrils were used at 5, 10, and 20 μM final concentrations. Endpoint determination was at 24, 48, and 72 h following treatment. In experiments examining Aβ42 toxic effects on neurons incubated with WT glia versus no glia, neurons were harvested from the same dissection and cultured under the same conditions until treatment with Aβ42. Upon Aβ42 treatment, the coverslips with neurons were transferred to a new 24-well plate. Conditioned media from a parallel set of co-cultures was filter sterilized and used to set up the Aβ42 treatment concentration in the new 24-well plate. Toxicity studies were also performed with 24 h exposure to glutamate and staurosporine (Sigma). Staurosporine was applied to cultures at concentrations of 0.003, 0.01, 0.03, 0.1, 0.3, and 1 μM, and glutamate was added at concentrations of 0.005, 0.05, 0.5, 5, 50, and 500 μM. Appropriate vehicle controls were used in all experiments. Six to eight separate experiments were performed with a minimum of three replicates for each experiment.

2.5. Cell toxicity assays

Neurotoxicity was assessed by measuring ATP using a ViaLight Plus kit (Cambrex). Cell lysis reagent (40% in media) was applied directly to the neuron-containing coverslips, after removal from co-culture dishes. Neuron lysate was transferred to a 96-well plate and reactions were carried out according to the manufacturer's instructions. Results are expressed as percent survival of Aβ42-treated cultures, with vehicle-treated controls corresponding to 100% survival. Although neurotoxicity as assessed by MTT and ATP assays for the present co-culture model are virtually identical [47], only the results from the ATP assay are reported here to ensure that the report of direct effects of Aβ on the MTT assay did not influence the assessment of neurotoxicity [44]. For both the ATP and MTT assays, a reduction in metabolic activity is an indicator of cellular toxicity. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Roche) assay colorimetrically measures conversion of MTT into formazan through succinate dehydrogenase activity in functional mitochondria [55]. The ATP assay uses luciferase, which catalyzes the formation of light from ATP and luciferin. The emitted light intensity is linearly related to the ATP concentration. Selected results were further confirmed by propidium iodide staining for apoptotic nuclei, as previously described (data not shown) [32]. For statistical analysis, an unpaired Student's *t*-test with unequal variance was used.

3. Results

3.1. Oligomeric Aβ42, but not fibrillar Aβ42 induced a dose-dependent increase in neurotoxicity independent of apoE genotype

In all apoE genotypes, oligomeric Aβ42 induced a significant dose-dependent increase in neurotoxicity. Dose- and time-dependent effects are shown in Fig. 1 comparing 24, 48 or 72 h treatment with 5, 10, or 20 μM oligomeric and fibrillar Aβ42 in co-cultures of WT neurons with glia from either WT (Fig. 1A) or apoE-KO (Fig. 1B) mice. Based on these time course data, Fig. 2 compares the effect of 48 h treatment with 5, 10, or 20 μM oligomeric and fibrillar Aβ42

