Specific Amyloid β Clearance by a Catalytic Antibody Construct*

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Background: Naturally occurring catalytic antibodies (catabodies) can hydrolyze peptide bonds.

Results: A catabody engineered from innate immunity principles hydrolyzed amyloid β (Aβ) specifically, dissolved Aβ aggregates, and cleared brain Aβ deposits without evident toxicity.

Conclusion: The catabody could potentially be developed as a therapy for Alzheimer disease.

Significance: The innate catabody repertoire may be a source of useful catabodies to toxic amyloids.

Classical immunization methods do not generate catalytic antibodies (catabodies), but recent findings suggest that the innate antibody repertoire is a rich catabody source. We describe the specificity and amyloid β (Aβ)-clearing effect of a catabody construct engineered from innate immunity principles. The catabody recognized the Aβ C terminus noncovalently and hydrolyzed Aβ rapidly, with no reactivity to the Aβ precursor protein, transthyretin amyloid aggregates, or irrelevant proteins containing the catabody-sensitive Aβ dipeptide unit. The catabody dissolved preformed Aβ aggregates and inhibited Aβ aggregation more potently than an Aβ-binding IgG. Intravenous catabody treatment reduced brain Aβ deposits in a mouse Alzheimer disease model without inducing microgliosis or microhemorrhages. Specific Aβ hydrolysis appears to be an innate immune function that could be applied for therapeutic Aβ removal.

According to the “amyloid hypothesis,” soluble and fibrillar amyloid β peptide (Aβ) aggregates contribute causally in the pathogenesis of Alzheimer disease (AD). The aggregates activate microglial inflammatory processes, exert direct neurotoxic effects, and disrupt the brain anatomic architecture (1). In addition to deposits of Aβ(1–42) (Aβ42) that damage the brain parenchyma, accumulation of Aβ(1–40) (Aβ40) in blood vessel walls causes microvasculature-related neuroinflammation and compromised blood-brain barrier (BBB) integrity (2), resulting in cerebral amyloid angiopathy (CAA) in nearly all AD patients (3). Intravenous administration of brain-penetrating Aβ-binding monoclonal IgGs was proposed for AD therapy (4–6). Such IgGs exert competing favorable and unfavorable effects (7, 8). Whereas the Aβ-IgG immune complexes are cleared via the Fc receptor-dependent uptake pathway by phagocytic cells (the microglia) (4), the activated cells release inflammatory mediators and neurotoxic factors (5, 9). Moreover, Aβ-binding monoclonal IgGs clear parenchymal Aβ42, but they induce increased Aβ40 deposition in blood vessel walls, enhancing the incidence of microhemorrhages and CAA (6, 7) thought to be correlated with cognitive impairments (10). Reminiscent of the exogenous IgG effect, the appearance of Aβ-binding autoantibodies in the cerebrospinal fluid of AD patients correlates with exacerbated CAA (11).

Catalytic antibodies (catabodies) hold potential for digesting the antigen into harmless soluble fragments with no dependence on accessory inflammatory cells. Conventional immunization procedures based on acquired immunity principles do not produce catabodies with hydrolytic rates sufficient for medical use. Recent studies suggest that catalysis is an innate property of the germ line immunoglobulin variable (V) domains that have developed over the course of Darwinian evolution (12). Degradation of several self-antigens by catabodies in autoimmune disease was reported (12, 13). The innate V domain rep-
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ertoire expressed prior to contact with an antigen is very large, containing diverse light and heavy chain V domains (\(V_L\) and \(V_H\) domains) that hold potential for specific recognition of individual antigenic epitopes. We reported the catalytic immunoglobulin V domain (IgV) construct 2E6 isolated from a human IgV library (14). Here we present evidence showing that the IgV degrades and clears Aβ specifically with no evidence of microglial activation or microhemorrhages.

MATERIALS AND METHODS

Antibodies—IgV clones 2E6 and MMF6 were isolated from an IgV library cloned in pHeN2 vector from peripheral blood lymphocytes of three lupus patients without amyloid disease (14). The two IgVs contain the same C-terminal \(V_{1_2}\) domain and different N-terminal \(V_{1_1}\) domains (GenBank\textsuperscript{TM} accession numbers FJ231715 and KF018653, respectively). The mutant IgV 2E6 gene was synthesized by Mutagenex Inc. (Hillsborough, NJ) by replacing the VL1 domain framework regions (FRs; Kabat residues 1–23, 35–49, 57–88, and 98–107) with the corresponding FRs of IgV MMF6 and \(V_{1_1}\) domain and cloned into pHeN2 vector (NcoI/XhoI sites). IgV expression in culture supernatants after 24-h induction with isopropyl-

