Published in final edited form as:

J Neuroimmune Pharmacol. 2008 September ; 3(3): 187–197. doi:10.1007/s11481-008-9114-6.

Deglycosylated Anti-A β Antibody Dose–Response Effects on Pathology and Memory in APP Transgenic Mice

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These data were presented at the Society for Neuroscience 36th annual meeting, October 15th 2006, Atlanta, GA, USA.

Conflict of Interest Disclosure We do wish to declare a competing financial interest. Arnon Rosenthal and Jan Grimm were employees of Rinat Labs, Pfizer, and Jaume Pons is currently an employee of Rinat Labs, Pfizer. Rachel Karlnoski has consulted for Rinat Labs, Pfizer. Rinat holds the patents for the antibodies used in the studies presented here.

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Abstract

Anti-A β antibody administration to amyloid-depositing transgenic mice can reverse amyloid pathology and restore memory function. However, in old mice, these treatments also increase vascular leakage and promote formation of vascular amyloid deposits. Deglycosylated antibodies with reduced affinity for Fc γ receptors and complement are associated with reduced vascular amyloid and microhemorrhage while retaining amyloid-clearing and memory-enhancing properties of native intact antibodies. In the current experiment, we investigated the effect of 3, 10, or 30 mg/kg of deglycosylated antibody (D-2H6) on amyloid pathology and cognitive behavior in old Tg2576 mice. We found that low doses of deglycosylated antibody appear more efficacious than higher doses in reducing pathology and memory loss in amyloid precursor protein (APP) transgenic mice. These data suggest that excess antibody unbound to antigen can interfere with antibody-mediated A β clearance, possibly by saturating the FcRn antibody transporter.

Keywords

Alzheimer's disease; amyloid; angiopathy; microglia; transgenic mice; immunization; hemorrhage; A β ; immunotherapy; behavior

Introduction

Passive immunization with anti-A β antibodies has been shown to reduce A β load and reverse cognitive decline while increasing cerebral amyloid angiopathy (CAA) and microhemorrhage in transgenic models of amyloid deposition (Wilcock et al. 2004a, b, c; Racke et al. 2005; Pfeifer et al. 2002). Although the mechanism/s responsible for the redistribution of A β is unclear, we have recently discovered that deglycosylation of the Fc portion on the anti-A β antibody significantly reduces the severity of CAA and microhemorrhage compared to native antibodies when passively administered to Tg2576 mice, albeit with slightly lower clearance of parenchymal amyloid deposits (Wilcock et al. 2006a, b; Carty et al. 2006).

Fc γ receptor and complement activation are two important mechanisms that initiate phagocytosis by microglia and macrophages. We and others have shown evidence for microglial involvement in the removal of amyloid using both intracranial and systemic administration of anti-A β antibodies (Bacskaï et al. 2001; Chauhan et al. 2004; Wilcock et al. 2003, 2004a, b; Bard et al. 2000, 2003). Observations of reactive microglia surrounding blood vessels that are both highly burdened with A β plaque and positive for microhemorrhage following passive immunization with anti-A β antibodies suggest that activated microglia bind opsonized amyloid and migrate to the vasculature to dispose of the material (Wilcock et al. 2004b). This evidence suggests that activated microglia may exacerbate the CAA and microhemorrhage.

Deglycosylation of the carbohydrate side chains on the Fc portion of an anti-A β antibody greatly reduces its affinity for Fc γ receptors, particularly Fc γ RIIb and Fc γ RIIIa, on effector cells like microglia, yet the antibodies are as capable of binding to A β as the intact antibody (Ravetch 1997; Gessner et al. 1998). Deglycosylation also impairs the antibody's ability to bind complement (Winkelhake et al. 1980). Therefore, one explanation for our prior results is that, by deglycosylating the A β antibody, we are able to mitigate specific A β clearance mechanisms that contribute to the accumulation of CAA and microhemorrhage. An alternative, however, is that our prior observations are primarily due to a slower rate of A β clearance, which, by avoiding saturation of normal vascular efflux pathways, minimized the accumulation of vascular deposits. One means of resolving the qualitative (specific mechanism) versus quantitative (slower removal) explanations of the effects of antibody deglycosylation on vascular amyloid accumulation would be to increase the rate of amyloid removal with deglycosylated antibodies. If higher rates of amyloid removal by deglycosylated antibody increase vascular deposits like native antibodies, then the quantitative explanation would be the most likely explanation.

