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Intracranial Adeno-Associated Virus-Mediated Delivery of Anti-Pan Amyloid β, Amyloid β40, and Amyloid β42 Single-Chain Variable Fragments Attenuates Plaque Pathology in Amyloid Precursor Protein Mice

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Accumulation of amyloid β protein (Aβ) aggregates is hypothesized to trigger a pathological cascade that causes Alzheimer's disease (AD). Active or passive immunizations targeting Aβ are therefore of great interest as potential therapeutic strategies. We have evaluated the use of recombinant anti-Aβ single-chain variable fragments (scFvs) as a potentially safer form of anti-Aβ immunotherapy. We have generated and characterized three anti-Aβ scFvs that recognize Aβ1–16, Aβx-40, or Aβx-42. To achieve widespread brain delivery, constructs expressing these anti-Aβ scFvs were packaged into adeno-associated virus (AAV) vectors and injected into the ventricles of postnatal day 0 (P0) amyloid precursor protein CRND8-transgenic mice. Intracranial delivery of AAV to neonatal mice resulted in widespread neuronal delivery. *In situ* expression of each of the anti-Aβ scFvs after intracerebroventricular AAV serotype 1 delivery to P0 pups decreased Aβ deposition by 25–50%. These data suggest that intracranial anti-Aβ scFv expression is an effective strategy to attenuate amyloid deposition. As opposed to transgenic approaches, these studies also establish a "somatic brain transgenic" paradigm to rapidly and cost-effectively evaluate potential modifiers of AD-like pathology in AD mouse models.

Key words: **single-chain variable fragments; immunotherapy; Alzheimer's disease; adeno-associated virus; amyloid; Aβ**

Introduction

Numerous studies of Alzheimer's disease (AD) support the hypothesis that cerebral accumulation of amyloid β protein (Aβ) triggers pathological changes leading to cognitive dysfunction. Strategies to prevent Aβ aggregation by reducing Aβ levels or targeting pathogenic Aβ aggregates are being developed (Golde, 2003). Aβ immunotherapies are potential AD therapeutics that could work by reducing Aβ levels, enhancing clearance of Aβ aggregates, neutralizing toxic aggregates, or combining some of these mechanisms. In amyloid precursor protein (APP) transgenic mice, active Aβ immunization and passive immunization with anti-Aβ monoclonal antibodies (mAbs) reduces cerebral Aβ deposition, neuritic dystrophy, and gliosis and improves cognitive deficits (Schenk et al., 1999; Bard et al., 2000; Bennett and Holtzman, 2005). A clinical trial using aggregated Aβ1–42 in combination with QS-21 adjuvant was halted when ~6% of the vaccinated subjects developed meningoencephalitis (Orgogozo et al., 2003; Gilman et al., 2005). However, patients who generated anti-Aβ antibodies had reduced cerebrospinal levels of tau and showed a slower cognitive decline (Gilman et al., 2005; Masliah et al., 2005). Because passive immunization with anti-Aβ antibodies is theoretically safer than active vaccination with Aβ peptides, several humanized anti-Aβ mAbs are currently being evaluated in early-phase clinical trials.

Studies have suggested that to reduce Aβ deposition, it is not necessary to use intact anti-Aβ antibodies (Bacskai et al., 2002; Matsuoka et al., 2003; Wilcock et al., 2003; Tamura et al., 2005). It may be possible to avoid potential side effects simply by targeting Aβ with high-affinity binding agents that lack immune effector functions. We have identified multiple anti-Aβ mAbs that are effective as passive immunogens in APP mice (Levites et al., 2006a) and generated recombinant single-chain variable fragments (scFvs) from the hybridomas expressing these anti-Aβ mAbs. We have injected adeno-associated virus serotype 1 (AAV1) vectors encoding anti-Aβ40-specific, anti-Aβ42-specific, and anti-pan-Aβ scFvs into neonatal CRND8 mice brains and examined their effects on Aβ levels and plaque deposition. Such studies establish a novel rapid, flexible, and cost-effective paradigm for evaluating potential gene- or protein-based modifiers of pathology in AD mouse models.

Materials and Methods

AAV construction and preparation

AAV was prepared by standard methods. Briefly, AAV vectors expressing the scFv under the control of the cytomegalovirus enhancer/chicken β-actin promoter, a woodchuck post-transcriptional regulatory element,

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and the bovine growth hormone, poly(A), were generated by plasmid transfection with helper plasmids in HEK293T cells. Forty-eight hours after transfection, the cells were harvested and lysed in the presence of 0.5% sodium deoxycholate and 50 U/ml Benzonase (Sigma, St. Louis, MO) by freeze thawing, and the virus was isolated using a discontinuous iodixanol gradient and affinity purified on a HiTrap HQ column (Amersham Biosciences, Arlington Heights, IL). The genomic titer of each virus was determined by quantitative PCR.