\textit{IN}) treated with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP; Sigma-Aldrich) was radiolabeled with \(^{125}\text{I}\) at Tyr\(^{10}\). Aβ40 forms particulate aggregates slowly compared with Aβ42. Routine hydrolysis tests were conducted using \(^{125}\text{I}\)-Aβ40 (\(30,000\) cpm) treated with nIgV or aIgV preparations (3–24 h) in PBS containing 0.1 mM CHAPS and 0.1% bovine serum albumin, followed by trichloroacetic acid precipitation to separate the intact peptide from fragments (14). Reported values are means ± S.D. (2 or 3 replicates). Formic acid was added to 60% (v/v) prior to FPLC gel filtration of \(^{125}\text{I}\)-Aβ40 (50,000 cpms; \(~0.2\) mM) digested with IgVs (2.5 \(\mu\)g/ml, 24 h; Superdex peptide column (GE Healthcare), run in 60% formic acid, 0.5-mL fractions). The nominal peptide mass was computed from the retardation volumes of these markers: aprotinin (6,512 Da), vasoactive intestinal peptide (3,326 Da), \(\text{N}-\text{tert-butoxycarbonyl-O-benzyl-Glu-Ala-Arg-7-amino-4-methylcoumarin (721 Da)}\), and Ala-7-amino-4-methylcoumarin (246 Da). Apparent \(K_m\) and turnover number (\(k_{\text{cat}}\)) were estimated from initial hydrolysis rates measured at a constant \(^{125}\text{I}\)-Aβ40 amount (\(~300,000\) cpms) mixed with increasing Aβ40 concentrations (0.1 nM to 25 \(\mu\)M), assuming an IgV mass of 30 kDa (14).

Aβ42 (90 \(\mu\)g) was labeled with \(^{125}\text{I}\) using 1,3,4,6-tetrachloro-3\(\alpha\),6\(\alpha\)-diphenylglycouril-coated tubes (Pierce), and free \(^{125}\text{I}\) was removed (Sep-Pak Vac C18 cartridge, Waters (Milford, MA)). The acetonitrile-eluted \(^{125}\text{I}\)-Aβ42 was dried, mixed with non-radiolabeled Aβ42 (1 mg), treated with HFIP for 1 h, lyophilized, and stored (\(~80^\circ\text{C}\)). After reconstitution in dimethyl sulfoxide, the \(^{125}\text{I}\)-Aβ42 (5 mM) was diluted to a concentration of 50 \(\mu\)M in PBS and incubated for 92 h (37 °C), and fibrillar Aβ42 was collected by centrifugation (16,000 \(\times\) g, 20 min). The radioactivity content of fibrillar \(^{125}\text{I}\)-Aβ42 was 2.3 \(\times\) 10\(^7\) cpms/\(\mu\)mol of Aβ. Fibrillar \(^{125}\text{I}\)-Aβ42 (2.2 nmol/0.1 ml) was incubated with nIgV samples in PBS containing 0.1 mM CHAPS. Formic acid was added to 60% (v/v) in the supernatant collected by centrifugation (16,000 \(\times\) g, 20 min), and FPLC gel filtration in formic acid was conducted as before. The pelleted \(^{125}\text{I}\)-Aβ42 was dissolved in PBS containing 60% formic acid and analyzed by FPLC gel filtration similarly.

For epitope identification, non-radioactive Aβ40, Aβ(1–16), Aβ(17–42), or Aβ(19–40) (Anaspec, Fremont, CA) was included as a competitive inhibitor in the \(^{125}\text{I}\)-Aβ40 hydrolysis
reaction mixture (0.2 μg/ml nIgV 2E6; 3 h, 37 °C). Hydrolysis of soluble FLAG-tagged APP751 (83 kDa, 0.1 μM; Origene Technologies, Rockville, MD), glutathione S-transferase (GST)-tagged amylin (153 kDa, 0.1 μM), and GST-tagged zinc finger protein 154 (43 kDa, 0.1 μM; Abnova, Taipei, Taiwan) treated with 2.5 μg/ml nIgV (24 h) was estimated by SDS electrophoresis and staining with horseradish peroxidase-conjugated anti-FLAG or anti-GST IgG antibodies (Sigma-Aldrich) (16). Minor low mass protein bands visible in the amylin hydrolysis reaction mixtures in addition to the major intact amylin band (153 kDa) are impurities from the starting protein preparation. Biotin-conjugated transthyretin (TTR) (1.5 mol of biotin/mol of TTR monomer; conjugation done as described (14)) was preaggregated for 5 days in 100 mM sodium acetate, pH 4.2, 5 mM sodium phosphate, 68 mM NaCl, 51 mM KCl containing 1 mM EDTA, followed by buffer exchange to PBS containing 0.1 mM CHAPS by ultrafiltration (Amicon Ultra-4 centrifugal filter, 10 kDa cut-off). TTR aggregation was confirmed by development of turbidity (400 nm) and increased thioflavin T (ThT) fluorescence (λex = 440 nm; λem = 485 nm; photomultiplier tube, 600 V) compared with the starting non-aggregated TTR (turbidity, 0.61 ± 0.01 and 0.02 ± 0.01, respectively; ThT fluorescence, 144.9 ± 3.2 and 17.4 ± 6.9, respectively). The preaggregated TTR (0.1 μM) was incubated with 40 μg/ml IgG (60 h), and the reaction mixtures were boiled in SDS and analyzed by electrophoresis (16.5% gels). Under these conditions, the physiological tetramer TTR species and amyloid TTR aggregates dissociate into the TTR monomer band (14 kDa), and a faint dimer band is detected (19).