Here we examine the rate of A β clearance from the brain by using three doses of deglycosylated anti-A β antibody in old Tg2576 mice. Paradoxically, we found that long-term systemic administration of low doses of deglycosylated anti-A β antibody is more effective at reducing amyloid deposits and reversing cognitive deficits in old amyloid precursor protein (APP) transgenic mice when compared to animals treated with intermediate and higher doses of antibody.

Materials and methods

Experimental design

All procedures performed in this experiment were approved by the institutional animal care and use committee at the University of South Florida. APP Tg2576-derived (Hsiao et al. 1996) mice were bred in our facility at the University of South Florida and genotyped using previously described methods (Holcomb et al. 1998; Gordon et al. 2002). Importantly, we have intentionally bred out the retinal degeneration 1 mutation from this colony to avoid the inclusion of occasional mice that are blind due to homozygous inheritance of this mutation contributed by the SJL/J background (Alamed et al. 2006). Twenty Tg2576 mice (19 months of age) were assigned to one of four groups as follows: three groups received the deglycosylated antibody (D-2H6 A β _{35–40} IgG2b; Rinat Neurosciences, Palo Alto, CA, USA) at 3 mg/kg, 10 mg/kg, or 30 mg/kg in saline vehicle. The fourth group received a control IgG antibody against drosophila amnesiac protein (AMN) at a concentration of 10 mg/kg (mouse monoclonal anti-drosophila amnesiac protein IgG2b; Rinat Labs, Pfizer, Palo Alto, CA, USA). Each group had a sample size of five mice. All mice were given weekly intraperitoneal injections of the appropriate antibody and dose for 12 weeks. A fifth group of age-matched, nontransgenic mice was used as a control also.

Deglycosylation of 2H6

An IgG2b antibody generated against A β _{33–40} was deglycosylated by enzymatic removal of N-linked glycans by incubating at 37°C for 1 week with peptide-N-glycosidase F (QA-

Bio, San Mateo, CA, USA; 0.05 U/mg of antibody) in 20 mM Tris-HCl pH 8.0; 0.01% Tween. The deglycosylated antibody was purified by Protein A chromatography, and endotoxin was removed by Q-Sepharose. Completeness of deglycosylation was verified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and protein gel electrophoresis (Carty et al. 2006).

Behavioral paradigm

Following 3 months of treatment, the mice were subjected to a 2-day behavioral paradigm consisting of the radial-arm water maze paradigm (RAWM) followed by an open-pool, visible-platform task. The radial arm water maze task was performed as previously described (Wilcock et al. 2004a, b, c). Briefly, the maze contained 6 arms submerged in water radiating out of an open central area, with a hidden escape platform located at the end of one of the arms (Alamed et al. 2006). On day 1, 15 trials were performed in three blocks. The start arm was varied for each trial, with the goal arm remaining constant for both days. For the first 11 trials, the platform was alternately visible, then hidden below the surface of the water, and then remained hidden for the last four trials. Day 2 of testing was similar except that the platform was hidden for all trials. The number of errors (incorrect arm entries) was measured in a 1-min time frame. Mice failing to make an arm choice in 15 s were assigned one error. In order to minimize the influence of individual trial variability, each mouse's errors for three consecutive trials were averaged, producing five data points for each day which were analyzed statistically by analysis of variance (ANOVA) using StatView (SAS Institute, North Carolina). Next, the mice were run in a 1-day, open-pool task with a visible platform to test swimming ability and eyesight. Any mice demonstrating impaired ability to swim or to see were excluded from behavioral analyses. All mice displayed competent swimming ability and eyesight.