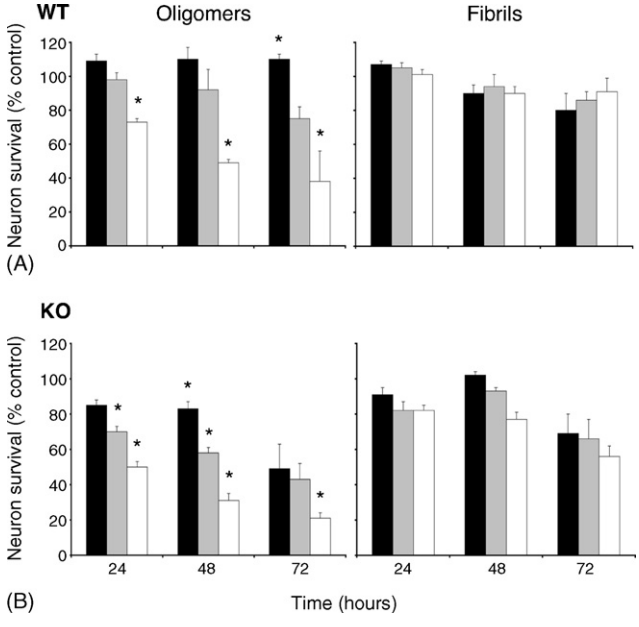

Fig. 1. Oligomeric Aβ42, but not fibrillar Aβ42, induced a dose- and time-dependent increase in neurotoxicity in the presence of WT glia (A) and KO glia (B). Cortical neurons from WT C57BL/6 mice were co-cultured with glial (∼95% astrocytes) cells from WT or apoE-KO mice. Aβ42 oligomers or fibrils were added to cultures at 5 μM (■), 10 μM (■) and 20 μM (□) and incubated for 24, 48, and 72 h. Results are expressed as percent survival of Aβ42-treated cultures with vehicle-treated controls corresponding to 100% survival. Neurotoxicity was assessed using the ATP assay as described in Section 2. * Significant difference between oligomers and fibrils at equivalent dose and time ($p < 0.05$).

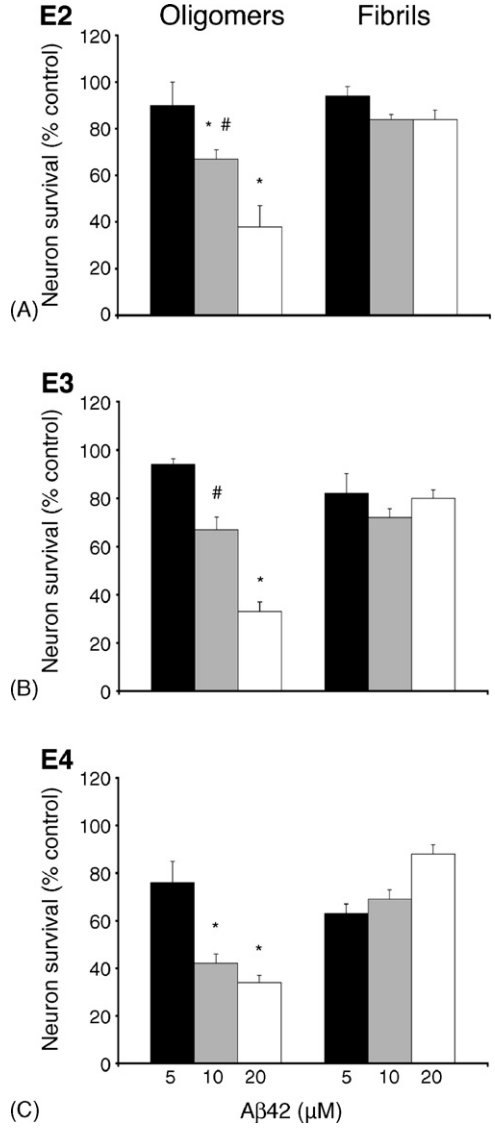

Fig. 2. Oligomeric Aβ42, but not fibrillar Aβ42, induced a dose-dependent increase in neurotoxicity in the presence of apoE2-TR glia (A), apoE3-TR glia (B) and apoE4-TR glia (C). Cortical neurons from WT C57Bl/6 mice were co-cultured with glia from apoE2-, E3-, or E4-TR mice and exposed to 5 μM (■), 10 μM (▩), and 20 μM (□) Aβ42 oligomers or fibrils for 48 h. Results are expressed as percent survival of Aβ42-treated cultures with vehicle-treated controls corresponding to 100% survival. Neurotoxicity was assessed using the ATP assay as described in Section 2. *Significant difference between oligomers and fibrils at equivalent dose ($p < 0.05$). #Significant difference between E4 and E2 or E3 at equivalent dose ($p < 0.05$).

on co-cultures of neurons from WT mice with glia from apoE2-TR (Fig. 2A), apoE3-TR (Fig. 2B) and apoE4-TR (Fig. 2C) mice. Oligomeric Aβ42 induced a significant dose-dependent increase in neurotoxicity in apoE2-TR, apoE3-TR, and apoE4-TR glial co-cultures. Neurotoxicity was quantified by measuring ATP levels as described in Section 2. Results are expressed as means ± S.E.M. for percent change from vehicle-treated control cultures for each dose and time point.