Anti-amyloid Assays—Preaggregated fibrillar Aβ42 (starting Aβ42 peptide concentration, 20 μM; prepared as described for 125I-Aβ42) was treated with nIgV (24 h, 37 °C) in PBS, 0.1 mM CHAPS, and 1% dimethyl sulfoxide. ThT (5 μM) was added, and fluorescence emission was measured after 30 min. Inhibition of fibrillization was determined similarly by treating non-aggregated Aβ42 (20 μM) with the nIgVs. The data were corrected for background ThT fluorescence of the same antibody without Aβ42 (<5−19 fluorescence units). For potency comparisons, ThT fluorescence for reaction mixtures containing nIgV 2E6 or IgG1 59 was expressed as a percentage of the value of the control antibody with the same scaffold structure (64–94 fluorescence units; nIgV MMF6 and IgG1 SKT03 directed to gp120) (20). Dissolution of preaggregated fibrillar Aβ42 (5 μM) treated with an equal volume of IgV-containing tissue culture supernatant was monitored by transmission electron microscopy using a JEOL 1400 microscope at 120 kV (5 μl of reaction mixture adsorbed for 1 min on glow-discharged 300-mesh Formvar carbon grids followed by three 30-s washes with water, negative staining for 1 min with 1% uranyl acetate in water, and another three 30-s washes with water). Oligomers were prepared by incubating Aβ42 (50 μM) in phenol red-free Ham’s F-12 medium (4 °C, 24 h) (21). The oligomer preparation (total Aβ peptide concentration 40 μM) was treated (24 h, 37 °C) with nIgV 2E6 or MMF6 (3 μg/ml) in Ham’s medium/PBS containing 0.1 mM CHAPS (4:1, v/v). SDS and β-mercaptoethanol were added (final concentrations, 2% and 0.46 M, respectively), and the reaction mixtures were analyzed by SDS-gel electrophoresis without prior boiling. The Aβ species mass (monomers, oligomers, and proteolytic fragments) was computed by comparison with reference proteins (1.4–27 kDa and 14–97 kDa ladders; Bio-Rad). Oligomer disappearance was monitored by densitometry of the SDS-stable trimer, tetramer, and high mass oligomer bands (45 kDa band, 64–84 kDa smear) following staining of gel blots with a mixture of mouse anti-Aβ monoclonal IgG 6E10, IgG 4G8 (both from Covance, Princeton, NJ), and IgG 6D4 (MyBioSource, San Diego, CA) (directed to Aβ(1−17), Aβ(17−24), and the Aβ C terminus, respectively). Freshly dissolved Aβ42 without prior oligomerization was mixed immediately with the nIgVs (3 μg/ml) to test the nIgV effect on oligomer accumulation over 24 h of incubation at 4 °C. Experimental and control antibody effects were studied using equivalently analyzed reaction mixtures (including equivalent gel staining and imaging procedures). Aβ42 binding by IgV 2E6 and IgG1 59 was tested by immunoblotting of oligomerized Aβ42 electrophoresed in SDS gels (1 μg of Aβ42/lane). Bound IgV was visualized by staining with mouse anti-c-Myc IgG followed by peroxidase-conjugated anti-IgG. The procedure was validated previously to detect IgV-Aβ immune complexes (14, 15). Bound IgG1 59 was detected by staining with peroxidase-conjugated anti-mouse IgG (22).

Brain Aβ Removal—In the first study, nIgV 2E6 or MMF6 (1 μg/2 μl of PBS) was injected into the right brain hemisphere neocortex of 5XFAD mice (5 months old). These mice express mutant human presenilin 1 (M146L and L286M mutations) and wild type Aβ produced from mutant human Aβ precursor protein (APP) (K670N, M671L, I716V, and V717I mutations associated with familial AD (23)). Another age-matched mouse group received similar PBS injections. Coronal brain sections of the right and left hemispheres obtained 7 days thereafter were stained with a mixture of anti-Aβ monoclonal IgGs 6E10 and 4G8, and the Aβ deposits were quantified (BIOQUANT Image Analysis Corp., Nashville, TN) (24). The Aβ plaque burden is defined as the percentage area occupied by the stained reaction product in a 640 × 480-μm rectangle surrounding the injection site (estimated in five 40-μm sections spaced 200 μm apart). The relative Aβ burden in the injected right hemisphere was computed as a percentage of the Aβ plaque burden in the corresponding non-injected left hemisphere neocortical area within the same sections from the same mouse.

In the second study, the algalV proteins purified by metal affinity chromatography were injected intravenously on day 0 and day 3 (50 μg of algalV in PBS/injection) in TgSwDI mice (n = 8/group, 7–9 months old). The mice express human Aβ with mutations outside the IgV-sensitive epitope and scissile bond region (E22Q and D23N, corresponding to the E693Q and D694N mutations in APP770) (25, 26). In addition to diffuse plaques, the TgSwDI mice develop vascular Aβ deposits at an early age compared with other human Aβ-expressing mouse models. Cortical Aβ burden was quantified on day 10 in five randomly selected sections as before using an MBF StereoInvestigator. The analyzed neocortical area was dorsomedial from the cingulate cortex and extended ventrolaterally to the rhinal fissure within the right hemisphere (measured field 700 × 700 μm). Left brain hemispheres after removing the olfactory bulb were homogenized as described (24). The hemispheres were weighed and homogenized (10%, w/v) in 20
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mm Tris base, 250 mm sucrose, 1 mm EDTA, 1 mm EGTA, 100 mm phenylmethylsulfonyl fluoride, 5 µg/ml pepstatin A, and a 25-fold dilution of Complete protease inhibitor mixture as recommended by the supplier (Roche Applied Science). Total Aβ levels were determined after solubilizing particulate Aβ in the homogenates mixed with cold formic acid (1:2.2 (v/v)) in duplicate using the Aβ 3-plex Ultrasensitive immunoassay kit (Meso Scale Discovery, Gaithersburg, MD). Soluble Aβ levels were measured similarly by ELISA in the soluble fractions of brain homogenates after treatment with cold 0.4% diethylamine containing 100 mm NaCl (24). The difference between total and soluble Aβ brain contents represents particulate Aβ dissolved by formic acid. Cerebral microhemorrhages were measured by staining with 5% potassium ferrocyanide in 10% hydrochloric acid for 30 min (Perl’s iron stain; 20 sections/animal, 40-µm sections spaced 400 µm apart throughout the brain) (24). Cortical microgliosis was assessed according to a semiquantitative scale in 15–20 similarly obtained coronal sections per animal, stained with antibody to Iba-1 (0, a few resting microglia; 1+, a few activated microglia; 2+, a moderate number of activated/phagocytic microglia; 3+, numerous activated/phagocytic microglia) (24).