Enzyme-linked immunosorbent assay analysis of serum A β

Serum was diluted and incubated in 96-well microtiter plates (MaxiSorp; Nunc, Roskilde, Denmark), which were precoated with antibody 6E10 (Biosource, Camarillo, CA, USA) at 5 μ g/ml in phosphate-buffered saline buffer, pH 7.4. The detection antibody was biotinylated 4G8 (Signet, Dedham, MA, USA) at a 1:5,000 dilution. Detection was done using a streptavidin-horseradish peroxidase conjugate (Amersham Biosciences, Arlington Heights, IL, USA), followed by tetramethylbenzidine substrate (Sigma-Aldrich, St. Louis, MO, USA). Standard curves of A β ₁₋₄₀ (American Peptide, Sunnyvale, CA, USA) scaling from 6 to 400 pM were used. To our knowledge, the detection of serum A β is not affected by its presence in an immune complex with D-2H6, and the measurement reflects both free and antibody-bound A β . Enzyme-linked immunosorbent assay (ELISA) group averages for serum A β levels were analyzed using a one-way ANOVA followed by Fischer's least significant difference (LSD) means.

ELISA analysis of serum anti-A β antibodies

The anti-A β antibody was dissociated from endogenous A β in serum as described previously (Li et al. 2004). Briefly, serum was diluted in dissociation buffer (0.2 M glycine HCl and 1.5% bovine serum albumin, pH 2.5) and incubated at room temperature for 20 min. The sera were pipetted into the sample reservoir of a Microcon centrifugal device

(10,000 molecular weight cutoff; YM-10; Millipore, Bedford, MA, USA) and centrifuged at 8,000×g for 20 min at room temperature. The sample reservoir was then separated from the flow-through, placed inverted into a second tube, and centrifuged at 1,000×g for 3 min. The collected solution containing the antibody dissociated from the A β peptide was neutralized to pH 7.0 with 1 M Tris buffer, pH 9.5. The dissociated sera were assayed by ELISA for antibody titer. A β _{1–40} (Global Peptide, Fort Collins, CO, USA)-coated 96-well microtiter plates (Max-iSorp; Nunc) were incubated with dissociated serum samples. A biotinylated goat-anti mouse IgG (Vector Laboratories, Burlingame, CA, USA) at a 1:5,000 dilution followed by peroxidase-conjugated streptavidin (Amersham Biosciences, Piscataway, NJ, USA) was used to detect serum anti-A β binding activity. Group averages for circulating anti-A β antibody levels were analyzed using a one-way ANOVA followed by Fischer's LSD means.

Tissue preparation

On the day the mice were killed, they were overdosed with 100 mg/kg of Nembutal sodium solution (Abbott laboratories, North Chicago IL, USA). The mice were perfused intracardially with 25 ml of 0.9% saline. The right brain hemisphere was dissected and stored for later analysis. These dissections were rapidly frozen in dry ice and stored at –80°C. The left hemisphere was removed and immersion-fixed in freshly prepared 4% paraformaldehyde for 24 h, then passed through 10%, 20%, and 30% sucrose solutions for 24 h each.

Histology

Horizontal sections of 25 μ m thickness were collected using a sliding microtome and stored in Dulbecco's phosphate-buffered saline + azide. A series of eight sections spaced approximately 600 μ m apart were stained immunohistochemically for A β (6E10 mouse monoclonal anti-A β , Biosource; 1:30,000) to determine the degree of A β removal and for CD45 (rat monoclonal anti-CD45, Serotec, Raleigh, NC, USA; 1:5,000) to determine the extent of microglial activation. A series of tissue sections 600 μ m apart were stained using 0.2% Congo red solution in NaCl-saturated 80% ethanol. Another set of sections was stained for hemosiderin using 2% potassium ferrocyanide in 2% hydrochloric acid (Prussian Blue) for 15 min followed by a counterstain in a 1% neutral red solution for 10 min.

