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Passive (Amyloid-β) Immunotherapy Attenuates Monoaminergic Axonal Degeneration in the *AβPPswe/PS1dE9* Mice

Ying Liu[a], Michael K. Lee[a,b,*], Maria M. James[a], Donald L. Price[a,c], David R. Borchelt[a,1], Juan C. Troncoso[a,c] and Esther S. Oh[a,d,e,*]
[a]*Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[b]*Department of Neuroscience and Institute for Translational Neuroscience, University of Minnesota-Twin Cities, Minneapolis, MN, USA*
[c]*Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[d]*Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[e]*Department of Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*

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Abstract. The role of amyloid-β (Aβ) in the neurodegeneration of Alzheimer's disease remains controversial, to a large extent because of the lack of robust neurodegeneration in mouse models of AD. To address this question, we examined the effects of Aβ antibodies in the recently described monoaminergic (MAergic) axonal degeneration in *AβPPswe/PS1dE9* mice. To determine if Aβ accumulation is directly involved in degeneration of MAergic axons, we examined the effects of passive anti-Aβ antibody (7B6) administration on Aβ pathology and MAergic degeneration in *AβPPswe/PS1dE9* mice. Injections of monoclonal antibody (mAb) 7B6 into mice (6 to 9 months of age) resulted in a modest reduction of Aβ load in the brains of *AβPPswe/PS1dE9* mice. In addition, 7B6 treated *AβPPswe/PS1dE9* mice had significantly higher densities of MAergic axons in both cortex and in hippocampus as compared to untreated mutant mice. For example, 7B6 treated mice showed almost 2-fold greater densities of serotonergic (5-HT) axons in the cortex compared to saline treated mice. Similar findings were observed in the catecholaminergic (TH) axons. Our results demonstrate that lowering of Aβ levels via passive Aβ immunotherapy ameliorates ongoing degenerative processes, supporting a causal link between Aβ and neurodegeneration.

Keywords: Amyloid-β, axon, immunotherapy, neurodegeneration, serotonergic

INTRODUCTION

Amyloid plaques, consisting of amyloid-β (Aβ) peptide, along with the presence of neurofibrillary tangles (NFTs) and progressive degeneration of neuronal processes and synapses, are the pathological hallmarks of Alzheimer's disease (AD) [1]. The role of Aβ in AD-associated neurodegeneration, however, remains controversial, to a large extent because of the lack of robust neurodegeneration in mouse models of Aβ amyloidosis [2–4].

Recently, we demonstrated that a progressive degeneration of monoaminergic (MAergic) neurons occurs with progressive deposition of Aβ in the *AβPPswe/PS1dE9* mouse model [5]. In these mice, degeneration of cortical and hippocampal MAergic axons becomes apparent prior to 12 months of age,

[1] D.R. Borchelt's present address: University of Florida, Gainesville Florida, USA.

*Correspondence to: Esther S. Oh, MD, 558 Ross Research Building, 720 Rutland Ave., Baltimore, MD 21205, USA. Tel.: +1 410 955 5632; Fax: +1 410 955 9777; E-mail: eoh9@jhmi.edu. Michael K. Lee, PhD, Department of Neuroscience and Institute for Translational Neuroscience, University of Minnesota-Twin Cities, 2102 6th S.E. Minneapolis, MN 55455, USA. Tel.: +1 612 626 8472; Fax: +1 612 626 2032; E-mail: mklee@umn.edu.

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followed by loss of neuronal cell bodies by 18 months of age [5]. These results indicate that similar to the loss of MAergic neurons occurring in human [6–10], degeneration of MAergic axons followed by loss of neurons can be reproduced in a mouse model. Further, because MAergic neuropathology occurs at the earliest stages of AD [7, 10], a mouse model of cerebral amyloid pathology can model early neurodegenerative stages of AD [5]. While MAergic degeneration in *AβPPswe/PS1dE9* mice occurs with the accumulation of Aβ in the target fields of the MAergic afferents, it is unknown if accumulation of Aβ is the primary factor driving the initial degeneration of MAergic axons. In addition, Aβ deposition initiates other pathological changes which may be responsible for axonal and neuronal degeneration. This question is particularly relevant since therapeutic interventions being developed to lower Aβ deposition may not prevent neurodegeneration and therefore fail to slow the progression of AD in humans [11]. However, it is possible that the pathological effects of Aβ are not reversible at later stages of AD.

Herein, we determined if Aβ is indeed responsible for the onset of MAergic neurodegeneration and whether neurodegenerative effects of Aβ can be attenuated at "preclinical" stages of disease in the *AβPPswe/PS1dE9* mice. We show that passive Aβ immunization of *AβPPswe/PS1dE9* mice resulted in modest reduction in brain amyloid burden and significant attenuation in the loss of MAergic afferents. Our results demonstrate that the extent of MAergic neurodegeneration is highly sensitive to Aβ accumulation and support the view that lowering Aβ accumulation could be neuroprotective at early neuropathological stages.