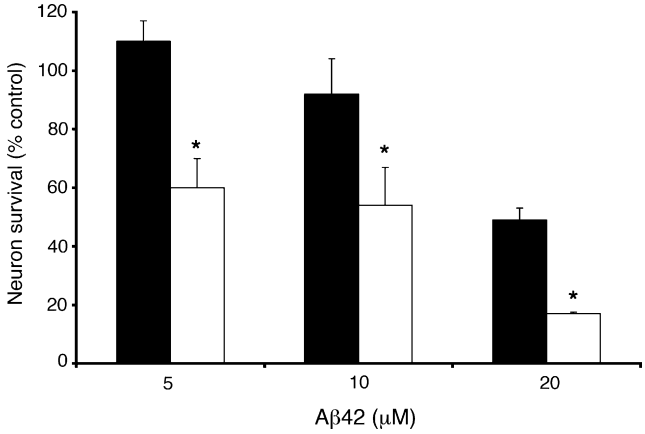

Fig. 3. Aβ42 oligomer-induced neurotoxicity is higher in the absence of glia. WT cortical neurons either alone (□) or in co-culture with WT glia (■) were treated with Aβ42 oligomers (5, 10, or 20 μM) for 48 h. *Significant difference between presence and absence of glia ($p < 0.04$).

3.2. Aβ42 oligomer-induced neurotoxicity is higher in the absence of glia

Glia, under basal as well as activated states, have an important paracrine role in regulating neural homeostasis. To determine the trophic or toxic effects of glia in our acute *in vitro* co-culture model, WT neurons were cultured with or without WT glia. The effect of 48-h treatment with 5, 10, or 20 μM oligomeric Aβ42 on WT neurons in the absence of glia or the presence of WT glia is shown in Fig. 3. Neurotoxicity was significantly increased in the absence of glia compared to the presence of WT glia, an effect that was seen at all Aβ42 concentrations ($p < 0.04$).

3.3. Neurons co-cultured with apoE4-expressing glia showed the highest oligomeric Aβ42-induced neurotoxicity

Fig. 4 directly compares the effect of apoE and apoE isoform on oligomer-induced neurotoxicity. Co-cultures were treated with 10 μM oligomeric Aβ42 for 48 h. Under these conditions, oligomeric Aβ42 did not induce a significant increase in neurotoxicity in co-cultures of WT neurons with WT glia. Conversely, in the presence of apoE-KO glia and apoE2-, apoE3-, and apoE4-TR glia, oligomeric Aβ42 caused significant neurotoxicity. Finally, the presence of glia from apoE4-TR mice caused a significant increase in neurotoxicity compared to co-cultures with glia from apoE-KO and apoE2- and apoE3-TR mice.

3.4. ApoE genotype does not affect staurosporine or glutamate neurotoxicity

We next determined whether the effect of apoE genotype on oligomeric Aβ42-induced neurotoxicity was specific to Aβ42, or a general synergism with neurotoxic insult. In dose–response studies with staurosporine, a general protein

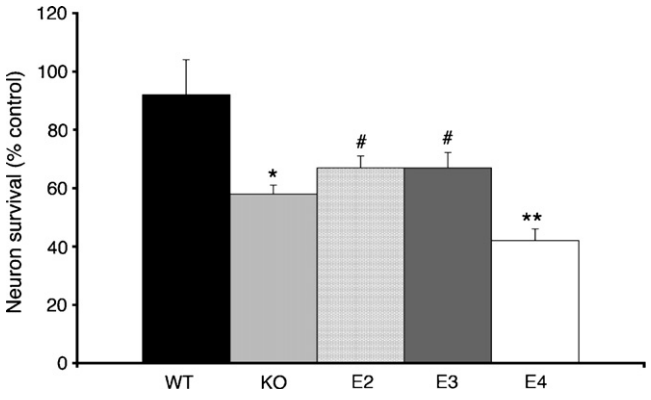

Fig. 4. Neurons co-cultured with apoE4-expressing glia showed the highest oligomeric Aβ42-induced neurotoxicity. Cortical neurons from WT C57BL/6 mice were co-cultured with glia (~95% astrocytes) from WT (■), apoE-KO (▩), apoE2-TR (▩), apoE3-TR (▩), or apoE4-TR (□) mice. Oligomeric Aβ42 (10 μM) was added to cultures and incubated for 48 h. Results are expressed as percent survival of Aβ42-treated cultures with vehicle-treated controls corresponding to 100% survival. Neurotoxicity was assessed using the ATP assay as described in Section 2. *Significant difference between WT and apoE-KO ($p < 0.04$). **Significant difference between apoE-KO and apoE4 ($p < 0.04$). #Significant difference between E4 and E2 or E3 ($p < 0.05$).