Quantification of Congo red, CD45, and A β staining were performed using the Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA) software to analyze the percent area occupied by positive stain. One region of the frontal cortex and three regions of the hippocampus were analyzed (to ensure that there was no regional bias in the hippocampal values). For Congo red, both parenchymal and vascular positive stain was included in the "total" Congo red value. Subsequently, parenchymal amyloid deposits were manually deselected to yield a percent area restricted to vascular Congo red staining (Wilcock et al. 2006a, b). To estimate the parenchymal area of Congo red, we subtracted the vascular Congo red values from the total Congo red values. For the hemosiderin staining, the numbers of Prussian-blue-positive deposits were counted over the cortex and hippocampus on all sections, and the average number of hemosiderin deposits per section was calculated. To assess possible treatment-related differences in pathology, the histochemical values for each

treatment group were analyzed by one-way ANOVA followed by Fischer's LSD means comparisons using StatView (SAS Institute).

Results

To study the dose response of deglycosylated antibodies on fibrillar and diffuse A β removal, cerebral amyloid angiopathy, microhemorrhage, and cognition, we used transgenic mice containing a double mutation, K670N and M671L, in the human APP gene under the control of a hamster prion promoter (Hsiao et al. 1996). We passively immunized 19-month-old APP mice with deglycosylated C-terminal (aa35–40) anti-A β antibodies (D-2H6) for 12 weeks with one of the following doses; 3, 10, or 30 mg/kg. Each treatment group had five mice that successfully underwent testing for behavioral, biochemical, and neuropathological tests.

Dose-dependent increases in serum A β and circulating anti-A β antibody titer

Antibody titer in the final serum bleeds displayed a dose-dependent response where the highest dose of deglycosylated antibody, 30 mg/kg, showed the greatest anti-A β IgG titer at approximately 2,500 nM, while the 10 mg/kg dose was intermediate at 1,500 nM, and the 3 mg/kg dose had the lowest titer at 250 nM (Fig. 1a). Anti-A β antibodies were not detected in the APP transgenic mice treated with the control antibody or in the nontransgenic mice. Similar to antibody titer, serum levels of circulating total A β displayed a dose-dependent response, with total serum A β increasing in parallel with increasing doses of deglycosylated antibody (Fig. 1b).

Reversal of cognitive deficits with low doses of deglycosylated anti-A β antibodies

After 12 weeks of antibody treatment, behavioral analysis using the RAWM showed spatial reference memory deficits in the control APP transgenic group treated with the anti-drosophila amnesiac antibody when compared to the non-transgenic mice (Fig. 2). The control APP transgenic group performed the maze with the greatest number of errors, and the non-transgenic mice performed with the least errors. On the second day of testing, the mice treated with 3 mg/kg of deglycosylated antibody performed equivalently to the nontransgenic mice with performance errors near 0.5 per trial (Fig. 2). The APP transgenic mice treated with 10 or 30 mg/kg doses of deglycosylated antibody performed at an intermediate level between the nontransgenic mice and APP transgenic mice treated with control antibody and were not significantly different from either group.

