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Antibodies raised against a structurally defined Aβ oligomer mimic protect human iPSC neurons from Aβ toxicity at sub-stoichiometric concentrations

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Abstract

Anti-Aβ antibodies are important tools for identifying structural features of aggregates of the Aβ peptide and are used in many aspects of Alzheimer's disease (AD) research. Our laboratory recently reported the generation of a polyclonal antibody, $pAb_{2AT\text{-}L}$, that is moderately selective for oligomeric Aβ over monomeric and fibrillar Aβ and recognizes the diffuse peripheries of Aβ plaques in AD brain tissue but does not recognize the dense fibrillar plaque cores. This antibody was generated against 2AT-L, a structurally defined Aβ oligomer mimic composed of three Aβ-derived β-hairpins arranged in a triangular fashion and covalently stabilized with three disulfide bonds. In the current study, we set out to determine if $pAb_{2AT\text{-}L}$ is neuroprotective against toxic aggregates of Aβ and found that $pAb_{2AT\text{-}L}$ protects human iPSC-derived neurons from $Aβ_{42}$-mediated toxicity at molar ratios as low as 1:100 antibody to $Aβ_{42}$, with a ratio of 1:25 almost completely rescuing cell viability. Few other antibodies have been reported to exhibit neuroprotective effects at such low ratios of antibody to Aβ. ThT and TEM studies indicate that $pAb_{2AT\text{-}L}$ delays but does not completely inhibit $Aβ_{42}$ fibrillization at sub-stoichiometric ratios. The ability of $pAb_{2AT\text{-}L}$ to inhibit $Aβ_{42}$ toxicity and aggregation at sub-stoichiometric ratios suggests that $pAb_{2AT\text{-}L}$ binds toxic $Aβ_{42}$ oligomers and does not simply sequester monomeric $Aβ_{42}$. These results further suggest that toxic oligomers of $Aβ_{42}$ share significant structural similarities with 2AT-L.

Introduction

Research over the past two decades has identified oligomers as the most toxic form of Aβ [1–6]. Aβ oligomers contribute to Alzheimer's disease (AD) pathogenesis by causing neurodegeneration and inflammation in the brain [1–3,6]. Identifying and targeting these species in AD has proven difficult because of the heterogeneity and

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metastability of Aβ oligomer populations and the low abundance of oligomers in AD pathology compared to monomeric and fibrillar Aβ [1–5,7,8]. Although methods exist to isolate oligomers from AD brain tissue, these methods cannot provide high-resolution structural data and can potentially alter the structures of the oligomers during the isolation or characterization processes [1,2,5,8]. No high-resolution structures of Aβ oligomers isolated from AD brain tissue have been elucidated. Techniques have emerged to stabilize Aβ oligomers formed *in vitro*, some of which have provided high-resolution structures, but the biological relevance of these structures remains to be determined [4,5,7,9,10]. Aβ oligomers can exhibit a variety of morphologies and aggregation states, some of which ultimately form Aβ fibrils like those observed in the brains of AD patients, but tendency to fibrillize does not necessarily correlate with toxicity [1–5,7].

Anti-Aβ antibodies are valuable tools for determining the relevance of various Aβ oligomers in AD pathology and are used in many aspects of AD research. Hundreds of antibodies have been generated against various fragments and species of Aβ. Some target linear epitopes – specific residues of Aβ, while others target conformational epitopes present in oligomeric or fibrillar Aβ which may or may not be residue specific as well. These antibodies have been used for biomarker detection, characterization or isolation of Aβ aggregates, studying the relationship between Aβ structure and toxicity, and a few have been approved for use in AD therapeutics research [1–5,11,12]. Although some antibodies have been developed that target oligomeric Aβ, the epitopes of most of these antibodies are structurally undefined. Antibodies developed against homogenous, structurally defined Aβ oligomers can provide stronger evidence for the relevance of certain Aβ oligomer structures in AD pathogenesis [2–4,6,11].

Our laboratory has synthesized covalently stabilized Aβ oligomer mimics, elucidated their structures at high-resolution, and used them to develop antibodies [4,10,13,14]. These oligomer mimics are composed of peptides designed to mimic β-hairpin conformations in toxic Aβ oligomers. We recently reported the high-resolution structure of one of our Aβ oligomer mimics, 2AT-L (Fig 1). 2AT-L is a toxic trimer composed of three β-hairpins derived from Aβ arranged in a triangular fashion and covalently stabilized with three disulfide bonds [14]. We also reported the generation and study of a polyclonal antibody raised against 2AT-L. In this initial report, we found that pAb2AT-L exhibited moderate selectivity for early Aβ aggregates, preferentially staining the diffuse Aβ surrounding plaque cores, exhibiting selectivity for early Aβ42 aggregates *in vitro*, and immunoprecipitating Aβ42 from a complex mixture of brain proteins. Furthermore, these observations suggested that Aβ assemblies in brain tissue and prepared *in vitro* share structural similarities with 2AT-L.