Mice

All animal husbandry procedures performed were approved by the Mayo Clinic Institutional Animal Care and Use Committee in accordance with National Institutes of Health guidelines under protocol A29803. To generate CRND8 mice, male CRND8 mice containing a double mutation in the human APP gene (KM670/671NL and V717F) (Chishti et al., 2001) were mated with female B6C3F1/Tac mice that were obtained from Taconic (Germantown, NY). Genotyping of Tg2576 and CRND8 mice was performed by PCR as described previously (Levites et al., 2006a). All animals were housed three to five to a cage and maintained on *ad libitum* food and water with a 12 h light/dark cycle.

mRNA isolation, cDNA synthesis, amplification of cDNAs encoding variable heavy and variable light regions, and construction of scFvs

mRNA was isolated from hybridoma cell lines using an mRNA isolation kit (Qiagen, Chatsworth, CA). cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) and random hexamers. The cDNA was then poly(G)-tailed with terminal transferase (New England Biolabs, Beverly, MA). cDNAs encoding the variable heavy (V_H) and variable light (V_L) chains were amplified using anchor PCR with a forward poly(C) anchor primer and a reverse primer specific for a constant region sequence of IgG2a (for pan Ab) and IgG1 for Ab40.1 and Ab42.2, as described by Gilliland et al. (1996). PCR products were then sequenced using the same primers, and the consensus V_H and V_L subunits were determined. cDNAs encoding scFvs of three anti-Aβ antibodies were constructed by ligating the V_H and V_L cDNAs in V_H-linker-V_L orientation separated by a Gly_4Ser_3 linker. Nonspecific scFv (scFv ns) was randomly obtained from a phage library (Medical Research Council, Cambridge, UK) and showed no affinity to Aβ.

Fibrillar Aβ pulldown assays

One milliliter of conditioned media from HEK293T cells transiently transfected with pSecTag plasmids encoding the anti-Aβ scFv was incubated with 10 μg of fibrillar Aβ40 or Aβ42 [fibrillar Aβ (fAβ)] at 4°C for 1 h. The fibrils were then spun down and resuspended in SDS-PAGE loading buffer. The presence of scFv was determined by Western blot with rabbit anti-His (Bethyl Laboratories, Montgomery, TX). To determine the Aβ40 binding properties of scFv secreted into the media, capture ELISA was used with Aβ40 peptide as capture and anti-c-myc-HRP (1:2000; Invitrogen, San Diego, CA) as detection.

Neonatal injections. The following procedure was adapted from Passini and Wolfe (2001). Briefly, postnatal day 0 (P0) pups were cryoanesthetized on ice for 5 min. Two microliters of AAV–scFv were injected intracerebroventricularly into both hemispheres using a 10 ml Hamilton syringe with a 30 gauge needle. The pups were then placed on a heating pad with their original nesting material for 3–5 min and returned to their mother for further recovery.

Analysis of Aβ in the brain. The following antibodies against Aβ were used in the sandwich capture ELISA: for brain Aβ40, Ab9 capture with Ab40.1-HRP detection; for brain Aβ42, Ab42.2 capture with Ab9-HRP detection. Biochemical Aβ analysis and immunohistochemical analyses were performed as described by Levites et al. (2006a).

Measurement of scFv levels in the brain and Aβ–scFv complex in the plasma. The levels of scFv expressed in the brain were evaluated after immunoprecipitation with anti-His antibody from a radioimmunoprecipitation assay (RIPA) brain extract, followed by Western blotting and detection with anti-c-myc antibody. A recombinant control protein, Positope (Invitrogen), with the c-myc tag was used as a standard to calculate the amount of c-myc-tagged scFv. Immunoprecipitation efficacy of ~40% was determined by spiking a noninjected brain lysate with 100 μl of conditioned media from scFv-transfected cells and comparing the amount of scFv immunoprecipitated with the amount present in the original media. Densitometric analysis of the c-Myc-positive bends was performed using Odyssey software version 1.2. To measure the Aβ–scFv complex in the plasma, ELISA was performed with a mAb against the free end of an Aβ peptide as capture (for scFv9, mAb40.1; for scFv40.1 and scFv42.2, mAb9) and anti-c-myc–HRP as detection.

Statistical analysis

One-way ANOVA followed by the Dunnett's multiple-comparison tests were performed using the scientific statistic software GraphPad Prism (version 4; Graph Pad, San Diego, CA).

Results

Construction and characterization of the scFvs

scFvs were cloned from hybridomas expressing an anti-Aβ1–16 mAb9 (IgG2ak), anti-Aβ40-specific mAb40.1 (IgG1k), and anti-Aβ42-specific mAb42.2 (IgG1k). The parent antibodies show high specificity for Aβ, recognize amyloid plaques, and effectively attenuate amyloid deposition when administered to young Tg2576 mice (Levites et al., 2006a). The amino-acid sequences of scFv9, scFv40.1, scFv42.2 (derived from anti-Aβ1–16 mAb9, Aβx-40-specifc mAb40.1, and anti-Aβx-42-specific mAb42.2, respectively) are shown in Figure 1*A* along with a non-Aβ-binding scFv ns used as a control.