Total A β immunoreactivity is reduced following treatment with deglycosylated antibodies

A β immunostaining detects both compact and diffuse amyloid deposits. In old APP transgenic mice, the pattern of total A β immunoreactivity resembles that of human Alzheimer's disease pathology. In the frontal cortex, we observed a typical pattern of diffuse A β staining, as well as compact, intensely stained deposits that were positive for amyloid when stained with Congo red or thioflavine-S (Fig. 3a). In the hippocampus, A β deposits were concentrated around the hippocampal fissure and the CA1 region (Fig. 3b), with fewer deposits throughout the remainder of the hippocampus. The appearance of A β immunoreactivity after administration of different doses of deglycosylated antibody was

presented in the frontal cortex (Fig. 3c, e, g) and hippocampus (Fig. 3d, f, h). Quantification of the percent area occupied by positive staining showed a reverse dose response trend where the lowest dose significantly reduced total A β in the frontal cortex by 48%, while the intermediate dose and high doses were less effective at reducing A β (Fig. 4). A similar trend was observed in the hippocampus, although the reductions caused by the lowest dose were not significant (Fig. 4).

Deglycosylated anti-A β immunization reduces fibrillar A β

Congo red binds preferentially to the beta-pleated sheet conformation of fibrillar A β and is a standard method for detecting compact amyloid plaques. Congo red histology in the frontal cortex of mice treated with deglycosylated antibody is presented in Fig. 5. Both the quantity and size of fibrillar, compact plaques in the parenchyma can be appreciated. In many fields, an apparent increase in vascular deposits could also be observed and are indicated with arrows in Fig. 5. Quantification of the total area occupied by Congo red stain revealed significant reductions with both the 3 and 10 mg/kg doses in the frontal cortex (Fig. 6a). Quantification of the parenchymal amyloid deposits showed that the 3-mg/kg dose resulted in the greatest reduction of compact plaque by approximately 70%, followed by the intermediate and highest doses of 10 and 30 mg/kg with a 40% reduction (Fig. 6b). There was an increase in vascular amyloid in both regions (Fig. 6c). Compared to the control group, all three doses of deglycosylated antibody resulted in a significant elevation of vascular Congo red, albeit the vascular deposits were still considerably fewer than the parenchymal amyloid deposits. It is important to recognize that variability is high when the fractional stained areas are as low as found for vascular deposits in this experiment (less than 0.25% of area). The 30 mg/kg dose increased CAA to the same extent as the 3 mg/kg dose without producing a comparable reduction in compact amyloid.

Deglycosylated antibody administration does not cause an increase in microhemorrhage

Prussian blue histochemistry is one classic method for demonstrating iron in tissues. Hemosiderin (iron storage granules) may be present in areas of hemorrhage or may be deposited in tissues with iron overload. After 3 months of passive immunization with a deglycosylated C-terminal antibody, the average number of positive Prussian blue profiles per section was less than one for all antibody doses, despite the increase in vascular amyloid (Fig. 7). For comparison purposes, we have included in Fig. 7 our prior data obtained with the native antibody (2H6) at 10 mg/kg, which resulted in greater than three positive profiles per section (Wilcock et al. 2006a, b).

Microglial activation was measured with CD45 immunohistochemistry. CD45 is a protein tyrosine phosphatase that is expressed when microglia are activated. CD45 positive microglia are observed surrounding compact, Congo-red-positive amyloid plaques. One method of microglial activation is through the Fc γ receptor, a method that is greatly diminished with the deglycosylated antibody. In a time study conducted by Wilcock et al. (2004b), CD45 expression after passive administration of intact C-terminal anti-A β antibodies showed that CD45 expression is significantly up-regulated at the 1- and 2-month time point and returns to baseline at the 3-month time point. In this study, CD45 immunoreactivity showed no differences between the three doses of deglycosylated antibody

and the control group, which confirms that the deglycosylated antibody is not activating microglia at the 3-month time point (Table 1).

Discussion

We have previously shown that deglycosylation of an anti-A β antibody retains the antibody's ability to reduce parenchymal amyloid deposits, to reverse cognitive deficits, and to reduce the potentially adverse changes such as microglial activation, CAA, and microhemorrhage (Wilcock et al. 2006a, b; Carty et al. 2006). The data presented in the current experiment suggest that peripheral administration of high doses (>10 mg/kg) of deglycosylated anti-A β antibodies in old APP transgenic mice are not as effective at reducing A β or reversing cognitive deficits as lower doses. It is noted that the results at 10 mg/kg in the present study are somewhat less than we reported previously with this same antibody dose (Wilcock et al. 2006a, b). Possible explanations for this difference are the 30% greater duration of antibody administration in the prior study and/or small lot-to-lot variations in antibody activity, just as there are for enzyme activities.