kinase-C (PKC) inhibitor that results in apoptotic cell death, we observed a dose-dependent (0.003–1 μM) increase in neurotoxicity after 24 h of treatment. No significant differences in neurotoxicity were observed between neurons co-cultured with apoE-WT, -KO, or human apoE2-, E3-, and E4-TR glia (Fig. 5A). Glutamate, a non-specific agonist for NMDA receptors, induced dose-dependent (0.005–500 μM) excitotoxicity measured at 24 h (Fig. 5B). Again, no significant differences were seen among the various apoE co-culture pairings.

4. Discussion

One of the aims of the present study was to determine the effect of Aβ42 conformation on neurotoxicity. Utilizing two distinct aggregation protocols developed in our lab, we consistently generate homogenous preparations of Aβ42 oligomers or fibrils [75]. These distinct assemblies are derived from chemically identical and structurally homogeneous starting material and allow for well-controlled comparative structure–function studies. In co-cultures of WT neurons with glia expressing different apoE genotypes, oligomeric Aβ42, but not fibrillar Aβ42, induced a dose-dependent increase in neurotoxicity independent of apoE genotype (Figs. 1 and 2). In addition, Aβ42 oligomer-induced neurotoxicity is higher in WT neurons cultured without glial cells compared to co-cultures of WT neurons with WT glia (Fig. 3). In these primary co-cultures, neurons are more resistant to both oligomeric and fibrillar Aβ42-induced toxicity compared to data from the Neuro-2A neuronal cell line. We previously demonstrated in this cell line that treatment with