In the current study, we set out to determine if $pAb_{2AT\text{-}L}$ is neuroprotective against toxic aggregates of Aβ. We chose to use human iPSC-derived neurons for these experiments because they are a better model for the human neurons affected by AD than immortalized mammalian cell lines or primary rodent neurons and are a valuable research tool because living human neurons cannot be harvested for research [15]. iPSC-derived neurons are neurons created from human stem cells that have been

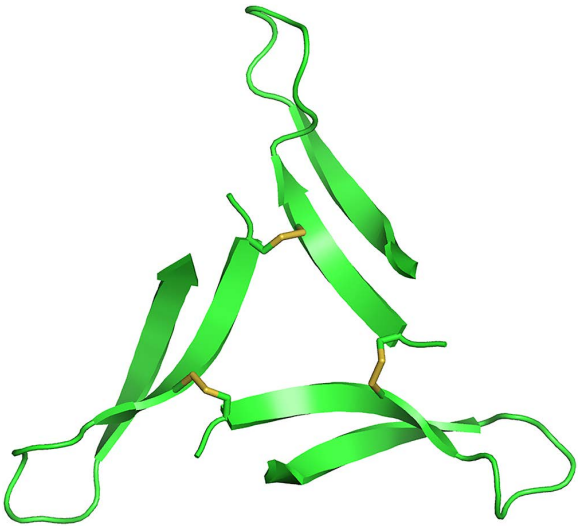

Fig 1. X-ray crystallographic structure of the Aβ oligomer mimic, 2AT-L (PDB 7U4P).

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treated to induce neuronal differentiation. We thus examined the effects of $A\beta_{42}$ on iPSC-derived neurons in the presence of various concentrations of $pAb_{2AT\text{-}L}$. We further studied the ability of $pAb_{2AT\text{-}L}$ to mitigate the production of pro-inflammatory cytokines induced by $A\beta_{42}$ in an immortalized human-derived microglia cell line, HMC3. Finally, we examined the effects of $pAb_{2AT\text{-}L}$ on $A\beta_{42}$ aggregation through thioflavin T (ThT) fluorescence assays and transmission electron microscopy (TEM).

Results

$pAb_{2AT\text{-}L}$ protects human iPSC-derived neurons from $A\beta_{42}$-mediated toxicity

Human iPSC-derived neurons are increasingly being used to study neurodegenerative diseases. Simple methods for generating iPSC-derived neurons have recently emerged, enabling the use of human neurons in research [16]. To investigate whether $pAb_{2AT\text{-}L}$ can mitigate the neurotoxicity of Aβ, we studied the effect of recombinant $A\beta_{42}$ on i^3Neurons in the presence and absence of $pAb_{2AT\text{-}L}$. i^3Neurons (i^3 = integrated, inducible, and isogenic) are glutamatergic cortical neurons that were differentiated from human iPSCs containing a doxycycline-inducible neurogenin-2 transgene (Ngn2 iPSCs) [17,18]. Treatment with doxycycline causes overexpression of neurogenin-2 which rapidly converts these iPSCs into neurons [17,18]. The Ngn2 iPSCs were designed for use in AD research [18], but to our knowledge, the cytotoxicity of recombinant Aβ toward i^3Neurons has not been studied.

We generated i^3Neurons by differentiating Ngn2 iPSCs using the protocols of Ward and coworkers [17] and measured their viability after exposure to $A\beta_{42}$ through two metrics — ATP production (metabolic activity) and LDH release (membrane integrity). In cell-based cytotoxicity assays, the IC50 of $A\beta_{42}$ is typically in the low-micromolar range [19–21]. To determine if $A\beta_{42}$ is cytotoxic to i^3Neurons, we first treated the cells with recombinant $A\beta_{42}$ at concentrations ranging from 25 μM to 98 nM and assayed for viability after 48 hours. Treatment with $A\beta_{42}$ reduced ATP production and increased LDH release in a concentration-dependent manner, with 25 μM $A\beta_{42}$ completely preventing measurable ATP production (Fig 2). Generally, concentrations of $A\beta_{42}$ above 3 μM caused significant reduction in ATP production, increase in LDH release, and morphological changes in the i^3Neurons. We subsequently determined that, at this concentration of $A\beta_{42}$, a greater degree of LDH release occurred by 72 hours than by 48 hours.