Before testing the effects of the scFv *in vivo*, we characterized the anti-Aβ scFvs expressed from HEK293T cells. Anti-Aβ scFvs are detected both in the 1% Triton cell lysate and in the conditioned media after transient transfection (Fig. 1*B*). The ~28 kDa band detected on an SDS-PAGE gel with an anti-His antibody represents monomeric scFvs secreted from the cells. ScFvs can also be visualized in the cell by immunocytochemistry with an anti-6XHis antibody (data not shown). To show that the scFvs bind Aβ, we used a fAβ pulldown assay (see Materials and Methods). After fAβ42 pulldown, an ~29 kDa band was detected from the conditioned media of cells transfected with scFv9 and scFv42.2 but not scFv40.1, whereas after fAβ40 pulldown, a 29 kDa band is detected from the conditioned media of cells transfected with scFv9 and scFv40.1 but not scFv42.2 (Fig. 1*C*). In addition, when conditioned media was loaded on an Aβ40-coated ELISA plate and the bound scFv was detected with HRP-conjugated anti-myc antibody, the media from scFv9- and scFv40.1-transfected cells gave a significant signal. When the same media was administered to an ELISA plate coated with Aβ42, a significant signal was only seen from scFv9- and scFv42.2-secreting cells (Fig. 1*D*), confirming the pulldown data. These scFvs were also able to detect amyloid plaques on paraffin sections from brains of old Tg2576 mice (Fig. 1*E*). Collectively, these data demonstrate that the three anti-Aβ scFvs maintain the binding properties of the parent mAbs.

Intracranial expression of green fluorescent protein and anti-Aβ scFv using AAV1 transduction of the neonatal brain

Injection of AAV1 into the cerebral ventricles of newborn mouse pups has been reported to result in widespread neuronal transduction and life-long expression of the packaged gene (Passini et al., 2003). We therefore bilaterally injected into the cerebral lateral ventricles of P0 Swiss-Webster mice, AAV1 encoding humanized green fluorescent protein (hGFP) (2×10^{10} genome particles/ventricle). GFP expression was detected by green fluorescence at 3 weeks and 10 months after injection (Fig. 2*A*). The most striking expression was seen in the neuronal cell layers of hippocampal CA1 to CA3 region, choroid plexus, and ependymal cells lining the ventricle. hGFP-positive signals were also detected