Brain entry of intravenously injected IgV in B6SJLF1/J mice (Jackson Laboratory, Bar Harbor, ME) was studied using 125I-labeled algV 2E6 prepared by the chloramine-T method followed by gel filtration in PBS containing 0.1 mm CHAPS and 1% BSA (Econo-Pac® 10DG column, Bio-Rad; specific activity, 3.7 × 10⁶ cpm/µg algV) (27). Essentially all recovered radioactivity was present in the algV bands identified by electrophoresis and autoradiography. Following injection of 125I-labeled algV into the tail vein (1.1 µg/110 µl/mouse, 6.3 × 10⁶ cpm), the radioactivity/g of whole blood from the retroorbital plexus and whole brain obtained at euthanasia was measured using a γ counter (n = 3 mice/time point). The nominal half-life was computed from single phase decay kinetics (cpm = (cpmmax × exp(−kt)) + cpm0 × exp(−kt)), where k is the decay constant and t₀ = ln2/λ, and cpm and cpmmax correspond to the observed radioactivity values at varying time points and the extrapolated maximum radioactivity value obtained by curve fitting, respectively.

Statistical Analysis—p values were from the unpaired two-tailed Student’s t test.

RESULTS

IgV 2E6 Hydrolytic Properties—Recombinant IgV 2E6 is a single-chain heterodimer of Vl domains (Fig. 1A, inset) that expressed Aβ hydrolyzing activity following purification of the IgV by acid elution from a metal affinity column (algV 2E6) (14). Per unit IgV 2E6 mass, the Aβ hydrolytic activity of the native IgV secreted into the bacterial supernatant was substantially superior to the acid-purified algV and at least comparable with nepriysin (Table 1), an enzyme with promiscuous hydrolytic activity not restricted to Aβ that is a proposed therapeutic agent for AD (28). Nanogram IgV 2E6 amounts in the unfractionated culture supernatant hydrolyzed radiolabeled 125I-Aβ40 with negligible contribution from bacterial proteases, shown by lack of hydrolytic activity of supernatants containing non-catalytic IgV MMF6 with the same scaffold structure as IgV 2E6 and mutated IgV 2E6 containing the IgV MMF6 frame-work regions (Fig. 1, A–C). Sub-Ångstrom conformational transitions can reduce the catalytic activity of IgVs (14) and enzymes (29). The superior catalytic efficiency of native IgV compared with the algV suggests compromised catalytic site integrity due to acid-induced conformational perturbations. For most substrate specificity and anti-amyloid tests, we used the highly catalytic IgV 2E6 fractionated partially by ion exchange chromatography at neutral pH (nIgV 2E6). The procedure removed small amounts of bacterial proteases in the supernatants without appreciable loss of catalytic activity due to conformational perturbations (Fig. 1, D–F; hydrolytic activity of the nIgV and native IgV in the bacterial supernatant, respectively, 211,555 and 181,827 cpm/µg). The previously characterized algV 2E6 with lesser activity was employed for certain confirmatory anti-amyloid tests. Like the algV (14), the native IgV in the culture supernatant was composed of the catalytically active 30-kDa intact Vl₁–Vl₂ IgV construct and an inactive co-purifying fragment containing the C-terminal Vl₂ domain (Fig. 1G). A bound divalent metal is required for maintenance of IgV 2E6 catalytic activity (18). Some anti-amyloid tests were conducted using nIgV 2E6 that had been irreversibly inactivated by EDTA chelation of the bound metal (Fig. 1H).