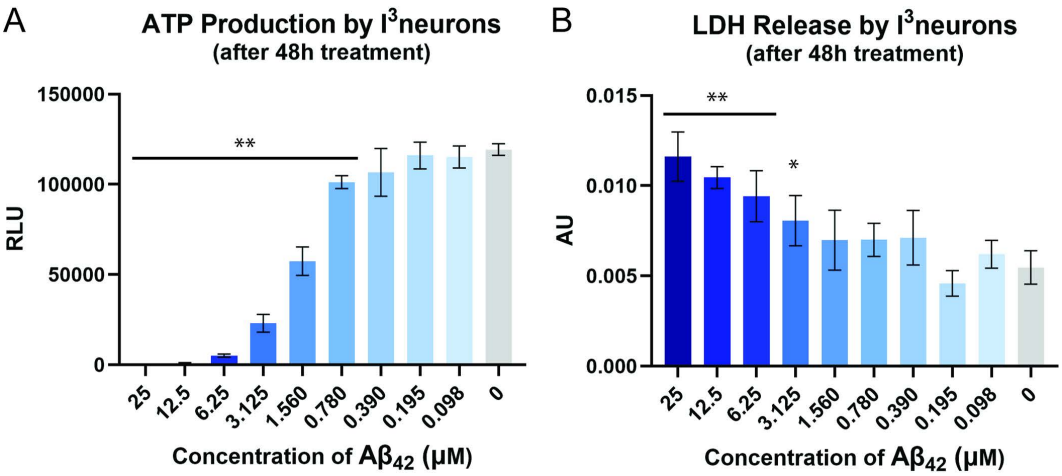

Fig 2. $A\beta_{42}$ toxicity in i³Neurons. Graphs of **(A)** ATP production and **(B)** LDH release by i³Neurons in the presence of varying concentrations of $A\beta_{42}$ (n = 6 technical replicates). ** Significantly different ($p < 0.01$) from i³neurons treated with PBS (vehicle for $A\beta_{42}$). *Significantly different ($p < 0.05$) from i³neurons treated with PBS (vehicle for $A\beta_{42}$).

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For subsequent assays, i³Neurons were treated with 5 μM $A\beta_{42}$ and $pAb_{2AT\text{-}L}$ at concentrations ranging from 200 to 6 nM in triplicate for 72 hours. A positive control of 5 μM $A\beta_{42}$ without $pAb_{2AT\text{-}L}$ and a negative control of phosphate buffered saline pH 7.4 (PBS) were also included. After 72 hours of treatment, we measured the relative amounts of ATP and LDH produced by the neurons. $pAb_{2AT\text{-}L}$ exhibited significant protective effects on the i³Neurons at or above 50 nM —a 1:100 molar ratio of antibody to $A\beta_{42}$ (Fig 3). The protective effects of $pAb_{2AT\text{-}L}$ were concentration dependent with 200 nM (a 1:25 molar ratio of antibody to $A\beta_{42}$) almost completely rescuing the cells from $A\beta_{42}$-mediated toxicity (Fig 3). ATP production was restored to levels consistent with the negative control at 200 nM $pAb_{2AT\text{-}L}$ (Fig 3A). LDH release was reduced to levels just above the negative control at 200 nM $pAb_{2AT\text{-}L}$ (Fig 3B).

We compared the protective effects of $pAb_{2AT\text{-}L}$ to those of the commercially available anti-Aβ antibodies 6E10 and 4G8; these antibodies are the most widely used in AD research [22]. 6E10 is a monoclonal antibody that targets residues 1–16 of Aβ, and 4G8 is a monoclonal antibody that targets residues 17–24 of Aβ [23]. 6E10 and 4G8 have been shown to bind monomeric, oligomeric, and fibrillar Aβ, with 6E10 having some preference for monomeric and oligomeric over fibrillar. 6E10 has previously been shown to disaggregate Aβ fibrils and increase Aβ neurotoxicity against SH-SY5Y neuroblastoma cells [24]. In the same study, 4G8 had no effect on cell viability or the aggregation state of Aβ [24]. To our knowledge, the protective effects of either of these antibodies against $A\beta_{42}$ have not been studied in iPSC-derived neurons.