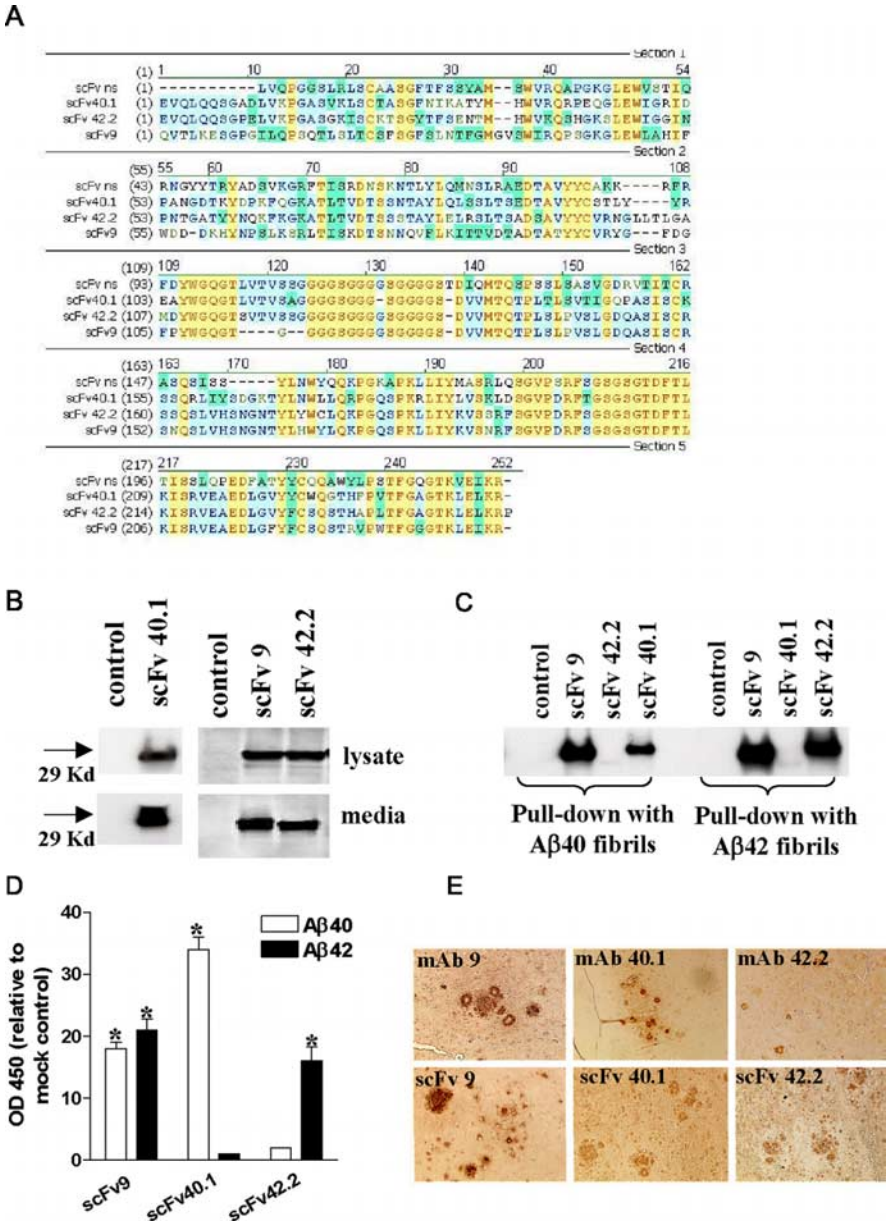

Figure 1. Expression and binding properties of anti-Aβ scFvs. HEK293T cells were transiently transfected with scFv9, scFv40.1, and scFv42.2 in pSecTag. ***A***, The sequence alignment of the anti-Aβ scFvs. ***B***, Western blot of a 1% Triton lysate and conditioned media, detected with anti-His primary antibody and anti-rabbit-HRP secondary antibody, showing expression of the anti-Aβ scFvs. ***C***, Western blot of a pull down of conditioned media with fAβ, detected with anti-His primary antibody and anti-rabbit-HRP secondary antibody, showing that the anti-Aβ scFvs maintain the binding selectivity of the parent antibodies. ***D***, Conditioned media from scFv9-, scFv40.1-, and scFv42.2-transfected cells was tested in an ELISA with Aβ40 or Aβ42 as capture and anti-c-myc–HRP as detection. *$p < 0.01$ versus control. ***E***, Paraffin sections of Tg2576 mice brains were stained with conditioned media from scFv-transfected cells (bottom) and anti-His primary antibody or with a corresponding parent anti-Aβ mAb (top). Representative plaque staining is shown. Magnification, 200×.

in the periventricular areas and the frontal cortex. Injection of 10-fold higher titers of AAV1–hGFP (total 4×10^{11} genome particles) resulted in localized green fluorescence in the choroid plexus and in a single layer of cells around the ventricle (data not shown). In pups injected at P1 or P2, the transduction of AAV1, as visualized by hGFP expression, was dramatically reduced with expression localized to the periventricular region (data not shown). GFP expression is more readily detected in neuronal cell bodies 3 weeks after injection but redistributes into neuronal processes by 10 months of age. No toxic side effects or mortalities after the operation were observed in CRND8 mice injected with AAV1–hGFP at any stage of the experiment.

Having confirmed the ability of AAV1 to mediate widespread delivery of a transgene to P0 mouse pups, we injected newborn P0 CRND8 mice as well as nontransgenic littermates with AAV1 vectors encoding the various anti-Aβ scFvs (2×10^{10} genome particles/ventricle). Three weeks after the injection, scFv expression was detected by immunohistochemistry with anti-His antibody throughout the brain (Fig. 2*B*). The distribution of each anti-Aβ scFv was similar to each other and to hGFP, thus demonstrating that widespread delivery of the transgene was achieved using AAV1 vectors. Cell body staining was noted despite scFv being a secreted protein; a general increase in the background was also observed, possibly attributable to secreted scFv or scFv present in neuronal processes. The amount of anti-Aβ scFv expressed in the brain at steady state was determined in the nontransgenic littermates 3 months after injection, as described in Materials and Methods. The levels of scFv9, scFv40.1, and scFv42.2 were 1.1 ± 0.42, 2.4 ± 0.9, and 2.0 ± 0.2 pmol/g, respectively.

Anti-Aβ scFv reduces Aβ deposition in CRND8 mice

Initial studies were performed with the anti-pan Aβ scFv9 and the anti-Aβ42-specific scFv42.2. Control mice were injected with AAV1–hGFP. After the P0 injection, CRND8 mice were killed at 5 months, and Aβ levels were analyzed in the brain. Both anti-Aβ scFvs significantly attenuated Aβ40 and Aβ42 levels in SDS-soluble and SDS-insoluble, formic acid (FA)-soluble extracts (Fig. 3). ScFv9 and scFv42.2 reduced SDS and FA Aβ40 and Aβ42, respectively, and appeared to decrease immunoreactive Aβ loads as well (Fig. 3). A second, more complete study was then conducted in CRND8 mice. After P0 injection of AAV expressing scFv9, scFv42.2, and scFv40.1, brain Aβ levels were analyzed in CRND8 mice at 3 months of age. In addition to a PBS injection control, we also used an AAV1 expressing a scFv ns, which has no affinity to Aβ as an additional control group. Aβ levels in scFv ns-treated mice were not significantly different from control mice injected with PBS (Fig. 4*B*,*C*). In all scFv-treated mice, plaque loads were decreased significantly (Fig. 4*A*,*B*). Aβ40 and Aβ42 levels in the SDS-soluble fraction were also reduced significantly by all scFvs (Fig. 4*C*) as follows: scFv9 (25 and 20% reduction in Aβ40 and Aβ42, respectively); scFv40.1 (40% reduction in both Aβ40 and Aβ42); and scFV42.2 (30 and 20% reduction in Aβ40 and Aβ42, respectively). The largest effect was demonstrated by scFv40.1, which was possibly

