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Anti-Aβ antibodies incapable of reducing cerebral Aβ oligomers fail to attenuate spatial reference memory deficits in J20 mice

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ABSTRACT

Compelling genetic evidence links the amyloid precursor protein (APP) to Alzheimer's disease (AD). A leading hypothesis proposes that a small amphipathic fragment of APP, the amyloid β-protein (Aβ), self-associates to form soluble assemblies loosely referred to as "oligomers" and that these are primary mediators of synaptic dysfunction. As such, Aβ, and specifically Aβ oligomers, are targets for disease modifying therapies. Currently, the most advanced experimental treatment for AD relies on the use of anti-Aβ antibodies. In this study, we tested the ability of the monomer-preferring antibody, m266 and a novel aggregate-preferring antibody, 1C22, to attenuate spatial reference memory impairments in J20 mice. Chronic treatment with m266 resulted in a ~70-fold increase in Aβ detected in the bloodstream, and a ~50% increase in water-soluble brain Aβ — and in both cases Aβ was bound to m266. In contrast, 1C22 increased the levels of free Aβ in the bloodstream, and bound to amyloid deposits in J20 brain. However, neither 1C22 nor m266 attenuated the cognitive deficits evident in 12 month old J20 mice. Moreover, both antibodies failed to alter the levels of soluble Aβ oligomers in J20 brain. These results suggest that Aβ oligomers may mediate the behavioral deficits seen in J20 mice and highlight the need for the development of aggregate-preferring antibodies that can reach the brain in sufficient levels to neutralize bioactive Aβ oligomers.

Aside from the lack of positive effect of m266 and 1C22 on cognition, a substantial number of deaths occurred in m266- and 1C22-immunized J20 mice. These fatalities were specific to anti-Aβ antibodies and to the J20 mouse line since treatment of wild type or PDAPP mice with these antibodies did not cause any deaths. These and other recent results indicate that J20 mice are particularly susceptible to targeting of the APP/Aβ/tau axis. Notwithstanding the specificity of fatalities for J20 mice, it is worrying that the murine precursor (m266) of a lead experimental therapeutic, Solanezumab, did not engage with putatively pathogenic Aβ oligomers.

1. Introduction

Alzheimer's disease (AD) is characterized by amyloid deposition, neurofibrillary tangles, synaptic loss, neuronal loss, reactive gliosis and memory impairment. Several transgenic human amyloid precursor protein (hAPP) mouse models reproduce certain features of AD and have been used to assess the efficacy of therapeutic interventions, including the use of anti-Aβ antibodies. Early studies demonstrated that the anti-Aβ monoclonal antibody (mAb), 3D6 (raised to $A\beta_{1-5}$), reduced cortical Aβ burden by ~86% in PDAPP mice (Bard et al., 2000), whereas when PDAPP mice were treated with the mid-region specific mAb, m266, it had little effect on Aβ deposition but dramatically increased circulating levels of antibody-bound Aβ (DeMattos et al., 2001). Subsequent studies using other anti-Aβ mAbs and mouse models also demonstrated significant reductions in amyloid burden (Levites et al., 2006; Schroeter et al., 2008). In addition, several studies found that passive administration of certain anti-Aβ antibodies protected or restored cognition in hAPP mice (Basi et al., 2010; Dodart et al., 2002; Karlnoski et al., 2008; Kotilinek et al., 2002; Oddo et al., 2006; Wilcock et al., 2004a,b, 2006; Zago et al., 2012). Specifically, m266, was shown to improve object recognition memory in 11 month PDAPP mice 24 h after a single antibody administration (Dodart et al., 2002). The mechanism of this striking short-term improvement is uncertain, but has been suggested to result due to either: (1) direct neutralization of soluble toxic

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forms of cerebral Aβ, or (2) m266 sequestering Aβ in the bloodstream and causing an efflux of soluble toxic Aβ species into the blood.

Although mAbs can engage Aβ when administered to man, the success of mAb immunization in pre-clinical AD models has not translated well to humans (Blennow et al., 2012; Farlow et al., 2012; Salloway et al., 2012, 2014). Solanezumab, the humanized version of m266, is the only antibody with published results reporting benefit in humans. Even with Solanezumab the cognitive benefit was marginal, with extracted analysis of a phase III trial data revealing only a modest attenuation of cognitive decline in mild AD patients and no effect in individuals with moderate AD (Doody et al., 2014).

Despite millions of dollars being invested in clinical trials, there is limited published data on the preclinical testing of m266 on cognition, and to-date all such studies have used a single mouse model — PDAPP mice. Moreover, no prior published study directly tested the effects of m266 versus another anti-Aβ antibody, nor assessed the effect of treatment on cerebral Aβ oligomers. Here we tested m266 alongside a recently described aggregate-preferring antibody, 1C22 (O'Nuallain et al., 2014; Yang et al., 2015) employing the well-characterized J20 mouse model (Cheng et al., 2007; Chin et al., 2005; Karl et al., 2012; Mably et al., 2015; Palop et al., 2003; Roberson et al., 2007, 2011; Wright et al., 2013). The J20 model was chosen because it has important similarities to the PDAPP mouse model which is the only model in which m266 has ever been tested. Specifically, both the PDAPP and J20 mice express similar APP copy numbers, the transgene has a similar minigene structure and both are under the control of the PDGF promoter (Hsia et al., 1999). In prior studies we tested the performance of J20 mice and littermate Wt controls from the same colony as used here at 3 different ages (4, 8, and 12 months) in 5 different tasks: (1) open field, (2) spontaneous alternation Y-maze, (3) radial arm maze (RAM), (4) novel object recognition, and (5) contextual fear conditioning (Mably et al., 2015). In agreement with several other reports (Karl et al., 2012; W.S. Kim et al., 2013; Wright et al., 2013) we found that J20 mice exhibited hyperactivity which waned with increasing age and decreased performance on the RAM which became more prominent with increasing age (Mably et al., 2015). However, J20 and Wt mice performed at comparable levels on Y-maze, contextual fear conditioning and novel objection recognition. In an attempt to mimic current human trials in individuals with mild AD, mice began immunotherapy at an age (9.5 months) when they had some amyloid deposition and mild impairment of spatial reference memory. We found that m266 bound to certain water-soluble forms of Aβ in the brain and dramatically elevated circulating levels of Aβ in blood, most of which was bound to antibody. In contrast, 1C22 bound to plaque Aβ and although it promoted an increase in the levels of circulating Aβ none of this was bound to 1C22. Despite clear evidence of engagement of m266 with Aβ monomer, and 1C22 with plaques, neither antibody affected cerebral Aβ oligomer levels or attenuated deficits in spatial reference memory. These results suggest that removal of Aβ oligomers may be necessary to overcome the spatial reference memory deficits evident in J20 mice. Furthermore, these findings indicate that in the complex milieu of brain removal of oligomers can only be achieved by antibodies with minimal reactivity to monomers or plaques.