In i³Neurons treated with 5uM $A\beta_{42}$, 6E10 and 4G8 did not protect against the neurotoxic effects of $A\beta_{42}$ at the concentrations tested. ATP production was not restored, nor were the levels of LDH reduced at any concentrations tested of these antibodies (200–6.25 nM). This observation is consistent with previous studies of 6E10 and 4G8 in SH-SY5Y cells [24,25]. To confirm that the protective effects exhibited by $pAb_{2AT\text{-}L}$ were not a general effect of rabbit IgG protein, we performed the same experiment with a generic rabbit IgG antibody. The generic rabbit IgG antibody did not have any protective effects against $A\beta_{42}$ in i3Neurons (S1 Fig.).

To study the protective effect of $pAb_{2AT\text{-}L}$ further, we also tested the ability of $pAb_{2AT\text{-}L}$ to inhibit $A\beta_{42}$-induced pro-inflammatory cytokine production by HMC3 microglia using Promega Lumit® immunoassays. Treatment with $A\beta_{42}$ causes HMC3 microglia to produce the pro-inflammatory cytokine IL-6 (S2 Fig.) [26–28]. IL-6 is also upregulated in AD animal models and human AD patients [29]. The two highest concentrations of $pAb_{2AT\text{-}L}$ tested, 200 nM and 100 nM, significantly reduced $A\beta_{42}$-induced IL-6 production by HMC3 microglia ($p < 0.01$) (S2 Fig.). At 200 nM $pAb_{2AT\text{-}L}$, IL-6 production was

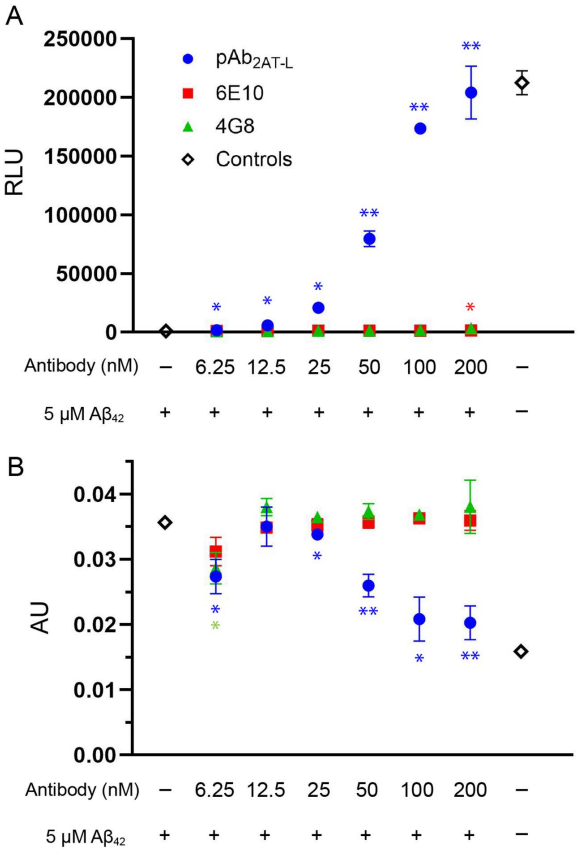

Fig 3. pAb_{2AT-L} protects i³Neurons from $Aβ_{42}$-mediated toxicity as measured by ATP production and LDH release. (A) ATP production by i³Neurons in the presence of $Aβ_{42}$ with varying concentrations of pAb_{2AT-L}, measured by the Promega CellTiter-Glo 2.0 assay (n = 3 technical replicates). (B) LDH release by i³Neurons in the presence of $Aβ_{42}$ with varying concentrations of pAb_{2AT-L}, measured by the CyQuant LDH assay (n = 3 technical replicates). ** Significantly different ($p < 0.01$) from i³neurons treated with just $Aβ_{42}$. *Significantly different ($p < 0.05$) from i³neurons treated with just $Aβ_{42}$.

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reduced by about 50% compared to the controls. This result corroborates that pAb_{2AT-L} sequesters toxic $Aβ_{42}$ species and protects cells at sub-stoichiometric concentrations.