MATERIALS AND METHODS

Mice

AβPPswe/PS1dE9 mice ($n = 13$) in B6/C3 hybrid background (Line 85) express a chimeric mouse/human (Mo/Hu) AβPP-695 with mutations (KM 593/594 NL) linked to familial AD. By 4-6 months of age, the *AβPPswe/PS1dE9* mice develop amyloid deposits in cerebral cortex and hippocampus [12]. The Institutional Animal Care and Use Committee of the Johns Hopkins University approved the studies, and they were conducted in strict compliance with the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals".

Immunotherapy

For this study, we used a clone of murine cells that produces a mAb 7B6 directed against the first 11 N-terminal amino acids of human Aβ [13]. This IgG_1 antibody recognizes native Aβ in tissue sections and Western blots of frozen brain tissues from cases of AD and from *AβPPswe/PS1dE9* mice. Each mouse in the study received 5 equally-spaced doses (approximately 3 weeks apart) of either 7B6 (250 μg) or phosphate buffered saline (PBS, pH = 7.4) starting at 6 months of age and ending at 9 months of age. In this investigation, we examined two cohorts of mice: PBS injected mice ($n = 7$); and 7B6 injected mice ($n = 7$).

Tissue preparation

All of the mice were sacrificed by intracardiac perfusion with PBS (pH = 7.4), and their brains were immersion-fixed in 4% paraformaldehyde. Brains were treated with 30% sucrose for cryoprotection, and stored frozen at –80 °C until sectioning. Frozen brains were cut serially (40 μm) on the sagittal plane. All animal procedures were performed in accordance with the Johns Hopkins Animal Care and Use Committee Guidelines.

Immunohistochemistry

Immunohistochemistry for Aβ was performed on 6 frozen parasagittal sections (40 μm) using the 6E10 antibody (Covance, U.S.), and detected with an anti-mouse antibody (VECTASTAIN Elite ABC kit, Vector Laboratories, Burlingame, CA). Sections were developed with 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA), and counterstained with hematoxylin. Immunohistochemical detection of serotonergic (5-HT) and catecholaminergic (TH) fibers was conducted on 4 frozen parasagittal sections (40 μm) each, with a polyclonal anti-5-HT antibody (Immunostar, Stillwater, MN), and a polyclonal anti-TH antibody (Novus Biologicals, Littleton, CO, USA). Primary antibodies were detected with an anti-rabbit antibody (VECTASTAIN Elite ABC kit, Vector Laboratories, Burlingame, USA), and developed with fast 3,3′-diaminobenzidine. In some cases, silver-gold intensification of immunoreactive fibers facilitated the identification of MAergic fibers.

Stereology

Area fraction

The area fraction of Aβ immunoreactivity in the hippocampus and cortex were quantified in six parasagittal sections from each mouse. The measurements of the area fractions were performed using a Stereo Investigator (MicroBrightField, Williston, VT) with a 40 × objective as described previously [13].

Stereology of densities of MAergic axons

The densities of MAergic afferents were determined by stereological length estimations using spherical probes (Space Balls) (Stereo Investigator, MicroBright Field, Williston, VT) [14] as described previously [5]. For this study, a virtual hemispheric probe (radius 10.00 mm, surface area 628 mm^2) was used. Because the densities of MAergic afferents show significant regional and rostro-caudal heterogeneities, our analysis focused on the selected subregions defined by the mouse brain in stereotaxic coordinates [15]. For each of the neurotransmitter markers, a total of 4 sections selected from the defined regions (lateral 0.96 mm to 2.04 mm) were processed for immunocytochemistry. The regions examined were the cortex (S1Tr +PtA+V2ML) and whole hippocampus (Dentate + CA1+ CA2/3).

Brain homogenation and ELISA for human Aβ

Brain homogenation

Frozen hemibrains were sequentially extracted using a three-step method [16]. Frozen brains were sonicated first in 1× PBS pH 7.6 with 1× protease inhibitor, followed by 2% SDS, and finally in 70% formic acid. At each step, sonication was followed by centrifugation at 100,000×*g* for 1 h at 4°C, and the supernatants were collected for ELISA.

ELISA

Samples were analyzed for $A\beta_{1–40}$ and $A\beta_{1-42}$ using Aβ [1–40] Human ELISA Kit and Human Aβ (Hu Ab42) High Sensitivity ELISA Kit (Invitrogen, USA), respectively. Samples were analyzed in duplicates.

Data analysis

Due to a small sample size, non-parametric (Mann-Whitney) comparison was used to compare Aβ area fractions and the MAergic axonal densities in the cerebral cortex and hippocampus of the mice that received 7B6 or PBS. Statistical analysis was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA.