Specificity—algV 2E6 cleaved the Aβ His14–Gln15 bond (14). nIgV treatment of 125I-Aβ40 generated a hydrolytic product with mass close to the predicted Aβ(1–14) radiolabeled product (Fig. 2, A and B; 1.654 Da; the C-terminal Aβ(15–40) fragment does not contain the radiolabel), indicating retention of the scissile bond specificity regardless of the IgV preparation method. APP fulfills an essential role in physiological neurotransmission (30). A single chain antibody fragment engineered from a catalytic Vl domain degraded APP (31), highlighting the risk of interference in APP function. Intact, full-length APP is found physiologically in two forms: the membrane-bound form and the soluble, secreted form (32, 33). nIgV 2E6 did not digest the purified soluble APP substrate detectably (Fig. 2C), suggesting that the Aβ region of soluble APP assumes an IgV-insensitive conformation. Sequence-independent recognition of a generic β-sheet amyloid epitope could explain Aβ degradation. nIgV 2E6, however, did not digest TTR amyloid aggregates (Fig. 2C). If the specificity for Aβ derives only from His14-Gln15 peptide unit recognition, the IgV is predicted to degrade other His-Gln-containing proteins. The nIgV did not degrade amphiphysin and zinc finger protein 154 containing the His-Gln unit at positions 110 and 111 and positions 57 and 58, respectively (Fig. 2C). In previous specificity studies (14), algV 2E6 did not degrade self-antigens and B cell superantigens containing numerous antibody-binding epitopes and protease-sensitive bonds. We analyzed synthetic Aβ peptides to assess the contribution of noncovalent epitope recognition in IgV specificity. Aβ(1–16) containing the His14-Gln15 dipetide unit did not inhibit 125I-Aβ40 hydrolysis by the nIgV, but the C-terminal Aβ(29–40), Aβ(17–42), and control full-length Aβ40 were equipotent inhibitors (Fig. 2D). The results indicate specific noncovalent recognition of the Aβ(29–40) epitope followed by hydrolysis at the remote His14-Gln15, with no requirement for the two Aβ(2) C-terminal residues (Fig. 2D, inset). In membrane-bound APP and its C99 fragment generated upon γ-secretase cleavage, the His14-Gln15 scissile bond is located in
the juxtamembrane extracellular protein region, and the Aβ(29–40) epitope is buried within the lipid bilayer (34).

Amyloid Dissolution—Aβ self-assembles into β-sheet fibrils and soluble oligomers. Treatment of prefibrillized Aβ42 with tissue culture supernatants containing IgV 2E6 but not IgV MMF6 dissolved the peptide fibrils nearly completely in two repeat experiments, leaving only sparse individual fibrils visible by electron microscopy (Fig. 3A, images 1–4). Likewise, a small concentration of the catalytically efficient ngV 2E6 preparation dissolved prefibrillized Aβ42, judged by the ThT-binding test for β-sheet-containing aggregates (Fig. 3B). The ngV 2E6 with lesser Aβ40 hydrolytic activity also dissolved prefibrillized Aβ42 but with lower potency compared to ngV 2E6 (Fig. 3C; 17-fold difference between dissolution potency, computed as the ratio of ngV and algV concentrations needed to reduce ThT binding by 20 fluorescence units compared with the value prior to incubation with the IgV; difference in Aβ hydrolytic

FIGURE 1. Hydrolytic properties of IgV 2E6. A, 125I-Aβ40 hydrolysis by un fractionated bacterial culture supernatants (1:10 diluted, 32–38 ng of IgV/assay). Each symbol represents an independent culture well. Hydrolysis by supernatants from control bacteria without the vector (bacteria alone) or with empty vector (pHEN2) was negligible. Inset, schematic IgV structure. Filled and unfilled boxes, FRs and CDRs, respectively. B, concentration-dependent 125I-Aβ40 hydrolysis by native IgV 2E6-containing bacterial culture supernatant. Hydrolysis by culture supernatants from control bacteria expressing IgV MMF6, bacteria without vector, or bacteria with empty vector was negligible. C, hydrolytically inactive mutant of IgV 2E6 with its FR1–FR4 segments replaced by the corresponding FRs of IgV MMF6 (125I-Aβ40 treated for 18 h with 1:30 diluted un fractionated culture supernatants). D, diagnostic anion exchange FPLC. Top, protein absorbance (A280) profiles for supernatants of bacteria transformed with the IgV 2E6 vector or empty vector. Bottom, the 125I-Aβ40 hydrolytic activity of native IgV 2E6 in the bacterial supernatant was recovered in the unbound fractions. The corresponding unbound fractions containing basal proteases secreted by bacteria harboring the empty vector displayed little or no hydrolytic activity (<3% hydrolysis). Aβ hydrolysis per unit IgV 2E6 mass by the pooled unbound fractions was comparable with the unfractionated bacterial supernatant (170,337 and 158,857 cpm/μg of IgV, respectively). E, ngV 2E6 preparation by a second anion exchange FPLC cycle. For details of the first FPLC cycle, see “Materials and Methods.” The bound fraction (gray) contained 160 mg of total protein and 1.1 mg of ngV 2E6, determined by immunoblotting for the c-Myc tag (retention volume, 26–34 ml), corresponding to ~10-fold catabody enrichment compared with the unfractionated culture supernatant. F, superior 125I-Aβ40 hydrolytic activity of ngV 2E6 from E compared with acid-purified algV. Control ngV MMF6 fractionated by anion exchange chromatography at neutral pH did not hydrolyze Aβ40. G, reducing SDS-electrophoresis of IgV 2E6. The bacterial culture supernatant contained the anti-c-Myc antibody-stained intact 30-kDa IgV 2E6 VL1-VL2 heterodimer and an 18-kDa IgV fragment containing the VL2 region (lane 1), along with other silver-stained non-IgV proteins (lane 2). The catalytically inactive 18-kDa fragment (14) was also present in the acid-purified algV 2E6 (lanes 3 and 4), stained with anti-c-Myc antibody and silver, respectively and the algV subjected to an additional ion exchange chromatography step (lanes 5 and 6, stained with anti-c-Myc and silver, respectively (14)). Lane 7, assembled IgG1 59 150 kDa band stained with silver. H, EDTA-inactivated ngV was treated with the metal chelator EDTA (10 mM, 24 h) or diluent, and EDTA was removed prior to the hydrolysis assay. The EDTA-inactivated ngV did not hydrolyze 125I-Aβ40 detectably (~161 cpm/h). Hydrolytic activity was measured at 0.15 μg ngV/ml over 18 h. Error bars, S.D.
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**TABLE 1**