ThT assay of $Aβ_{42}$ with pAb_{2AT-L}

To better understand the interactions between pAb_{2AT-L} and $Aβ_{42}$, we investigated the effects of pAb_{2AT-L} on $Aβ_{42}$ aggregation. First, we monitored the fibrilization of a 3 μM solution of recombinant $Aβ_{42}$ using thioflavin T in the presence and absence of pAb_{2AT-L}. In the absence of pAb_{2AT-L}, the ThT signal began to increase after one hour, plateauing after 2 hours (Fig 4). In the presence of pAb_{2AT-L}, $Aβ_{42}$ fibrillization occurred more slowly. At the lowest concentration of pAb_{2AT-L}, 210 nM, $Aβ_{42}$ fibrillization was delayed by about an hour. Increasing concentrations of pAb_{2AT-L} led to increasing delays in $Aβ_{42}$ fibrillization. At 800 nM pAb_{2AT-L}, ThT-positive aggregates of $Aβ_{42}$ did not appear to form until after five hours, indicating that pAb_{2AT-L} delayed fibrillization by about four hours.

TEM of $Aβ_{42}$ with pAb_{2AT-L}

We performed transmission electron microscopy (TEM) to visualize the effect of pAb_{2AT-L} on $Aβ_{42}$ aggregation. To do this, we prepared a 3 μM solution of lyophilized recombinant $Aβ_{42}$ dissolved in PBS and incubated in the presence or absence

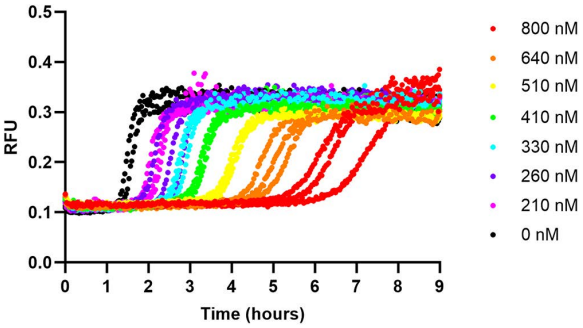

Fig 4. pAb_{2AT-L} delays fibril formation of $A\beta_{42}$. ThT fluorescence assay of 3 μM $A\beta_{42}$ in the presence of varying concentrations of pAb_{2AT-L} (210–800 nM).

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of 800 nM pAb_{2AT-L} for four hours. We then applied the solutions to TEM grids, stained with a 1% uranyl acetate solution, and imaged the grids. In the absence of pAb_{2AT-L}, $A\beta_{42}$ formed bundles of fibrillar structures (Fig 5). The protofibrils making up the bundles appear to be approximately 200–1000 nm in length and were often observed in the presence of spherical or amorphous aggregates. In the presence of pAb_{2AT-L} under the same conditions, $A\beta_{42}$ did not appear to form fibrillar structures (Fig 5). Instead, only amorphous aggregates were observed. We also applied a solution of pAb_{2AT-L} to TEM grids and observed solids. Collectively, these TEM studies corroborate the findings from the ThT studies that sub-stoichiometric pAb_{2AT-L} inhibits $A\beta_{42}$ fibrillization.