In the present study, the lowest dose (3 mg/kg) of D-2H6 reduced diffuse A β and fibrillar A β and reversed cognitive deficits to the greatest extent compared to higher doses of D-2H6 or control IgG. Similar results were presented by Gitter et al. (2002) at a scientific meeting. In their work, a dose response study using an intact mid-domain antibody administered for 5 months to PDAPP mice showed that the highest dose failed to reduce A β plaque burden and, at the same time, sequestered the most peripheral A β in the plasma, while the lower doses sequestered less A β in the serum and reduced total plaque burden to a greater extent. Similar to our study, plasma A β and IgG levels showed a dose-response relationship where the highest dose of antibody sequestered the highest levels of plasma A β . However, in this study as well as in Gitter's study, peripheral A β sequestration did not correlate to reductions in brain A β levels.

If the peripheral sink mechanism were playing a major role in A β clearance from the brain, one would predict the greatest reductions in A β would occur with the highest dose. One possible explanation for this discordance is that much of the increase in plasma A β results from retarded A β degradation due to antibody sequestration rather than simply increased clearance from the brain. Previous studies have established that A β has a very short half-life in the plasma. When free A β is injected intravenously into mice, it is cleared within 10 min (Ghisso et al. 2004). However, peripheral administration of anti-A β antibodies creates a stable mAb:A β complex in the plasma, and the complex is cleared slowly. The antibody-bound A β has a half-life of 5–7 days (Levites et al. 2006). Thus, the rapid rise in plasma A β observed is attributable to prolongation of the half-life of A β bound to antibody rather than increased efflux of A β from the brain. This phenomenon may be unique to APP transgenic mice since the over-expression of APP and the accumulation of A β are not specific to the central nervous system but also occur in several organ systems (Kawarabayashi et al. 2001). APP transgenic mice have higher endogenous circulating A β levels than humans (Kawarabayashi et al. 2001). Therefore, one might not observe the spike in plasma A β following immunization in humans, as reported by Hock et al. (2003).

Deglycosylation of anti-A β antibodies significantly reduces the affinity of the antibody for Fc γ receptors I, II, and III located on effector cells and the complement cascade initiator, C1q (Carty et al. 2006). Immunohistochemical analyses of APP brain sections after intracranial injections of intact or deglycosylated antibody confirmed that deglycosylation does not activate microglia as measured with antibodies against Fc γ RII/III and CD45 when compared to mice treated with control or native intact anti-A β IgG (Carty et al. 2006). In addition to intracranial studies, our lab performed a direct comparison of native and deglycosylated anti-A β antibodies in a long-term systemic study. After 4 months of treatment, reversal of cognitive deficits and reductions in fibrillar and diffuse A β were found with both the deglycosylated and intact antibody, although the deglycosylated antibody appeared slightly less active. However, the incidence of CAA and microhemorrhage were significantly reduced with the deglycosylated antibody compared to its native counterpart (Wilcock et al. 2006a, b). The less significant CAA caused by the deglycosylated antibody may be due to the catalytic disaggregation of A β fibrils or the neutralization of A β oligomers resulting in a sudden excess of free A β which may saturate normal drainage pathways (Solomon et al. 1997; Klyubin et al. 2005).

Although deglycosylation reduces the affinity of IgG for Fc γ receptors, the interaction of immunoglobulin with neonatal Fc receptor (FcRn), also known as the Fc transport receptor (FcTR), is not affected by the removal of carbohydrate side chains (Hobbs et al. 1992). The FcRn is structurally and functionally distinct from the Fc γ receptors (Ravetch and Bolland 2001; Brambell et al. 1964). The functional roles of the FcRn are to recycle IgG and transport IgG bidirectionally across epithelial barriers (Lencer and Blumberg 2005).