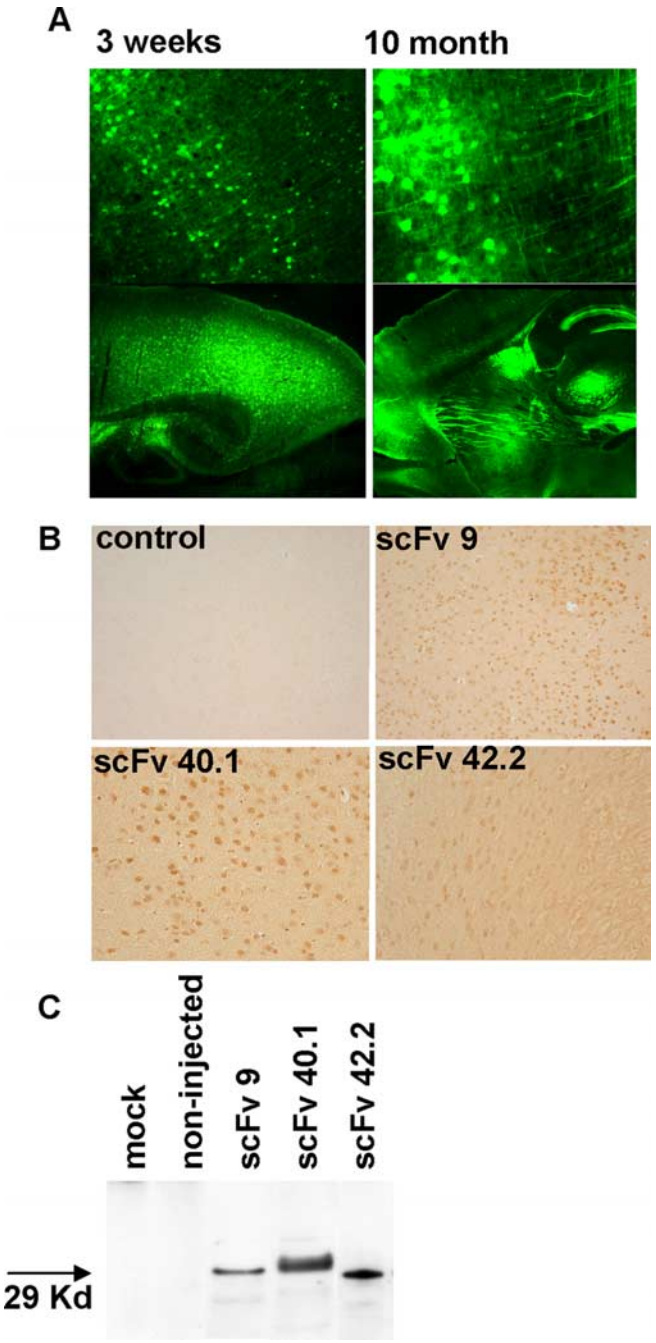

Figure 2. Expression of an anti-Aβ scFv in the neonatal mouse brain using AAV1. ***A***, P0 Swiss-Webster pups were injected intracerebroventricularly with AAV1–hGFP, a total of 4×10^{12} genomes. AAV expression in a mouse brain 3 weeks and 10 months after injection. Magnification: top, 40×; bottom, 200×. ***B***, Newborn CRND8 mice were injected intracerebroventricularly with AAV1 scFv. After 3 weeks, brain paraffin sections were analyzed for scFv expression using anti-His primary antibody and anti-rabbit secondary antibody. Magnification, 200×. ***C***, Representative Western blot of a rabbit anti-His immunoprecipitation from brains of scFv-treated CRND8 mice, detected with chicken anti-c-myc primary antibody and anti-chicken HRP secondary antibody, showing the anti-Aβ scFvs expressed in the brain.

attributable to a higher expression level in the mouse brain. In this study, there was insufficient Aβ present in the FA fraction (in 3-month-old mice) to make any reliable measurements. None of these studies showed any untoward side effects; no evidence for microhemorrhage was seen with Prussian Blue staining, and low levels of cerebral amyloid angiopathy (CAA) present in 5-month-old mice were decreased by scFv treatment (data not shown).