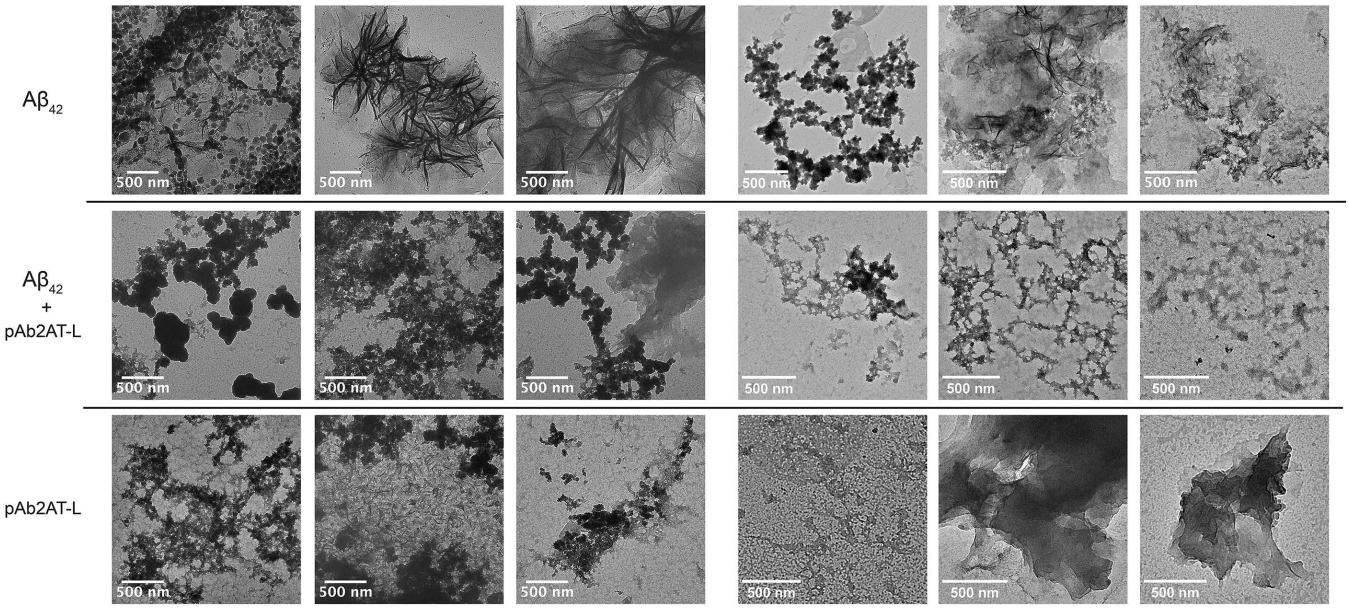

Fig 5. pAb_{2AT-L} inhibits $A\beta_{42}$ fibril formation as visualized by TEM imaging. Representative TEM images of 3 uM $A\beta_{42}$ (Top), 3 uM $A\beta_{42}$ and 800 nM pAb_{2AT-L} (middle), 800 nM pAb_{2AT-L} (bottom) from two separate TEM sessions (left and right). After four hours of incubation in PBS, samples were applied to carbon mesh copper grids and stained with uranyl acetate (UA) before imaging (the session on the left was stained with 2% UA and the session on the right was stained with 1% UA).

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Discussion

A11 and OC are the most commonly used polyclonal anti-Aβ antibodies in AD research. While OC binds fibrils and fibrillar oligomers of Aβ, A11 binds high-molecular weight (~40 kDa or larger) non-fibrillar oligomers of Aβ, as well as a variety of other amyloid oligomers [30,31]. Glabe and coworkers originally generated A11 against $Aβ_{40}$ oligomers conjugated to gold nanoparticles [30]. At equimolar concentrations or more, A11 protects SH-SY5Y cells from the toxicity of A11-positive $Aβ_{40}$ and $Aβ_{42}$ oligomers, but at lower concentrations A11 exhibits minimal inhibition of Aβ oligomer-mediated toxicity [30,32,33]. It is noteworthy that pAb_{2AT-L} at nanomolar concentrations substantially alters the toxicity and aggregation of Aβ at micromolar concentrations. There are few reports of antibodies affording significant neuroprotective effects at such low ratios of antibody to Aβ [34–42]. Lecanemab, a monoclonal anti-Aβ antibody that preferentially binds protofibrils of Aβ, has been shown to completely rescue PC12 cells from the toxic effects of $Aβ_{42}$ protofibrils at sub-stoichiometric ratios. For 1 uM $Aβ_{42}$ protofibrils, the ED50 of Lecanemab was 13 nM [40]. Additionally, some Aβ oligomer-specific antibodies and antibody fragments exhibit protective effects against toxic Aβ oligomers at sub-stoichiometric ratios [34–39,41]. The most protective of these exhibited protective effects on SH-SY5Y cells at ratios of antibody to $Aβ_{42}$ oligomers as low as 1:100 in some cases [36]. Most other anti-Aβ antibodies have not been reported to exhibit substantial protective effects on cells at less than equimolar concentrations of antibody to $Aβ_{42}$ [33,43–50].

The sub-stoichiometric activity of pAb_{2AT-L} against the toxicity and aggregation of $Aβ_{42}$ suggests that pAb_{2AT-L} can bind epitopes displayed on toxic $Aβ_{42}$ oligomers and is not simply sequestering monomeric $Aβ_{42}$ and preventing its aggregation. If pAb_{2AT-L} were only binding monomeric $Aβ_{42}$, only a small fraction of $Aβ_{42}$ would be sequestered at the concentrations of pAb_{2AT-L} that were tested, and this would not result in the significant reduction of $Aβ_{42}$-mediated toxicity. Instead, it appears that pAb_{2AT-L} must be binding non-fibrillar aggregates (oligomers) of $Aβ_{42}$. Although the data presented here do not definitively confirm that pAb_{2AT-L} does not bind fibrillar $Aβ_{42}$, the ThT and TEM data show that pAb_{2AT-L} delays $Aβ_{42}$ fibrillization which suggests that pAb_{2AT-L} binds $Aβ_{42}$ species before fibril formation. One of the limitations of the cell-based experiments is that during the 72 hours in which $Aβ_{42}$ is incubated with the i^3Neurons, a variety of aggregates form and thus we cannot be certain of the exact toxic species. Nevertheless, we previously showed that pAb_{2AT-L} has a moderate preference for binding oligomeric $Aβ_{42}$ over monomeric and fibrillar $Aβ_{42}$ [14], and the data presented here further support that conclusion.