RESULTS

To establish that the accumulation of Aβ is directly responsible for the MAergic degeneration in *Aβ PPswe/PS1dE9* mice [5], and to determine whether anti-Aβ therapy can attenuate progressive neurodegeneration in brain, we examined the effect of reducing Aβ pathology via systemic injections of anti-Aβ antibody (7B6) on the degeneration of MAergic axons. Previous studies showed that anti-Aβ antibody immunotherapy can reduce the Aβ burden [17]. However, because the *in vivo* models were not thought to exhibit overt progressive neurodegeneration [2], it is not clear whether immunotherapy can protect from MAergic degeneration.

Following treatment with 7B6, a monoclonal antibody (mAb) directed against Aβ 1-11, for 3 months, the brain sections were immunostained for Aβ (6E10) and the 6E10 immunoreactive area fraction were determined as indicators of total amyloid deposition. The results show that 7B6 mAb treatment leads to a modest reduction of Aβ deposition in the brain of *AβPPswe/PS1dE9* mice (Fig. 1). Biochemical analysis for cerebral Aβ levels in various soluble fractions show that while 7B6 administration did not significantly reduce PBS and SDS soluble Aβ levels, 7B6 treatment resulted in significant and reduction in the insoluble (formic acid) fraction of both $A\beta_{40}$ and $A\beta_{42}$ (Fig. 2).

To determine if administration of 7B6 can protect cortical and hippocampal MAergic afferents from degeneration, brain sections from the *AβPPswe/PS1dE9* mice receiving 7B6 or PBS ($n = 7$ each) were compared to examine the integrity of serotonergic (5-HT) and catecholarminergic (TH) afferents. The brain sections were immunostained for MAergic afferents and the density of the fibers in given brain region of interest were determined by unbiased stereology. The analysis of cortical 5-HT and TH fiber densities showed that in 7B6 treated mice, the 5-HT and TH afferent densities were 92% ($p = 0.001$) and 59% ($p = 0.007$) greater, respectively, than in the PBS treated mice (Fig. 3A). In the dorsal hippocampus, mice injected with 7B6 showed 138% ($p = 0.002$) greater 5-HT axonal densities as compared to the mice that received PBS. TH axonal densities were 28% ($p = 0.02$) greater with 7B6 treatment in the hippocampus (Fig. 3B). In addition, closer examination of the sections stained for

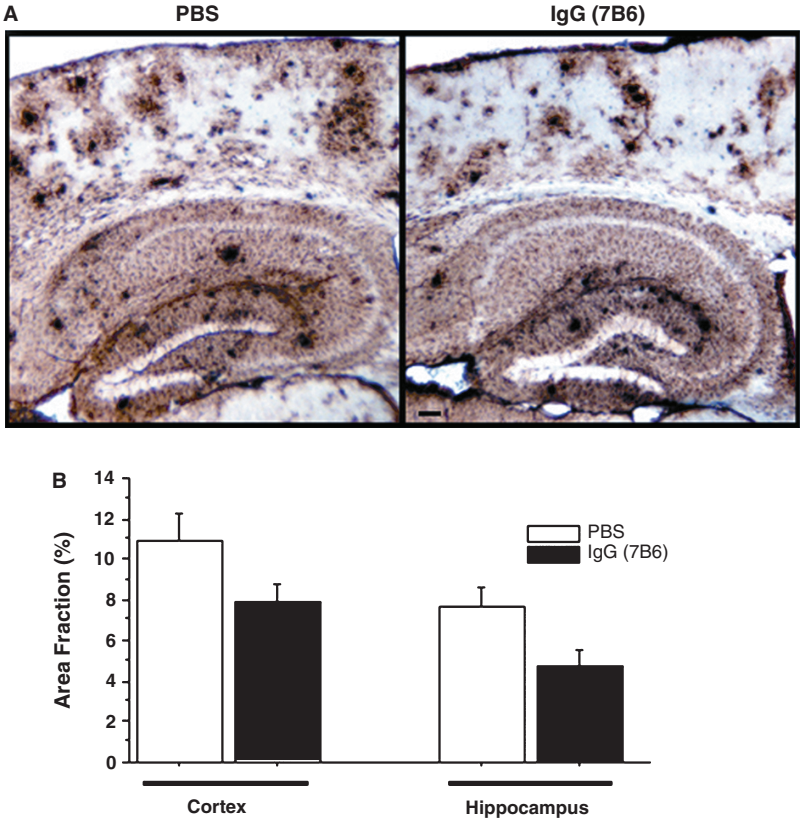

Fig. 1. Intraperitoneal injection (i.p.) of anti-Aβ antibody 7B6 reduces amyloid plaques in *AβPPswe/PS1dE9* mice. A) Representative images of amyloid plaque burden stained for Aβ with 6E10. Fewer amyloid plaques are seen in the mice that received 7B6 (right) compared to those that received PBS (left). B) Area fraction quantification of amyloid plaques in the cortex. There was 28% reduction in cortical Aβ deposits in the mice that received 7B6 as compared to the mice that received PBS with trends toward significance ($p < 0.07$). There was 38% reduction in hippocampal Aβ deposits in the 7B6 treated mice compared to the mice that received PBS, with trends towards significance ($p < 0.07$). Scale bar represents 100 μm.