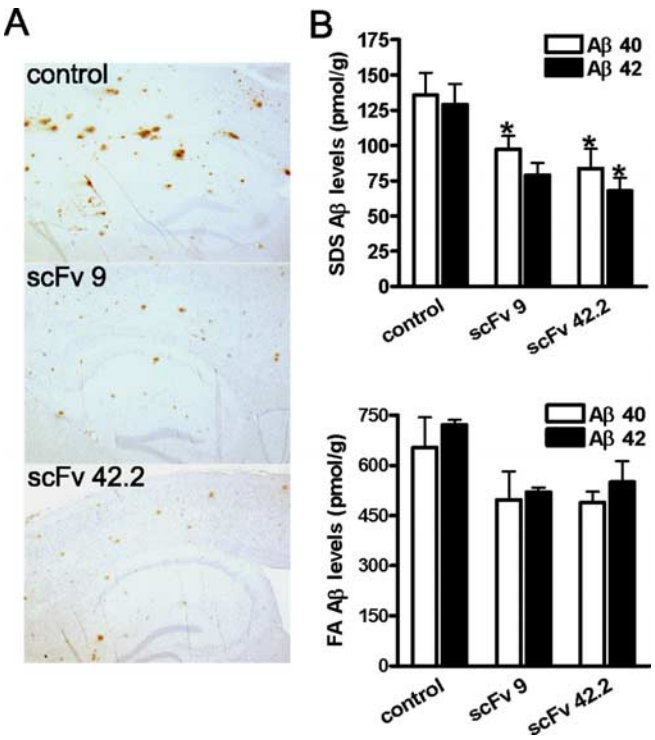

Figure 3. Anti-Aβ scFvs attenuate Aβ deposition in 5-month-old CRND8 mice. Newborn CRND8 mice were injected intracerebroventricularly with AAV1 scFv9 and scFv42.2. Control mice received AAV1–hGFP. Five months later, mice were killed after treatment; one hemibrain was processed for immunohistochemistry, and the other was processed for biochemical analysis. ***A***, Representative immunostained sections for amyloid plaques from brains of scFv-treated CRND8 mice. Magnification, 40×. ***B***, Aβ levels in the SDS-soluble and SDS-insoluble FA-soluble fractions analyzed by Aβ sandwich ELISA. $n = 5$; $^*p < 0.05$ versus control.

A complex of scFv bound to Aβ was detected in the plasma of CRND8 mice by ELISA with an antibody specific to a free end of Aβ as capture and anti-c-myc–HRP as detection. For scFv9, we used mAb40.1 as capture, and for scFv40.1 and scFv42.2, mAb9 was used as capture (Fig. 4*D*). The highest relative level of the scFv–Aβ complex was detected for scFv40.1. This result suggests that scFv alone, or in a complex with Aβ, is cleared from the brain to the plasma.

Discussion

These studies demonstrate that intracranial expression of multiple anti-Aβ scFvs are effective at reducing Aβ deposition in an AD mouse model. These findings are similar to a recent report in which AAV vectors were used to express a single anti-Aβ scFv in the cortex and hippocampus of APPSwe Tg2576 mice (Fukuchi et al., 2006), although no appropriate controls were used in that study. These data add to a growing body of findings demonstrating that an intact antibody is not required to effectively attenuate Aβ deposition in the brain. Although microhemorrhage has been associated with passive immunotherapy in some studies, it is not a consistent consequence of anti-Aβ mAb administration (Chauhan and Siegel, 2004; Wilcock et al., 2004; Racke et al., 2005; Levites et al., 2006a). No adverse effects were noted after scFv administration in this study. As in our previous studies, in which the parent mAbs were peripherally administered to APP mouse models (Levites et al., 2006a), CAA was reduced by these anti-Aβ scFvs, and there was no evidence for microhemorrhage. However, because anti-Aβ-induced microhemorrhage has only been reported in old APP mice with robust CAA (Pfeifer et al., 2002), it

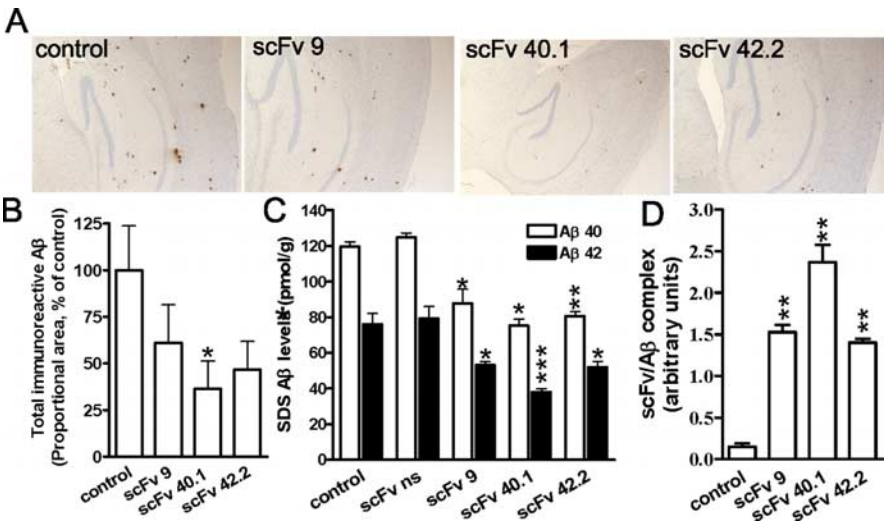

Figure 4. Anti-Aβ scFvs attenuate Aβ deposition in 3-month-old CRND8 mice. Newborn CRND8 mice were injected intracerebroventricularly with AAV1 expressing scFv9, scFv40.1, and scFv42.2. Control mice received AAV1–scFv ns or PBS. Three months later, mice were killed after treatment. One hemibrain was used for immunohistochemistry, and the other was used for biochemical analysis. ***A***, Representative immunostained sections for amyloid plaques from brains of scFv-treated CRND8 mice. Magnification, 40×. ***B***, Quantitative image analysis of amyloid plaque burden in the neocortex of scFv-treated CRND8 mice. *$p <$ 0.05 versus control. ***C***, Aβ levels in SDS-soluble extracts. ***D***, An Aβ–scFv complex in plasma was detected by ELISA with a capture antibody specific to the free end of Aβ (for scFv9, mAb40.1; for scFv40.1 and scFv42.2, mAb9) and anti-myc–HRP as detection. $n = 7$; *$p < 0.05$ versus nonspecific scFv; **$p < 0.01$ versus nonspecific scFv; ***$p < 0.005$ versus nonspecific scFv. Error bars indicate SEM.

is also possible that the lack of microhemorrhage in 5-month-old CRND8 mice might be attributed to the modest levels of CAA present. Such data suggest that delivery of anti-Aβ scFvs might be a safe therapeutic modality for the treatment or prevention of AD by using scFv derived from mAbs that are well characterized.