Our studies also revealed an important negative effect of m266 and 1C22, that is, ~20% of immunized J20 mice died. These studies follow on from our recent report that a mid-region anti-tau mAb also caused death in J20 mice (Mably et al., 2015), and suggest that J20 mice are particularly susceptible to targeting of the APP/Aβ/tau axis. To the best of our knowledge this is the first report that anti-Aβ antibodies can have deleterious effects on animal viability, and that monomer-preferring and aggregating-preferring mAbs are unable to reduce the levels of water-soluble Aβ oligomers in brain. How generalizable these results are to humans, or indeed other mouse models, is as yet unclear. Nonetheless, the fact that a lead experimental therapeutic did not reduce the levels of a putatively pathogenic form of Aβ might explain its very modest efficacy in the clinic.

2. Materials and methods

2.1. Mice and antibody administration

Mice were housed under a 12 h light:dark cycle (lights on 7 am, lights off 7 pm). Ad libitum food (standard chow; LabDiet, Richmond, IN) was provided unless otherwise indicated. Male hemizygous $hAPP_{Swe/Ind}$ mice (J20) were obtained from Jackson Laboratories (Bar Harbor, ME) and crossed with C57BL/6J female mice to produce hemizygous J20 mice or wild type (Wt) littermate controls. Only male hemizygous J20 mice and Wt littermate controls were used for the study, and were the F1 progeny of 7 male J20 mice. J20 mice over-express hAPP carrying the Swedish (KM670/671NL) and Indiana (V717F) mutations (Mucke et al., 2000). Pups were weaned at 20–21 days old, male progeny were tail snipped and genotyped. Mice were group housed (2–4 animals per cage) until 5 days prior to behavioral testing, and after this time mice were housed individually. Female PDAPP mice and Wt littermate controls were a kind gift from Janssen Alzheimer Immunotherapy (South San Francisco, CA). PDAPP mice arrived at 9 months and were housed (2–4 animals per cage) according to genotype. PDAPP mice over-express hAPP carrying the Indiana mutation (Games et al., 1995), PDAPP mice and littermates were on a hybrid background representing a combination of 3 strains: (1) Swiss–Webster, (2) C57BL/6J, and (3) DBA/2J. Five days prior to behavior testing, mice were individually housed. The 46-4-treated J20 and Wt mice reported here have previously been reported as a control for a separate immunization study that was conducted in parallel with the current study (Mably et al., 2015).

Exactly the same immunization paradigm was employed for all transgenic and Wt mice. Beginning at 9.5 months animals received 11 weekly intraperitoneal (i.p.) infusions of endotoxin-free antibody (250 μl of 1 mg/ml antibody in sterile PBS) (Fig. 1). To ensure that levels of circulating antibody were kept at a maximum, throughout behavioral testing mice received an additional 3 250 μg antibody injections (Fig. 1). Antibody infusions took place in the afternoon (2–5 pm). On days where behavioral testing had taken place, antibody infusions were carried out at least 2 h after training. Injections were administered by a person other than the investigator carrying out the behavioral testing. The investigator carrying out the behavioral testing was blind to the treatment groups. All animal procedures were approved by the Harvard Medical School Institutional Animal Care and Use Committee (Protocol number 04869).

2.2. Antibodies used for passive immunization studies

Three IgG_1 antibodies were used in this study: (i) monoclonal antibody (mAb) 1C22 which was raised in-house and preferentially

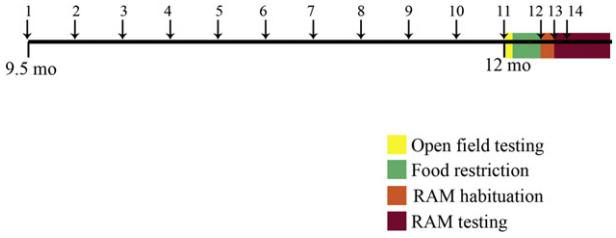

Fig. 1. Immunization paradigm and behavioral testing. Mice began receiving antibody injections (250 μg, i.p) at 9.5 months of age. Animals received one weekly antibody administration for 10 weeks, prior to behavioral assessment commencing at 12 months. Following the 11th antibody infusion testing in the open field arena took place. Five days later, mice received their 12th i.p infusion of antibody; the following day habituation to the RAM began. The 13th and 14th i.p infusions of antibody took place on the final day of RAM habituation and on the 2nd day of RAM training, respectively. All antibody injections were carried out in the afternoon (2–5 pm). Injections on days during behavioral testing were done at least 2 h after testing. Blood, CSF and brain samples were collected immediately following completion of behavioral testing.