The unique structure of the 2AT-L antigen used to generate pAb_{2AT-L} is likely responsible for the sub-stoichiometric activity of this antibody. 2AT-L was designed to mimic an Aβ oligomer, specifically a trimer. It lacks the N-terminus of Aβ, presents residues 17–36 in a β-hairpin conformation consisting of residues 17–23 and 30–36 in β-strand conformations and residues 24–29 in a loop conformation. These properties of 2AT-L are likely reflected in some of the epitopes bound by the antibodies present in the polyclonal mixture comprising pAb_{2AT-L}. Because pAb_{2AT-L} is a mixture, we cannot be sure which features of the antigen are reflected in the epitopes of the individual antibodies that bind the toxic oligomers formed by $Aβ_{42}$. Among the features that may be reflected is the β-hairpin conformation of the 17–36 segment of Aβ. β-Hairpins are the building blocks of several reported and proposed structures of toxic Aβ oligomers and an affinity for binding Aβ β-hairpin conformations might explain the protective effect afforded by pAb_{2AT-L} [4,7,14,51–57].

Materials and methods

Antibody generation

pAb_{2AT-L} was generated in rabbits immunized with 2AT-L conjugated to KLH by Pacific Immunology. The rabbit serum was collected, and affinity purified on an NHS-activated agarose resin column functionalized with 2AT-L.

$Aβ_{42}$ preparation

Recombinantly expressed $Aβ_{42}$ as the ammonium salt was purchased from rPeptide (catalog# A-1167–2) and received as lyophilized solid. The solid was dissolved in 2 mM NaOH to create a 1 mg/mL $Aβ_{42}$ solution, sonicated for 5 minutes, and

aliquoted into 0.02 µmol aliquots. The aliquots were then frozen, lyophilized, and stored at −80° C until use. Solutions of $A\beta_{42}$ were freshly prepared from the lyophilized aliquots for assays.

i³Neuron culture

i³Neurons were generated from Ngn2 iPSCs according to the protocols published by Ward and coworkers [17]. Ngn2 iPSCs were obtained from the Blurton-Jones laboratory at the Sue and Bill Gross Stem Cell Research Center. Briefly, Ngn2 iPSCs were thawed and plated in mTeSR media treated with 10 µM rock inhibitor. Cells were plated at a density of 500,000 cells per ml on a Matrigel coated 6-well plate (2 ml per well). Cells were incubated at 37 °C with 5% CO2 and cell media was aspirated and replaced daily with mTesR (without rock inhibitor) until the cell density reached 80% confluency. Cells were then passaged by aspirating the media, treating with accutase, centrifuging, and replating in fresh mTesR with rock inhibitor.

For differentiation, cells were passaged and resuspended in neuronal predifferentiation media (Knockout DMEM:F12, 1x N2, 1x non-essential amino acids, 10 ng/ml BDNF, 10 ng/ml NT3, 1 ug/ml mouse laminin, 2 ug/ml doxyclyine) with rock inhibitor (10 uM), plated in a new Matrigel coated 6-well plate at the same density in 2 ml of media per well, and incubated at 37 °C. The following two days media was aspirated and replaced. Cells were then either frozen in Knockout DMEM with 10% DMSO for later maturation or passaged and resuspended in neuronal maturation media (1:1 neurobasal A media and DMEM f:12, 1x B27 0.5x N2, 1x non-essential amino acids, 0.5x glutamax 10 ng/ml BDNF, 10 ng/ml NT3, 1 ug/ml mouse laminin, 2 ug/ml doxyclyine). Cells were plated for maturation on 96-well poly-D-lysine-coated plates at a density of 200,000 cells per ml. After one week of maturation, media was gently removed and replaced with maturation media containing the compounds of interest, but lacking doxycycline. The neurons were incubated for 72 hours before assaying according to manufacturer protocols.