5-HT and TH afferents show that axonal dystrophy was less prominent in the 7B6 treated mice compared to those that received PBS (Fig. 4A, B). Consistent with the previous study [5], the overall densities of the cholinergic afferents were not obviously affected in the *AβPPswe/PS1dE9* mice at this age. However, similar to that seen with MAergic afferents, dystrophic cholinergic terminals in the 7B6 treated mice were qualitatively less prominent than in the PBS treated mice (Fig. 4C).

DISCUSSION

Our results show that a modest reduction in Aβ pathology by passive immunotherapy can lead to significant protection of MAergic afferents from degeneration in the *AβPPswe/PS1dE9* mice, a robust model of cerebral amyloidosis. Since 7B6 is a mAb specific for human Aβ [13], the likely mechanisms for the protection of MAergic afferents is the reduction of brain Aβ accumulation, which appears to damage terminals and axons [5]. Consistent with this view, 7B6 treatment leads to modest attenuation of overt amyloid deposits (Fig. 1). Our biochemical analysis showed selective reduction of the FA soluble Aβ levels, but not of the PBS soluble Aβ levels with 7B6 treatment. However, in our hands, the levels of PBS soluble Aβ were in low abundance, which may have limited our ability to detect any real differences in the levels of the PBS soluble Aβ species. While our efforts to document changes in Aβ oligomers levels using commercial anti-oligomer antibodies were also not conclusive (not shown), fibrillar or protofibrillar Aβ are also thought to be associated with the formation of abnormal dystrophic axons and dendritic atrophy [18]. In addition, the toxic effects of the fibrillar Aβ may also be related to their possible roles as "reservoirs" for neurotoxic Aβ oligomers that are released from these sites