| Catalyst          | $K_m$  | $k_{cat}$ | $k_{cat}/K_m$ |
|-------------------|--------|-----------|---------------|
| algV              | $8.0 \times 10^{-5}$ | 0.3 ± 0.1 | 37.0 ± 6.0    |
| Native IgV        | 2.5 ± 0.5 | 8.2 ± 1.0 | 330 ± 66     |
| Neprilysin        | 2.2     |           |               |

Increasing Aβ40 (0.1 nM to 25 μM) containing a constant 125I-Aβ40 amount (~300,000 cpm) was treated with the acid-purified IgV (3.8 μg/ml) or native IgV in the bacterial culture supernatant (0.1 μg/ml, determined by c-Myc dot blotting). 

**activity of the two IgV preparations, 36-fold.** The control non-catalytic nlgV MMF6 and algV MMF6 preparations did not dissolve fibrillar Aβ. IgG 2E6 is a metal-dependent serine protease. The EDTA-treated nlgV and algV 2E6 preparations without Aβ hydrolitic activity also failed to dissolve fibrillar Aβ, suggesting catalysis as the dissolution mechanism. Enzymatic hydrolysis at the IgV-sensitive His14-Gln15 bond was described to generate the soluble, non-amyloidogenic, and non-neurotoxic Aβ(1–14) and Aβ(15–42) fragments (35). A soluble peptide fragment with mass approximating that of the anticipated Aβ(1–14) product was released upon nlgV 2E6 treatment of preformed fibrillar 125I-Aβ42 (Fig. 3D). The residual particulate fraction following nlgV 2E6 treatment contained only the non-degraded 125I-Aβ42 peak, shown by recovery of essentially all of its radioactivity content at a retention volume of 9.7 ml corresponding to the full-length peptide (not shown). Detection of SDS-stable aggregates in electrophoresis gels run is a diagnostic test for Aβ42 oligomerization (21) (Fig. 3E, inset, lane 1). Treatment with nlgV 2E6 reduced the content of the 3-mer Aβ(42) band (13 kDa), 4-mer Aβ(42) band (17 kDa), and higher mass Aβ(42) bands (45 kDa, 64–84 kDa) in prelabeled Aβ42, accompanied by the appearance of a 2.5-kDa product fragment stainable by anti-Aβ antibodies (Fig. 3E). The monomer Aβ42 band intensity does not serve as a useful index of monomer Aβ42 hydrolysis because the monomer can be generated by dissociation of certain Aβ42 oligomers at the SDS treatment step (36) after completion of the nlgV hydrolysis reaction. The appearance of minor 10 kDa and 19–27 kDa bands in the nlgV reaction mixture suggests the presence of disaggregation intermediates containing full-length Aβ along with variable product fragment amounts. The small mass Aβ(1–14) fragment generated by His14-Gln15 hydrolysis was not detected, consistent with the anomalous electrophoresis behavior of this peptide fragment (37). The 2.5-kDa product band probably corresponds to the Aβ(15–42) fragment.

We also tested the anti-amyloid effect of nlgV 2E6 using Aβ42 that had not been subjected to prior aggregation. Aβ42 aggregates rapidly. In diluent, ThT binding was measurable at the earliest time point studied (bar labeled 30 min) and was increased at 48 h, indicating continued peptide fibrillization (Fig. 4A). The ThT binding values for Aβ42 treated with diluent or control IgV MMF6 for 48 h were similar, but ThT binding was reduced significantly by treatment with nlgV 2E6 for the same duration (Fig. 4A). Likewise, the content of each SDS-stable oligomer species was reduced significantly by nlgV 2E6 treatment of Aβ42 that had not been subjected to prior oligomerization, accompanied by the appearance of the 2.5-kDa hydrolytic product (Fig. 4B). Small nlgV 2E6 concentrations were sufficient to inhibit the accumulation of Aβ42 fibrils and oligomers in the foregoing tests (0.13–4 μg/ml). Consistent with its lesser hydrolytic activity, the concentrations of an algV 2E6 preparation required to inhibit Aβ fibril and oligomer accumulation were larger (e.g. at 27 μg of algV/ml, inhibition by 63.3 ± 3.1 and 70.4 ± 18%, respectively). We compared the anti-amyloid effect of nlgV 2E6 with the reference Aβ-binding IgG (IgG1 59). The IgG contains V domains that clear brain Aβ in a mouse AD model (15). The amyloid inhibition effect was evident at 1 μg/ml nlgV 2E6, whereas a 30-fold larger concentration of the Aβ-binding IgG1 59 was without effect (Fig. 4C). No algV binding to the Aβ42 monomer or oligomers was evident by immunoblotting, whereas the reference IgG1 59 displayed Aβ binding activity (Fig. 4C, inset).

Previous ELISAs also failed to reveal measurable IgV-Aβ complexes, suggesting that hydrolysis of Aβ and product release is too rapid for detection of the IgV-Aβ complexes. Together, the data suggest catalytic Aβ degradation as the mechanism of IgV anti-amyloid effects without the participation of accessory phagocytic cells that clear immune complexes by the Fc receptor uptake pathway.