FcRn expression is found at the brain microvasculature and choroid plexus epithelium on the blood brain barrier (Schlachetzki et al. 2002). The expression of the FcRn in the blood brain barrier may mediate the 'reverse transcytosis' of IgG and immune complexes (ICs) in the brain-to-blood direction (Schlachetzki et al. 2002). In a study by Banks et al. (2002), anti-A β antibodies and albumin showed similar influx rates into the brain for the first hour after i.v. injection. However, in later time points, the influx rate of anti-A β antibody was significantly reduced to less than 1%. The reduction in influx rate was caused by antibody efflux mechanisms. In order for new antibody to enter the brain, antibody in the brain must depart. Zhang and Pardridge (2001) tested brain efflux mechanisms of IgG with intracranial injections of radiolabeled IgG2a and found that efflux mechanisms were saturated with the addition of excess Fc fragments or intact IgG molecules, resulting in complete suppression of efflux mechanisms. However, efflux mechanisms were not inhibited by high concentrations of F(ab)₂ fragments or albumin (Zhang and Pardridge 2001). These findings support the fact that the receptors necessary for IgG transport require the Fc portion on IgG to be active. Furthermore, Deane et al. (2005) found that the FcRn transport system is the main mechanism mediating the transcytosis of A β -anti-A β ICs from the brain to blood in old Tg2576 mice. This group also found that antibody-mediated A β clearance from the brain is abolished in old FcRn^{-/-} mice. Similar to Zhang and Pardridge, Deane and colleagues demonstrated that the addition of excess IgG inhibited the clearance of immune complexes from the brain. Therefore, high concentrations of immunoglobulin would saturate the FcRn receptors with antibody not bound to antigen, hinder the FcRn binding of A β -anti-A β immune complexes, and ultimately result in the inhibition of A β clearance from the brain.

The saturation of FcRn may explain why high doses of deglycosylated antibody are not as effective at reducing diffuse and fibrillar A β compared to antibodies administered at lower doses. The saturation of this efflux system may also result in A β building up along the brain microvasculature and lead to the formation of CAA. Ironically, our original purpose for conducting this study, to contrast qualitative versus quantitative explanations for the benefits of deglycosylation, could not be addressed because the high doses of antibody failed to clear more amyloid.

In summary, we have shown that the efficacy of passively administered deglycosylated antibodies depends upon drug dose. Our experiment demonstrated that the lowest dose of deglycosylated anti-A β antibody was the most efficacious at reducing total A β , fibrillar A β , and reversing cognitive deficits when tested in old APP transgenic mice. Antibody influx and efflux mechanisms may play a major role in A β clearance and in the efficacy of an antibody. Saturation of these mechanisms with uncomplexed antibody may greatly reduce antibody efficacy, emphasizing the requirement for precise titration of dose–response characteristics in human trials of anti A β immunotherapy.

Acknowledgments

This work was supported by National Institutes of Aging/NIH grants AG15490 (MNG), AG18478 (DM). RK is the Thorne Scholar in Alzheimer Research.

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Fig. 1.

Dose-related increases in serum levels of A β and anti-A β antibody after deglycosylated anti-A β antibody administration. **a** Average amounts of circulating A β in sera; **b** average amounts of circulating anti-A β antibodies 24 h following the final antibody injection in APP transgenic mice receiving either control antibody (*Cont*) or anti-A β antibody at doses of 3, 10, or 30 mg/kg. Nontransgenic (*NTg*) mice received no treatment. ** $P < 0.001$ compared to APP mice given control antibody injections

Fig. 2.