There are four rather curious aspects to our current data. First, one might predict that intracranial expression of the anti-Aβ scFv might lead to a more robust reduction in Aβ deposition. In previous studies, we and others have found that very little anti-Aβ mAb enters the brain after active or passive immunization in mice (Bard et al., 2000; DeMattos et al., 2002; Banks et al., 2005). We have never been able to detect the anti-Aβ mAbs using sensitive immunocytochemical staining methods, and biochemical methods indicated that <0.1% (~1 pm/g brain) of peripherally administered mAbs enter the brain. Moreover, the mAb–Aβ complex is very stable but rapidly cleared from the brain; thus, after passive administration, a very small amount of anti-Aβ mAbs enters the brain and is only present in a free state to bind Aβ (at least in mice with high levels of plasma Aβ) for short periods of time (~12 h) (Levites et al., 2006b). Because the neonatal injections of the AAV1 vectors results in widespread production of the anti-Aβ scFvs *in situ*, it is somewhat surprising that the reduction in Aβ deposition is similar to that seen with peripheral administration of the parent mAbs. The steady-state level of the anti-Aβ scFvs expressed in the brain in the current studies ranges from ~1.1 to 2.4 pmol/g. Given the scFv is expressed constitutively in the CNS, additional kinetics studies will be needed to determine its half life in the brain and to estimate what levels of expression will lead to optimal efficacy. Although this method likely delivers more anti-Aβ to the brain than peripheral administration, additional studies will be needed to determine whether the lack of a more robust reduction is attributable to a general limitation of the anti-Aβ immunotherapy approach, some limitation inherent to the scFvs we have used (e.g., stability, affinity or target epitope), or some limitation of the immunotherapy approach in the CRND8 mouse model that has an extremely rapid onset of Aβ deposition. Second, we find no significant difference in the ratio of the deposited Aβ40 and Aβ42, despite the fact that we are expressing different anti-Aβ scFvs that recognize total Aβ (scFv9, anti-Aβ1–16), Aβx-40 (scFv40.1), and Aβx-42 (scFv42.2). Again, these studies are similar to the data obtained using the parent antibodies as passive immunogens (Levites et al., 2006a). Given that shifting the ratio of Aβ40/Aβ42 has profound effects on Aβ deposition (Borchelt et al., 1996; McGowan et al., 2005), such data would suggest that the anti-Aβ antibodies and scFvs are not likely to be working by targeting monomeric Aβ. If they were targeting monomeric Aβ and shifting the ratio of Aβ40/Aβ42, one might predict that this would affect both the overall level and the ratio of the deposited Aβ. Third, we find that the most effective anti-Aβ scFv appears to be the one that selectively targets Aβ40. At the present time, we have insufficient data to precisely determine what makes one anti-Aβ scFv more effective than others, although it is interesting to note that the scFv40.1 may be expressed at higher levels both *in vitro* and *in vivo*. Fourth, we find evidence for a complex of Aβ and the anti-Aβ scFv in the plasma, suggesting that the anti-Aβ scFvs are transported out of the brain either as a preformed complex with Aβ or binds Aβ in the plasma. However, the presence of the anti-Aβ scFv–Aβ complex in the plasma does not result in an overall increase in plasma Aβ levels.

The ability to achieve widespread, apparently permanent expression of genes delivered intracerebroventricularly by AAV1 to P0 mice establishes a novel cost and time-effective paradigm in which to validate therapeutic targets or strategies in existing AD mouse models. Such data extend studies whereby AVV1-mediated delivery of a transgene is used to attenuate pathology in mouse models of lysosomal storage disease (Passini et al., 2003). We believe that a useful term for this technology is "somatic brain transgenics." Given the time and expense of creating transgenic mice to validate targets, the somatic brain transgenic technology is highly enabling and should advance the speed in which potential modifiers of AD pathology can be can be evaluated *in vivo*.

References

Bacskai BJ, Kajdasz ST, McLellan ME, Games D, Seubert P, Schenk D, Hyman BT (2002) Non-Fc-mediated mechanisms are involved in clearance of amyloid β *in vivo* by immunotherapy. J Neurosci 22:7873–7878.

Banks WA, Pagliari P, Nakaoke R, Morley JE (2005) Effects of a behaviorally active antibody on the brain uptake and clearance of amyloid beta proteins. Peptides 26:287–294.

Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, et al. (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 6:916–919.

Bennett DA, Holtzman DM (2005) Immunization therapy for Alzheimer disease? Neurology 64:10–12.

Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada CM, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M,

Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1–42/1–40 ratio in vitro and in vivo. Neuron 17:1005–1013.