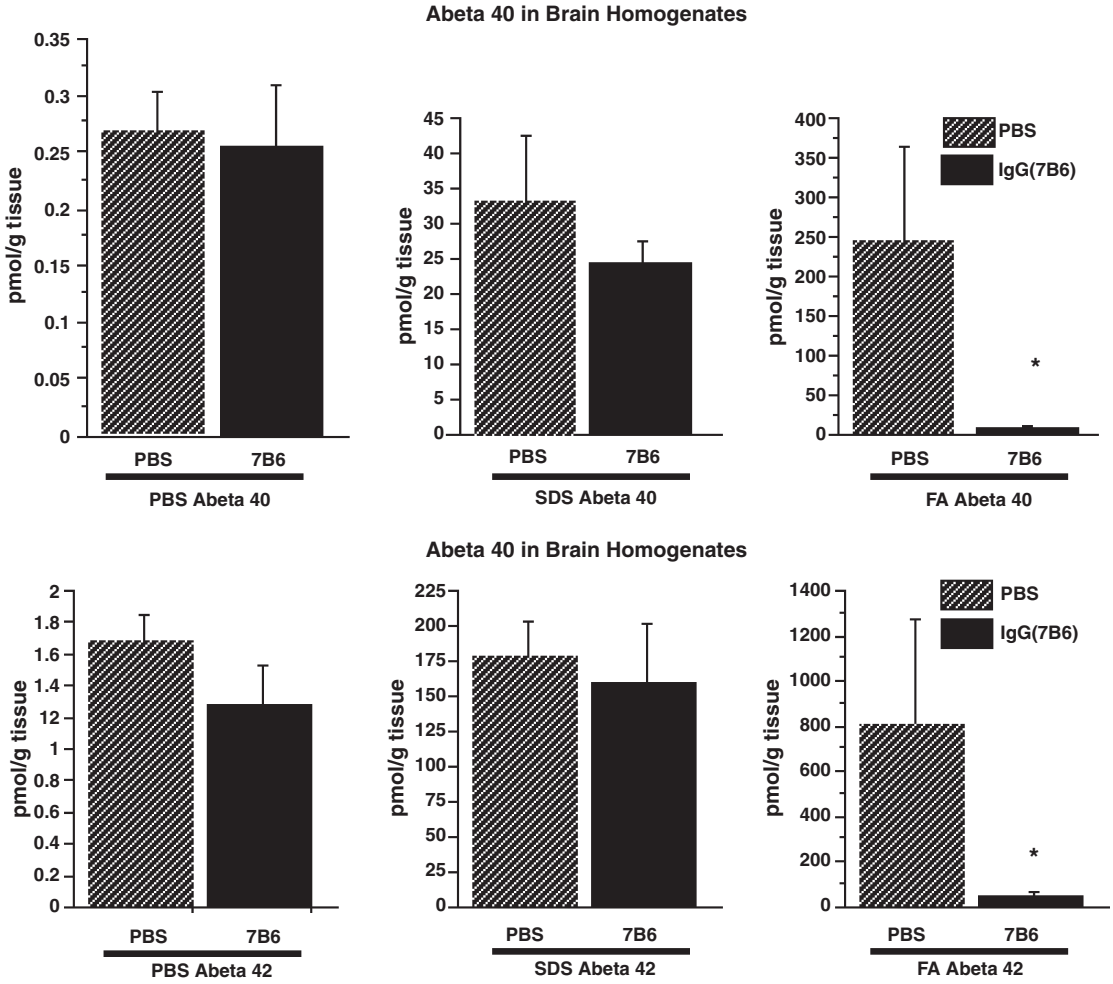

Fig. 2. Intraperitoneal injection (i.p.) of the anti-Aβ antibody 7B6 reduces $A\beta_{40}$ and $A\beta_{42}$ levels in the insoluble (formic acid) fraction of the *AβPPswe/PS1dE9* mouse brains. *AβPPswe/PS1dE9* mice were administered 250 μg of 7B6 or PBS injections at 3 week intervals from 6 to 9 months of age ($n = 7$ per group). Frozen hemibrains were processed by a three-step sequential extraction method, and Aβ levels were determined by ELISA. In the phosphate buffered saline (PBS) and sodium dodecyl sulfate (SDS) brain homogenate fractions, there were no significant differences in the $A\beta_{40}$ and $A\beta_{42}$ levels in the group that received 7B6 injections compared to the group that received PBS injections. There was a significant difference in the $A\beta_{40}$ levels of the formic acid (FA) fraction between the mice that received PBS (245 ± 117.9 pmol/g) and those that received 7B6 (8.94 ± 3.05 pmol/g) ($^*p < 0.007$). Similar reductions were seen in $A\beta_{42}$ levels between those that received PBS (807 ± 468 pmol/g) compared to those that received 7B6 (46.67 ± 18.11 pmol/g) ($^*p < 0.05$). Error bars represent s.e.m.

[19]. Recently, Koffie and colleagues demonstrated that Aβ oligomers surround amyloid plaques in the *AβPPswe/PS1dE9* mouse model, and exert their neurotoxic effects even far from the plaques [20]. Once the Aβ oligomers cause degeneration of MAergic axons, the MAergic cell bodies may suffer from the chronic loss of retrograde trophic support, including the loss of brain derived neurotrophic factor (BDNF) [21] and neurotrophin-3 (NT-3) signaling.

The second question is the site of the neuroprotection by immunotherapy, whether it is a local process targeting the distal MAergic axons/terminals in the forebrain or a more general effect affecting other regions of the brain. In our previous study [5], there was significant age-associated progressive axonal degeneration of the forebrain MAergic system in *AβPPswe/PS1dE9* mice detectable by 12 months of age. The loss of MAergic afferents occur in absence of any local Aβ deposits in the brainstem or intracellular accumulation of Aβ at this age [5]. Thus, MAergic neurodegeneration begins when Aβ deposits are limited to cortical/hippocampal region in these mice. Thus, we believe that the most likely neuroprotective sites are in the distal fields of MAergic projections