Low doses of deglycosylated antibody improve cognition after 12 weeks of treatment. Radial arm water maze performance is plotted as the mean number of errors per block of three trials over a 2-day trial period following 3 months of immunization with deglycosylated anti-A β . The graph shows the average number of errors for APP transgenic mice treated with either 3 mg/kg deglycosylated anti-A β antibody (*open circle, dashed line*), 10 mg/kg deglycosylated 2H6 anti-A β antibody (*open square, dashed line*), 30 mg/kg deglycosylated anti-A β antibody (*open triangle, dashed line*), control anti-AMN antibody (*closed square, solid line*), and nontransgenic mice (*closed circle, solid line*). * $P < 0.05$ between the 3 mg/kg treated group and the control IgG-treated group

Fig. 3. Total A β immunoreactivity is reduced after systemic administration of anti-A β antibodies. **a–h** A β immunohistochemistry from APP transgenic mice in the frontal cortex (**a**, **c**, **e**, and **g**) and CA1 region of the hippocampus (**b**, **d**, **f**, and **h**). Mice were treated with control antibody (anti-AMN 2908; **a** and **b**), 3 mg/kg deglycosylated anti-A β antibody (**c** and **d**), 10 mg/kg deglycosylated 2H6 anti-A β antibody (**e** and **f**), or 30 mg/kg deglycosylated anti-A β antibody (**g** and **h**). Both the number of parenchymal deposits and their intensity appear less

after immunother-apy. *Scale bar* in **a**=120 μ m for all panels. *CA1* CA1 pyramidal cell layer;
F fissure

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Fig. 4.

Total Aβ immunoreactivity is significantly reduced in the frontal cortex after systemic treatment with the 3 mg/kg dose of deglycosylated antibody. The graph shows the quantification of percent area of total Aβ staining in the frontal cortex (a) and hippocampus (b) after 12 weeks of treatment with control IgG (*CTRL*), 3 mg/kg, 10 mg/kg, and 30 mg/kg doses of deglycosylated anti-Aβ antibody. * $P < 0.05$ when compared to mice treated with control antibody

Fig. 5. Congo red staining in the frontal cortex after immunotherapy. **a** Anti-AMN control antibody; **b** 30 mg/kg dose D-2H6 anti-A β antibody; **c** 10 mg/kg; **d** 3 mg/kg dose. Scale bar in **a**=120 μ m. Arrows shown in **b**, **c**, and **d** indicate vessels that are positively stained for CAA

Fig. 6.

Lower doses of deglycosylated antibody cause greater decreases in fibrillar A β . **a** Quantification of percent area of total Congo red staining in the frontal cortex and hippocampus after 12 weeks of anti-A β antibody passive immunization in frontal cortex (*solid bars*) and hippocampus (*open bars*). **b** Quantification of parenchymal Congo red in the frontal cortex (*solid bars*) and hippocampus (*open bars*). **c** Quantification of vascular Congo red in the frontal cortex (*solid bars*) and hippocampus (*open bars*). * $P < 0.05$, ** $P < .001$ compared with control antibody

Fig. 7.

Incidence of microhemorrhage is not changed with any dose of deglycosylated antibody. The graph shows quantification of positive Prussian blue profiles per section in APP transgenic mice treated with control anti-AMN IgG, 3, 10, or 30 mg/kg doses of deglycosylated antibody for 3 months (mean±SEM). Incidence of microhemorrhage after intact 2H6 antibody at 10 mg/kg observed by Wilcock et al. (2006a, b) was much higher

Table 1

Percent area of CD45 expression on microglia determined by immunohistochemistry in APP transgenic mice following 3 months of passive immunization

Treatment	Anterior cortex	Hippocampus
Control anti-AMN antibody	2.9±0.2	2.7±0.6
3 mg/kg of D-2H6	2.5±0.4	2.9±0.3
10 mg/kg of D-2H6	2.7±0.1	2.5±0.2
30 mg/kg of D-2H6	2.3±0.3	2.3±0.2

Values are mean±SEM 1