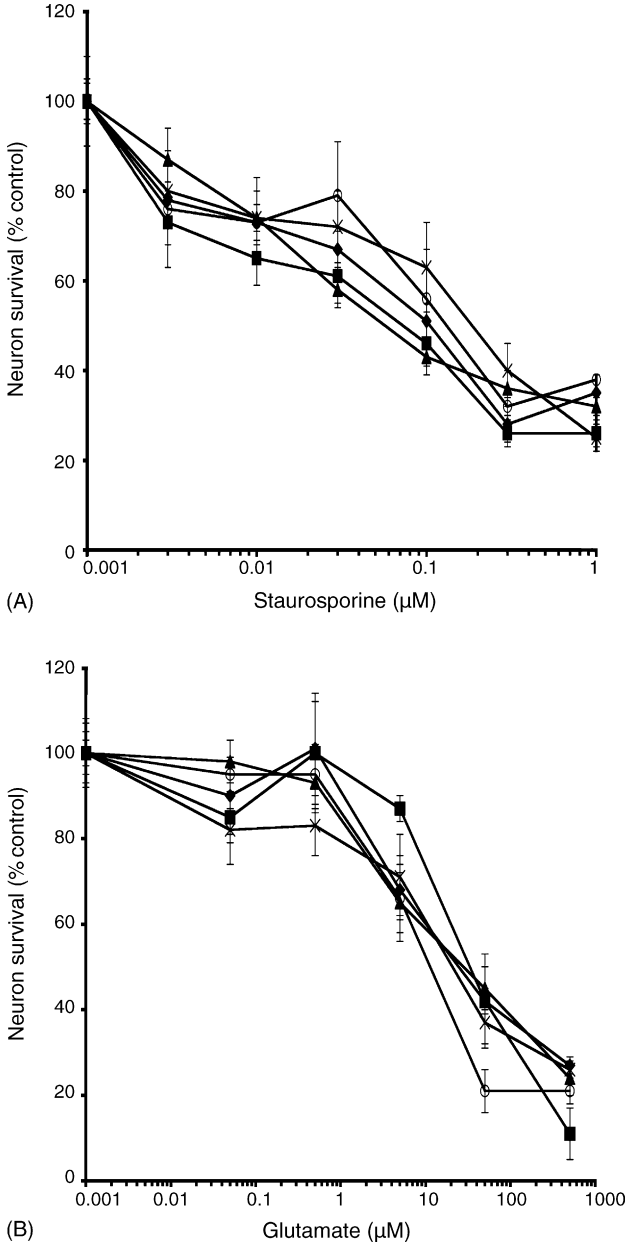

Fig. 5. ApoE genotype does not affect glutamate-induced (A) or staurosporine-induced (B) neurotoxicity. Neurotoxicity in WT mouse cortical neurons following 24-h treatment with increasing concentrations of (A) staurosporine, or (B) glutamate was assessed using the ATP assay as described in Section 2. Neurons in co-culture with glia from WT (■), apoE-KO (♦), apoE2-TR (▲), E3-TR (×), or E4-TR (◯) mice.

oligomeric Aβ42 for 20 h resulted in significant neurotoxicity at 10 nM (20%), 50% toxicity at 100 nM, and >80% toxicity at the maximal dose of 15 μM [9]. Fibrillar Aβ42-induced toxicity in the Neuro-2A cells exhibited a dose-dependent decrease in cell survival between 0.1 and 10.0 μM, with 50% toxicity at ~5 μM. In the present culture model, 10 μM oligomeric Aβ42 treatment for 48 h induced only 50% neurotoxicity in the absence of glia, the most vulnerable condition. In co-cultures, fibrillar Aβ42 does not induce significant toxicity at the time and doses measured. While these differences

in the neurotoxic response of Neuro-2A cells and primary neuron:glia co-cultures could reflect differences in experimental conditions, it is likely a result of the presence of glia in the co-culture model. *In vivo*, glia play a supportive role in neuronal homeostasis and in the present *in vitro* experiments, acute low dose oligomeric Aβ42 neurotoxicity was completely blocked by the presence of WT glia. This observation is a bit surprising as we have previously demonstrated that glial cultures treated with oligomeric Aβ42 for 24–72 h resulted in an increase in several inflammatory markers that could potentially be toxic to neurons, including iNOS, NO, TGFβ, TNFα, and IL-1β [86]. However, the neurotrophic or neurotoxic effects of glia likely result from the interaction of a number of factors, particularly acute versus chronic treatment, and results will vary with the *in vitro* or *in vivo* model used. In the present co-culture model, the presence of glia provides a protective effect from neurotoxicity induced by acute treatment with oligomeric Aβ42.

Compositional and structural differences in apoE vary with the material source (purified, recombinant, cultured astrocytes, CSF or plasma) and, like Aβ42, the conformation of apoE has been shown to result in functional heterogeneity *in vitro* [15,41,70]. We previously isolated and characterized the unique apoE-containing lipoprotein particles secreted by cultured glial cells and in human CSF [36]. As glia are the primary apoE-synthesizing cell type in the brain [4,11,16,17,57,58,61,63,76], the endogenous apoE-containing particles secreted by cultured glia are a physiologically relevant source for *in vitro* models of neural cell function. For these reasons, we co-cultured WT neurons with glia expressing no apoE (apoE-KO), mouse apoE (WT), or human apoE2, -E3, or -E4 from apoE-TR mice. Our results support the overall hypothesis that apoE4 potentiates oligomeric Aβ42-induced neurotoxicity and demonstrate both a loss of positive function for apoE (comparing neurotoxicity in co-cultures with WT apoE glia versus apoE-KO glia) and a gain of negative function for apoE4 (comparing neurotoxicity in co-cultures with apoE-KO glia versus apoE4 glia) (Fig. 4). ApoE isoform effects appear specific for Aβ42-mediated neurotoxicity, as neurotoxicity induced by treatment with staurosporine and glutamate showed no apoE isoform effect. While our previous studies and those from other investigators have demonstrated that apoE inhibits Aβ-induced neurotoxicity [32,45,54,87], the exact conformational species of Aβ42 mediating these effects was not known, nor addressed, and likely contained a mixture of oligomeric, globular, fibrillar, and aggregated fibrillar assemblies [75]. In the present study, the neurotoxic effects of homogenous conformational species of Aβ42 were studied together with the regulatory role of glial-derived human apoE.