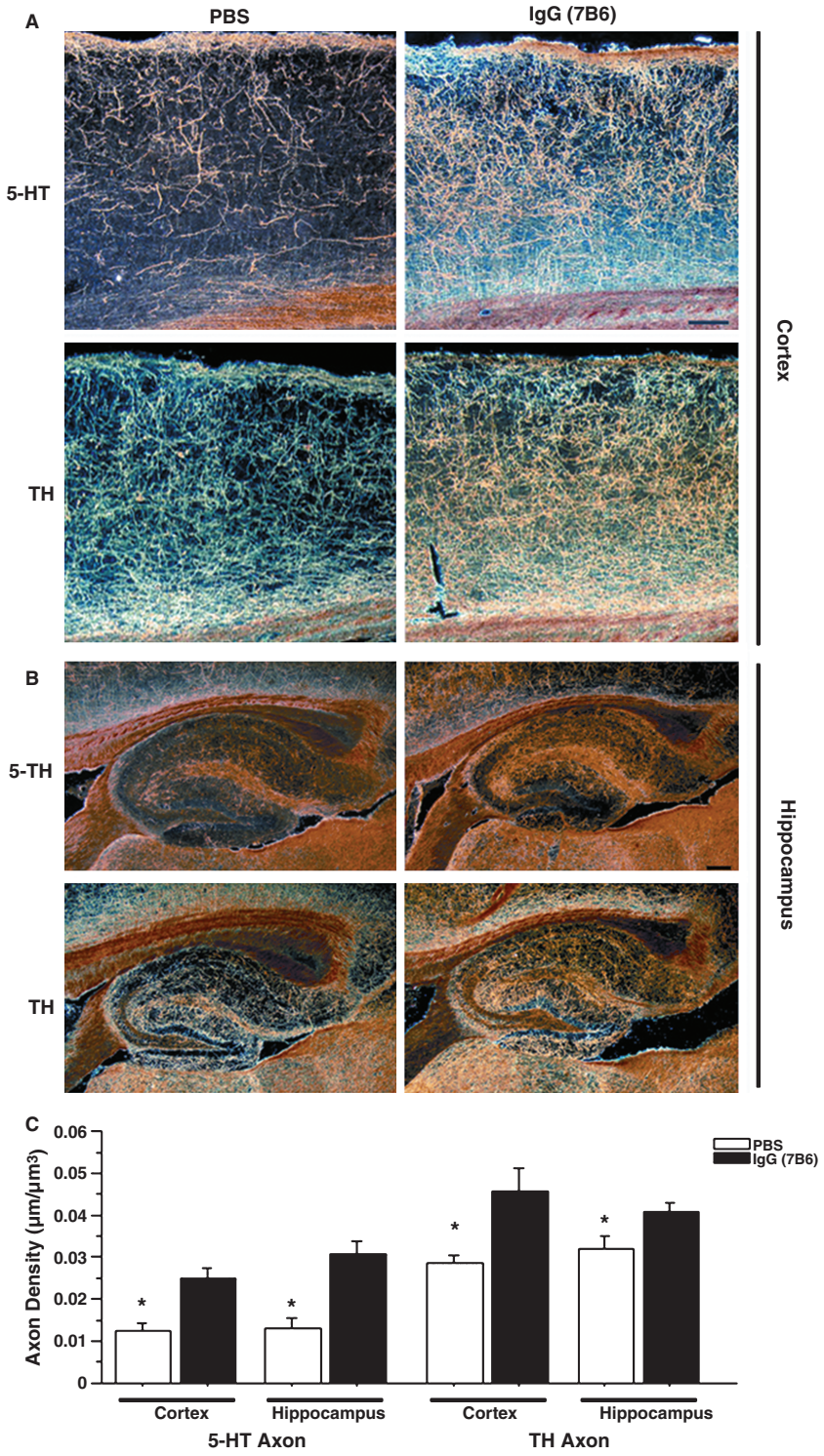

Fig. 3. Effects of 7B6 immunotherapy on the MAgeric system. Attenuation of (A) cortical and (B) hippocampal MAergic neurodegeneration in the 7B6 treatment group. C) Stereological analysis demonstrated greater cortical 5-HT axonal fiber density in the 7B6 group (0.025 ± 0.002 μm/μm^3) compared to the PBS group (0.013 ± 0.002 μm/μm^3) (*$p = 0.001$). Similar findings were seen in the hippocampus with greater 5-HT axonal fiber density in the 7B6 group (0.031 ± 0.003 μm/μm^3) compared to the PBS group (0.013 ± 0.002 μm/μm^3) (*$p = 0.002$). TH axonal fiber density was also greater in the cortex of the 7B6 group (0.046 ± 0.005 μm/μm^3) compared to the PBS group (0.029 ± 0.002 μm/μm^3) (*$p = 0.007$), as well as in the hippocampus of the PBS group (0.032 ± 0.003 μm/μm^3) compared to the 7B6 group (0.041 ± 0.002 μm/μm^3) (*$p = 0.02$). Scale bars represent 200 μm.