HMC3 culture. HMC3 microglia cells were purchased from ATCC (Cat# CRL-3304). Upon arrival, cells were stored at −80 °C for 2 days and then thawed in a bead bath at 37 °C. The cells were diluted in pre-warmed EMEM with 10% fetal bovine serum and centrifuged. The supernatant was aspirated, and the cell pellet was redissolved in 1 mL of EMEM and transferred to a 75 cm^2 cell culture flask containing 19 mL of media. The cells were incubated at 37 °C with 5% CO_2 until reaching 80% confluence (three to five days) before passaging with trypsin to a new cell culture flask. On the subsequent passage, the cells were plated on 96-well cell culture plates at a density of one hundred thousand cells per mL. incubated overnight before treatment. The cells were incubated for 72 hours before assaying with Promega Lumit® immunoassay kits according to manufacturer protocols.

Statistical analysis

At the request of a reviewer, statistical analysis has been applied to the cell assay data. Statistical comparisons were made using unpaired two-tailed t-tests on technical replicates. Relative luminescence unit (ATP and Il-6) or absorbance unit (LDH) values for each condition were compared to the control well values to yield *p* values. Raw assay values and calculated *p* values are listed in the supporting information (S1 and S2 Tables).

ThT assay

The ThT assay was performed on 3 µM $A\beta_{42}$ in PBS at pH 7.4 (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl) containing 10 µM ThT in the presence of a dilution series of $pAb_{2AT\text{-}L}$ (800–210 nM, 0nM). The assay was performed in triplicate in Corning® 96-well Half Area Black/Clear Flat Bottom Polystyrene NBS Microplates (product# 3881) at 25 °C under quiescent conditions. Briefly, a serial dilution series of 10x concentrations of $pAb_{2AT\text{-}L}$ was prepared in deionized water in a 96-well plate and aliquoted in quadruplicate across four rows of the 96-well plate. A 3.33 µM solution of $A\beta_{42}$ was then prepared in PBS at pH 7.4 (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl) containing 10 µM

thioflavin T (ThT) and added to all but one row of the pAb_{2AT-L} solutions. The plate was sealed with clear adhesive and fluorescence was immediately measured on a ThermoFisher Scientific Varioskan Lux plate reader at excitation/emission wavelengths of 440/485 nm and excitation bandwidth of 12 nm for one second. Measurements were acquired every two minutes for five hours. The data were plotted in GraphPad Prism.

TEM sample preparation and imaging

3 μM solutions of $A\beta_{42}$ were prepared in PBS at pH 7.4 with and without 800 nM pAb_{2AT-L}. Samples were incubated for three hours at room temperature without shaking to elicit fibril formation. After incubation, 5uL of sample was deposited on 200-mesh formvar/carbon-coated copper grid carbon-copper grids purchased from Electron Microscopy Sciences (catalog #FCF200-Cu-50) and left to dry for 15 minutes. The grids were then gently wicked, allowed to dry for another 5 minutes, and treated with 5uL of two percent uranyl acetate for 2 minutes for negative staining. The grids were then gently wicked, allowed to dry for 5 minutes, and then washed with 5 μL of nanopure water. After a minute, the water was wicked, and the grids were left to dry for 10 minutes before imaging. Samples were transferred to a JEOL 2100F TEM and imaged using a Schottky type field emission gun operating at 200kV. Images were recorded using a Gatan OneView CMOS camera at 4k x 4k resolution.

Supporting information

S1 File. Supporting information for: Antibodies raised against a structurally defined Aβ oligomer mimic protect human iPSC neurons from Aβ toxicity at sub-stoichiometric concentrations. S1 Fig – Rabbit antibodies and $A\beta_{42}$ in i^3Neurons. S2 Fig - HMC3 microglia cells. S3 Fig – rabbit IgG TEM images. S4 Fig – Indirect ELISA of pAb_{2AT-L}, 6E10, and 4G8 against recombinant $A\beta_{42}$. S5 Fig – Zoe images of i^3neurons. S1 Table – Raw values and statistical analyses of $A\beta_{42}$ cell assays. S2 Table – Raw values and statistical analyses of $A\beta_{42}$ and antibody cell assays. S1 Text – Procedures detailing sample preparation, generation and purification of pAb_{2AT-L}, cell culture and differentiation, cell assays, ELISAs, ThT assays, and TEM studies.
(DOC)

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Formal analysis: Sarah M. Ruttenberg, Rakia Dhaoui, Adam G. Kreutzer.

Funding acquisition: Adam G. Kreutzer, James S. Nowick.

Investigation: Sarah M. Ruttenberg, Rakia Dhaoui, Adam G. Kreutzer.

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