Amyloid Removal in Transgenic Mice—For in vivo clearance tests, we first analyzed brain sections 7 days after administering the nlgV 2E6 (1 μg) directly into the right brain neocortex of 5XFAD transgenic mice, an AD model characterized by robust accumulation of human Aβ in the brain parenchyma. Consistent with previous findings, the needle track was visible from increased Aβ deposition, an effect caused by physical trauma to the tissue (38). The needle track terminus was assumed to represent the delivery site of injected nlgV 2E6, nlgV MMF6, and control PBS. Decreased Aβ plaque burden surrounding the nlgV 2E6 delivery site was evident compared with the non-injected left brain neocortex of the same mice (Fig. 5, A and B). The Aβ clearance effect was limited to the immediate vicinity of the injection site, consistent with administration of a small IgV volume (2 μl). There was no decrease of local Aβ burden following nlgV MMF6 or PBS injection into the right brain neocortex compared with the non-injected left brain neocortex.

Small proportions of intravenously administered full-length IgG (39) and IgM (40) molecules are documented to permeate the BBB in mouse AD models. We observed restricted brain entry of intravenously injected 125I-labeled algV 2E6 in wild type mice (e.g. at 2 h, the radioactivity/g of brain tissue was 5.2 ± 0.8% of the radioactivity/g of peripheral blood; nominal algV half-life in brain and blood, 2.6 and 1.8 h, respectively; Fig. 6A). Aβ clearance following intravenous algV 2E6 administration was analyzed in the TgSwDI mouse model characterized by the predominant vasculotropic deposition of the transgene-encoded mutant Aβ40 peptide, which causes the CAA-like state (25, 26). These mice are suited for testing induction of microvascular dysfunction observed upon infusion of Aβ-binding monoclonal IgGs that penetrate the BBB (8, 41). Moreover, impaired Aβ peptide egress from the brain in the TgSwDI model is thought to minimize compensatory brain Aβ release incidental to peripheral Aβ clearance by Aβ-binding antibod-
ies, with the result that any observed brain Aβ clearance effect is probably due to antibodies that cross the BBB (42). Ten days after intravenous treatment with aIgV 2E6 (100 μg total IgV/mouse), the right hemisphere neocortical Aβ deposits were reduced significantly compared with the control aIgV MMF6 treatment, as judged by immunohistochemical staining of brain sections (28% reduction, p < 0.05; Fig. 6, B and C). The Aβ-clearing effect was confirmed from the reduced hippocampal Aβ deposits (Fig. 6D). ELISA measurements in whole left brain hemisphere extracts showed modest but significant reductions of the soluble and insoluble Aβ40 and Aβ42 levels in the aIgV 2E6-treated mice (Table 2), suggesting a widespread Aβ clearing effect. Aβ38 was not detected reliably at the brain extract concentrations tested (<16 and <0.5 pg/mg insoluble and soluble Aβ38, respectively). Treatment with algV 2E6 did not induce microglial activation (Fig. 7, A and B) or microhemorrhages (Fig. 7C). Together, the studies suggest significant brain Aβ clearance by the brief intravenous aIgV 2E6 treatment. In comparison, prolonged intravenous treatment with milligram Aβ-binding IgG amounts is required for brain Aβ clearance in various mouse models of Alzheimer disease (4, 6).

DISCUSSION

Two major brain Aβ clearance mechanisms were proposed for intravenously administered Aβ-binding monoclonal IgGs: the microglia-dependent removal of brain Aβ by BBB-pene-
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trating IgGs (e.g. bapineuzumab) (6–8) and increased Aβ egress from the brain induced by Aβ binding to IgGs in peripheral blood (e.g. solanezumab) (43). The catalytic IgV rapidly digested Aβ into non-amyloidogenic soluble fragments without forming stable immune complexes. As for enzymes, non-specific proteolysis is a significant risk. IgV 2E6 did not degrade substrates without structural similarity to Aβ, transthyretin amyloid containing the β-sheet amyloid motif, or the Aβ precursor protein, suggesting sufficiently specific Aβ removal. The same Fc receptor-dependent microglial interactions with Aβ-IgG immune complexes that result in beneficial Aβ clearance also hold potential for damaging neurons and other brain cells (5–8). Side-by-side toxicity studies of catalytic IgV and Aβ-binding IgGs remain to be conducted, but it is noteworthy