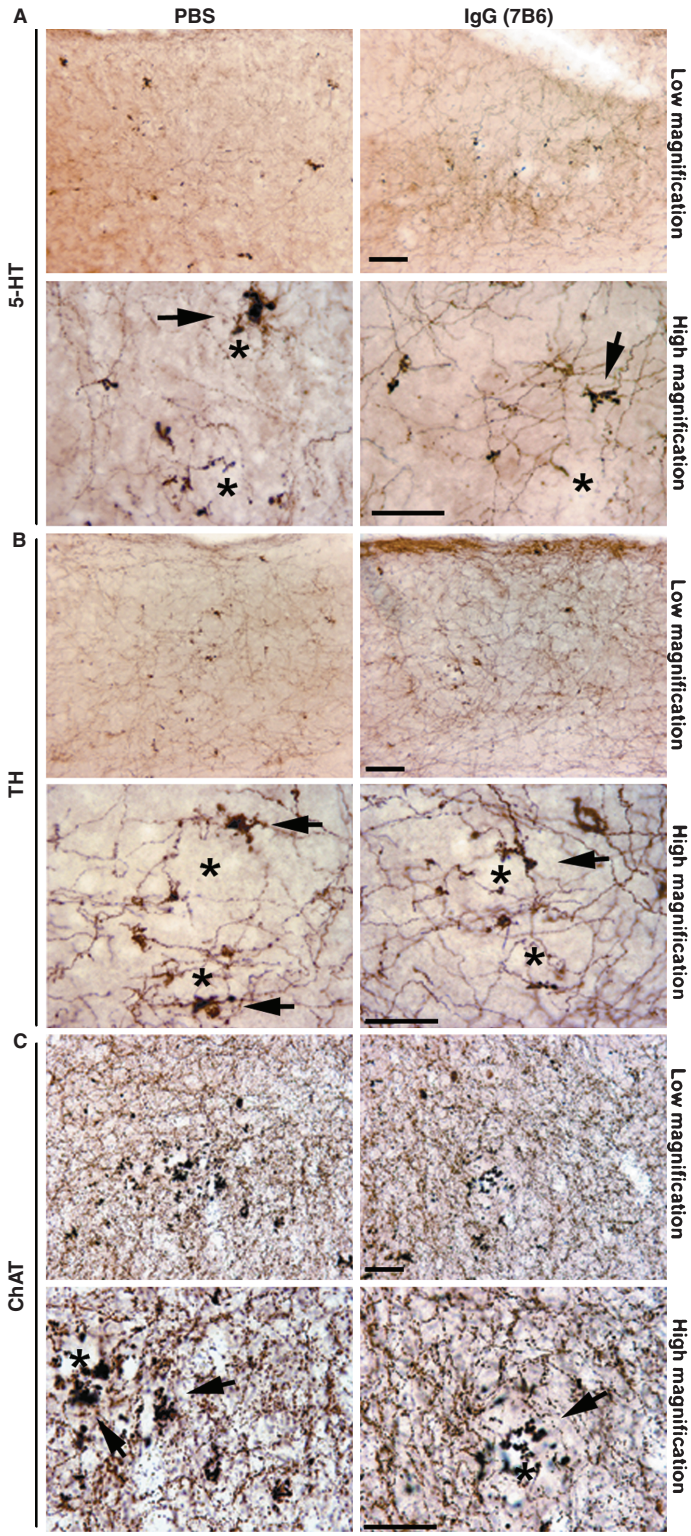

Fig. 4. Effects of 7B6 mAb treatments on local axonal dystrophy. A representative images of dorsal cortical regions from sagittal sections stained for 5-HT+(A), TH+(B), and ChAT+(C) afferent. Both low and high magnification images show prominent dystrophic neurites (arrows), particularly around amyloid deposits (*). However, the abundance and/or the size of dystrophic neurites are less prominent in 7B6 treated mice compared to the PBS treated controls. Scale bars: low magnification –100 μm, high magnification −20 μm.

to the forebrain where toxic Aβ has been removed, in part, by passive immunotherapy. Since we examined animals of the same age and at a single time point, we cannot determine whether the preservation of MAergic forebrain innervation in the treated group reflects the prevention of synaptic or axonal degeneration or enhanced regeneration of already injured fibers. However, since the age of mice used represent the initial stages of Aβ deposition, it is most likely that the administration of 7B6 acts predominantly to prevent the degeneration of MAergic axons.

Finally, an important question that arises from our present observations in *AβPPswe/PS1dE9* mice is their potential relevance to AD, a complex disease of the human brain. While Aβ appears to be a critical driver of the pathogenesis of the disease [22], there are many other pathological processes and changes that occur in AD [23]. Degeneration of MAergic system is noted during the clinical course of AD [6, 24]. Its importance comes from interactions with other neurotransmitters of AD in modulating learning and memory [25], and its involvement in behavioral and psychological signs of dementia [26]. Determination of the optimal treatment time point is critical in the development of therapeutic tools for AD. Our data with the *AβPPswe/PS1dE9* model show that starting immunotherapy in the early stages of Aβ accumulation protects the MAergic axonal system, however, there may still be some axonal degeneration compared to the non-transgenic mice due to the neurotoxic effects of the soluble Aβ. It would be important to determine how early immunotherapy can be started in order to maximally preserve the neurotransmitter systems. It is also important to determine how late in the disease stage immunotherapy would still be effective in reversing the course of the neurodegeneration or in facilitating regeneration, repair, and recovery. Different studies have shown that immunotherapy was able to promote recovery of neuritic dystrophy in older transgenic mouse models [27, 28], however, it was proposed that more mature dystrophic neurite may not recover as well as a newly developed neuritic dystrophy [28]. As our study provides a strong proof of concept that Aβ immunotherapy can protect MAergic system from degenerating in the setting of brain Aβ amyloidosis, we believe that our observation of protection of MAergic axons by Aβ immunotherapy has substantial implications for the prevention or therapy of AD. As more therapeutics will be targeted towards earlier stages of the disease with identification of subjects in the earliest stages of AD pathology, these issues will become more important in the future [29].

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REFERENCES

[1] Price DL, Sisodia SS (1998) Mutant genes in familial Alzheimer's disease and transgenic models. *Ann Rev Neurosci* **21**, 479-505.

[2] McGowan E, Eriksen J, Hutton M (2006) A decade of modeling Alzheimer's disease in transgenic mice. *Trends Genet* **22**, 281-289.

[3] Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT (1997) APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol* **56**, 965-973.