Chauhan NB, Siegel GJ (2004) Intracerebroventricular passive immunization in transgenic mouse models of Alzheimer's disease. Expert Rev Vaccines 3:717–725.

Chishti MA, Yang DS, Janus C, Phinney AL, Horne P, Pearson J, Strome R, Zuker N, Loukides J, French J, Turner S, Lozza G, Grilli M, Kunicki S, Morissette C, Paquette J, Gervais F, Bergeron C, Fraser PE, Carlson GA, et al. (2001) Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J Biol Chem 276:21562–21570.

DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM (2002) Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. Science 295:2264–2267.

Fukuchi K, Tahara K, Kim HD, Maxwell JA, Lewis TL, Accavitti-Loper MA, Kim H, Ponnazhagan S, Lalonde R (2006) Anti-Abeta single-chain antibody delivery via adeno-associated virus for treatment of Alzheimer's disease. Neurobiol Dis 23:502–511.

Gilliland LK, Norris NA, Marquardt H, Tsu TT, Hayden MS, Neubauer MG, Yelton DE, Mittler RS, Ledbetter JA (1996) Rapid and reliable cloning of antibody variable regions and generation of recombinant single chain antibody fragments. Tissue Antigens 47:1–20.

Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Boada Rovira M, Forette F, Orgogozo JM (2005) Clinical effects of Aβ immunization (AN1792) in patients with AD in an interrupted trial. Neurology 64:1553–1562.

Goel A, Colcher D, Baranowska-Kortylewicz J, Augustine S, Booth BJ, Pavlinkova G, Batra SK (2000) Genetically engineered tetravalent single-chain Fv of the pancarcinoma monoclonal antibody CC49: improved biodistribution and potential for therapeutic application. Cancer Res 60:6964–6971.

Golde TE (2003) Alzheimer disease therapy: can the amyloid cascade be halted? J Clin Invest 111:11–18.

Levites Y, Das P, Price RW, Rochette MJ, Kostura LA, McGowan EM, Murphy MP, Golde TE (2006a) Anti-Abeta42- and anti-Abeta40-specific mAbs attenuate amyloid deposition in an Alzheimer disease mouse model. J Clin Invest 116:193–201.

Levites Y, Smithson LA, Dakin R, Sierks M, McGowan E, Das P, Golde T (2006b) Insights into the mechanisms of action of anti-Abeta antibodies in Alzheimer's disease mouse models. FASEB J, in press.

Masliah E, Hansen L, Adame A, Crews L, Bard F, Lee C, Seubert P, Games D, Kirby L, Schenk D (2005) Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. Neurology 64:129–131.

Matsuoka Y, Saito M, LaFrancois J, Gaynor K, Olm V, Wang L, Casey E, Lu Y, Shiratori C, Lemere C, Duff K (2003) Novel therapeutic approach for the treatment of Alzheimer's disease by peripheral administration of agents with an affinity to β amyloid. J Neurosci 23:29–33.

McGowan E, Pickford F, Kim J, Onstead L, Eriksen J, Yu C, Skipper L, Murphy MP, Beard J, Das P, Jansen K, Delucia M, Lin WL, Dolios G, Wang R, Eckman CB, Dickson DW, Hutton M, Hardy J, Golde T (2005) Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. Neuron 47:191–199.

Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C (2003) Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. Neurology 61:46–54.

Passini MA, Wolfe JH (2001) Widespread gene delivery and structure-specific patterns of expression in the brain after intraventricular injections of neonatal mice with an adeno-associated virus vector. J Virol 75:12382–12392.

Passini MA, Watson DJ, Vite CH, Landsburg DJ, Feigenbaum AL, Wolfe JH (2003) Intraventricular brain injection of adeno-associated virus type 1 (AAV1) in neonatal mice results in complementary patterns of neuronal transduction to AAV2 and total long-term correction of storage lesions in the brains of beta-glucuronidase-deficient mice. J Virol 77:7034–7040.

Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, Mathews PM, Jucker M (2002) Cerebral hemorrhage after passive anti-Abeta immunotherapy. Science 298:1379.

Racke MM, Boone LI, Hepburn DL, Parsadainian M, Bryan MT, Ness DK, Piroozi KS, Jordan WH, Brown DD, Hoffman WP, Holtzman DM, Bales KR, Gitter BD, May PC, Paul SM, DeMattos RB (2005) Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid β. J Neurosci 25:629–636.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400:173–177.

Tamura Y, Hamajima K, Matsui K, Yanoma S, Narita M, Tajima N, Xin KQ, Klinman D, Okuda K (2005) The F(ab)′2 fragment of an Abeta-specific monoclonal antibody reduces Abeta deposits in the brain. Neurobiol Dis 20:541–549.

Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, Gordon MN, Morgan D (2003) Intracranially administered anti-Aβ antibodies reduce β-amyloid deposition by mechanisms both independent of and associated with microglial activation. J Neurosci 23:3745–3751.

Wilcock DM, Rojiani A, Rosenthal A, Levkowitz G, Subbarao S, Alamed J, Wilson D, Wilson N, Freeman MJ, Gordon MN, Morgan D (2004) Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. J Neurosci 24:6144–6151.