In terms of apoE isoform-specific modulation of oligomeric Aβ42-induced effects on neuronal viability, we have previously demonstrated that oligomeric Aβ42 and apoE4 act synergistically to impair LTP *in vitro* [84]. The key findings in the present study are a similar synergy between oligomeric Aβ42 and apoE4 on neurotoxicity *in vitro*. Several factors could contribute to the apoE isoform-dependent regulatory effects on oligomeric Aβ42-induced neurotoxicity. (1) In regard to apoE:Aβ42 complex formation, it has been established that *in vitro* Aβ42 has a greater affinity for apoE2 and apoE3 than for apoE4 [35,37,38,77,83]. In addition, the amount of SDS-stable apoE:Aβ42 complex formation is apoE3:oligomers > apoE3:fibrils > apoE4:oligomers > apoE4:fibrils [46]. These results are consistent with the hypothesis that apoE3 preferentially binds oligomers, the toxic species of Aβ42, and inhibits their toxicity likely through interactions with apoE receptors, either increasing clearance or altering signaling. Evidence for the latter is that apoE:Aβ42 complexes have been shown to differentially modulate cell-death pathways [5,13,42,59,89]. (2) ApoE isoforms differentially alter synthesis, clearance and neurotoxicity of administered oligomers and fibrils. As discussed above, apoE:Aβ42 complexes could alter apoE receptor binding and subsequent clearance, intracellular Aβ42 deposition, or cellular signaling of Aβ42 in an isoform- and conformation-dependent manner [10,15,22,35,41,46,64,65,69,70]. The role of apoE in intraneuronal Aβ accumulation is also suggested by several *in vitro* studies using smooth muscle cells (SMC). ApoE and Aβ co-localize in SMC from both brain vessels in amyloid affected brains and *in vitro* following acute incubation with Aβ [49,50,85]. In addition, apoE4 increased intracellular Aβ accumulation in SMC [52,53], an effect potentiated by TGFβ1 [51]. Exogenous apoE has also been shown to reduce Aβ42 levels by 20–30% in conditioned media primarily by altered synthesis and, to a lesser extent, via clearance and/or degradation of Aβ42 [30]. *In vitro*, several recent studies have demonstrated that apoE2 and E3 but not E4 protect neurons against cell death induced by non-fibrillar Aβ42 with no effect on fibrillar-induced toxicity [13,48]. We have also demonstrated that oligomeric Aβ42-induced neurotoxicity is significantly greater in Neuro-2A cells treated with exogenous apoE4 [48]. Recently, apoE4-dependent increases in Aβ42 levels have been demonstrated both *in vitro* [90] and *in vivo* [12]. (3) Another potentially relevant hypothesis is that the ε4 allele may be associated with lower apoE levels *in vitro* [68] and *in vivo* [71]. Lowered apoE levels in culture may not be adequate to facilitate clearance/degradation of administered Aβ42, resulting in the apoE4-mediated potentiation of neurotoxic effects. Alternatively, we have recently demonstrated that apoE3 and apoE4 recycle differently, and apoE4 can traffic into late endocytic compartments where it may promote intracellular Aβ42 accumulation and toxicity in neurons [41]. Previous characterization of the apoE-TR mice demonstrate comparable basal levels of expression of apoE2, apoE3 and apoE4, both *in vivo* and *in vitro* [10,78,79]. Although not measured in the present study, it is possible that oligomeric Aβ42 induced isoform-specific changes in apoE expression. (4) Aβ42-induced release of pro- and anti-inflammatory factors from glial cells [21,82] could also have an effect on neurotoxicity in co-cultures. We have previously demonstrated that apoE receptors are necessary for apoE3-mediated protection

against Aβ-induced, glial-mediated inflammation [25,39,40] and that oligomeric Aβ42 induces a greater inflammatory effect than fibrillar Aβ42 *in vitro* [86]. Here, we demonstrate *in vitro* a general protective role for glia in acute oligomeric Aβ42-induced neurotoxicity, an effect further modulated by the apoE genotype of the glial cell.

Collectively, the data from the present investigation provides evidence for a gain of negative function for apoE4, synergistic with oligomeric Aβ42 in mediating neurotoxicity. It will be of interest to delineate the cellular and molecular basis for apoE4-dependent regulation of oligomeric Aβ42-mediated neurotoxicity using this co-culture model.

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