[4] Irizarry MC, Soriano F, McNamara M, Page KJ, Schenk D, Games D, Hyman BT (1997) Abeta deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. *J Neurosci* **17**, 7053-7059.

[5] Liu Y, Yoo MJ, Savonenko A, Stirling W, Price DL, Borchelt DR, Mamounas L, Lyons WE, Blue ME, Lee MK (2008) Amyloid pathology is associated with progressive monoaminergic neurodegeneration in a transgenic mouse model of Alzheimer's disease. *J Neurosci* **28**, 13805-13814.

[6] Zweig RM, Ross CA, Hedreen JC, Steele C, Cardillo JE, Whitehouse PJ, Folstein MF, Price DL (1988) The neuropathology of aminergic nuclei in Alzheimer's disease. *Ann Neurol* **24**, 233-242.

[7] Rub U, Del Tredici K, Schultz C, Thal DR, Braak E, Braak H (2000) The evolution of Alzheimer's disease-related cytoskeletal pathology in the human raphe nuclei. *Neuropathol Appl Neurobiol* **26**, 553-567.

[8] Parvizi J, Van Hoesen GW, Damasio A (2001) The selective vulnerability of brainstem nuclei to Alzheimer's disease. *Ann Neurol* **49**, 53-66.

[9] Lyness SA, Zarow C, Chui HC (2003) Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: a meta-analysis. *Neurobiol Aging* **24**, 1-23.

[10] Grudzien A, Shaw P, Weintraub S, Bigio E, Mash DC, Mesulam MM (2007) Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. *Neurobiol Aging* **28**, 327-335.

[11] Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, Nicoll JA (2008) Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* **372**, 216-223.

[12] Jankowsky JL, Slunt HH, Ratovitski T, Jenkins NA, Copeland NG, Borchelt DR (2001) Co-expression of multiple trans-

genes in mouse CNS: a comparison of strategies. *Biomol Eng* **17**, 157-165.

[13] Tucker SM, Borchelt DR, Troncoso JC (2008) Limited clearance of pre-existing amyloid plaques after intracerebral injection of Abeta antibodies in two mouse models of Alzheimer disease. *J Neuropathol Exp Neurol* **67**, 30-40.

[14] Mouton PR, Gokhale AM, Ward NL, West MJ (2002) Stereological length estimation using spherical probes. *J Microscopy* **206**, 54-64.

[15] Paxinos G, Franklin KBJ (2001) *The Mouse Brain in Stereotaxic Coordinates*, Academic Press, San Diego.

[16] Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG (2001) Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci* **21**, 372-381.

[17] Brody DL, Holtzman DM (2008) Active and passive immunotherapy for neurodegenerative disorders. *Ann Rev Neurosci* **31**, 175-193.

[18] Tsai J, Grutzendler J, Duff K, Gan WB (2004) Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. *Nat Neurosci* **7**, 1181-1183.

[19] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* **14**, 837-842.

[20] Koffie RM, Meyer-Luehmann M, Hashimoto T, Adams KW, Mielke ML, Garcia-Alloza M, Micheva KD, Smith SJ, Kim ML, Lee VM, Hyman BT, Spires-Jones TL (2009) Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc Natl Acad Sci U S A* **106**, 4012-4017.

[21] Tong L, Balazs R, Thornton PL, Cotman CW (2004) Beta-amyloid peptide at sublethal concentrations downregulates brain-derived neurotrophic factor functions in cultured cortical neurons. *J Neurosci* **24**, 6799-6809.

[22] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-356.

[23] Geula C (2000) Pathological diagnosis of Alzheimer's disease. In *Early Diagnosis of Alzheimer's Disease*, Scinto LFM, Daffner KR, eds. Humana, New Jersey, p. 65.

[24] German DC, Nelson O, Liang F, Liang CL, Games D (2005) The PDAPP mouse model of Alzheimer's disease: locus coeruleus neuronal shrinkage. *J Comp Neurol* **492**, 469-476.

[25] Decker MW, McGaugh JL (1991) The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* **7**, 151-168.

[26] Garcia-Alloza M, Gil-Bea FJ, Diez-Ariza M, Chen CP, Francis PT, Lasheras B, Ramirez MJ (2005) Cholinergic-serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease. *Neuropsychologia* **43**, 442-449.

[27] Lombardo JA, Stern EA, McLellan ME, Kajdasz ST, Hickey GA, Bacskai BJ, Hyman BT (2003) Amyloid-beta antibody treatment leads to rapid normalization of plaque-induced neuritic alterations. *J Neurosci* **23**, 10879-10883.

[28] Brendza RP, Bacskai BJ, Cirrito JR, Simmons KA, Skoch JM, Klunk WE, Mathis CA, Bales KR, Paul SM, Hyman BT, Holtzman DM (2005) Anti-Abeta antibody treatment promotes the rapid recovery of amyloid-associated neuritic dystrophy in PDAPP transgenic mice. *J Clin Invest* **115**, 428-433.

[29] Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**, 119-128.