FIGURE 3. Dissolution of preformed Aβ aggregates by IgV 2E6. A, transmission electron micrographs of preformed Aβ fibrils (2.5 μM) treated for 24 h with diluent (image 1) or tissue culture supernatant containing IgV MMF6 (image 2) or IgV 2E6 (images 3 and 4). Scale bar, 200 nm. The dense Aβ plaques visible after diluent or IgV MMF6 treatment were absent after IgV 2E6 treatment, and only rare individual Aβ fibrils were evident (image 4 shows a magnified solitary fibril). IgV concentration was 0.13 μg/ml. B, ThT binding to prefibrillized Aβ42 following treatment with ngV 2E6 (24 h). ThT binding was reduced to levels below the starting fibrillar Aβ42 prior to incubation with the ngVs (dashed line, 103 ± 10 fluorescence units (FU)). Increased ThT binding is observed over 24 h of diluent and IgV MMF6 treatment due to continued Aβ fibrillization. ThT binding by Aβ42 immediately after dissolving the peptide in buffer was 68 ± 11. *, p < 0.05; **, p < 0.01. C, ThT binding to preformed fibrillar Aβ42 following treatment with algV 2E6 (24 h). ThT binding was reduced to levels below the starting fibrillar Aβ42 prior to incubation with the algVs (dashed line, 103 ± 10 fluorescence units). ThT binding by Aβ42 immediately after dissolving the peptide in buffer was 68 ± 11. *, p < 0.05. D, FPLC gel filtration profile of the supernatant following treatment of fibrillar 125I-Aβ42 with ngV 2E6. Resuspension of fibrillar 125I-Aβ42 in ngV 2E6 (10 μg/ml) resulted in release of the soluble 1654-Da fragment into the supernatant. The intact 125I-Aβ42 (4,629 Da) peak represents spontaneous peptide release that occurred at equivalent levels in reaction mixtures of fibrillar Aβ42 treated with PBS (not shown), ngV 2E6, and control ngV MMF6. Inset, time-dependent release of 1,654-Da product fragment into the supernatant by ngV 2E6 treatment. E, dissolution of preformed Aβ42 oligomers. Treatment of preoligomerized Aβ42 with ngV 2E6 but not ngV MMF6 (3 μg/ml, 24 h) depleted the SDS-stable Aβ trimers, tetramers, and high mass oligomers (45 kDa band, 64–84 kDa region). *, p < 0.005. **, p < 0.005. inset, Aβ42 oligomers stained with a mixture of Aβ-binding monoclonal antibodies after treatment with ngV MMF6 (lane 1) or ngV 2E6 (lane 2). Migration of protein standards is shown by mass values on the right. Error bars, S.D.
that the catalytic IgV dissolved fibrillar Aβ with no requirement for microglia, and there was no evidence for microglial activation or cerebral microhemorrhages in the IgV-treated mice.

The Aβ recognition properties of IgV 2E6 are similar to the previously documented single domain IgV cataybody fragment (clone 5D3) (14). While the present manuscript was under review, we reported that prolonged brain expression of IgV 5D3 by means of gene transfer induced significant Aβ clearance with no noticeable inflammatory or vascular effects (44). The gene transfer approach may eliminate the need for repeated antibody infusions, but its clinical use awaits long term safety analyses. In comparison, there are ample precedents for intravenous antibody therapy. A brain Aβ-clearing effect was evident after a brief intravenous catalytic IgV treatment in mice. Certain shortcomings of the IgV can be foreseen. First, like other antibody fragments devoid of the Fc region (45, 46), the IgV displayed a comparatively short blood half-life. Second, like nIgV 2E6 (14), IgV MMF6 showed reduced ThT binding after 48 h but not the Aβ-binding ligG1 59 (1 or 30 μg/ml) did not bind the Aβ monomer or oligomers determined by immunoblotting (lane 2). The 2.5-kDa product fragment is visible. C, superior inhibition of Aβ aggregation was evident, which was reduced significantly by treatment with nIgV MMF6 and IgG1 SKT03, respectively. **, p < 0.005; *, p < 0.03. Inset, Aβ42 oligomers stained with a mixture of Aβ-binding monoclonal antibodies following treatment with nIgV MMF6 (lane 1) or nIgV 2E6 (lane 2). The 2.5-kDa product fragment is visible. C, superior inhibition of nIgV 2E6 fibrillation by nIgV MMF6 compared with IgG1 59. Aβ42 fibrillation tested as in A was inhibited by nIgV 2E6 (1 μg/ml) but not the Aβ-binding IgG1 59 (1 or 30 μg/ml). Shown are ThT fluorescence data as a percentage of the values in the presence of control antibodies with the same scaffold structure as nIgV 2E6 and IgG1 59 (nIgV MMF6 and IgG1 SKT03, respectively), **, p < 0.005. Inset, IgV 2E6 at a concentration displaying readily detectable Aβ hydrolysis and anti-amyloid effects (4 μg/ml) did not bind the Aβ42 monomer or oligomers determined by immunoblotting (lane 4). Lane 5, control immunoblot after treatment of the same Aβ42 preparation with an equivalent control IgV MMF6 concentration. Lane 1, silver-stained trimer and tetramer bands in the Aβ42 preparation. Lanes 2 and 3, the Aβ42 oligomers stained with the Aβ-binding IgG1 59 and control nonimmune IgG1 SKT03, respectively. Error bars, S.D.

FIGURE 4. Reduced Aβ aggregation in the presence of nIgV 2E6. A, inhibition of Aβ42 fibrillation. freshly dissolved Aβ42 substrate treated with nIgV 2E6 (4 μg/ml) but not nIgV MMF6 showed reduced ThT binding after 48 h, **, p < 0.01 versus IgV MMF6. ThT fluorescence values of Aβ treated with nIgV MMF6 and diluent after 48 h were indistinguishable (p > 0.1). B, inhibition of Aβ42 oligomerization. The intensities of the SDS-stable trimer and tetramer were reduced by nIgV 2E6 treatment (24 h) of Aβ42 that had not been subjected to prior oligomerization compared with an equivalent nIgV MMF6 concentration (3 μg/ml). Higher order SDS-stable oligomers were less abundant than in preoligomerized Aβ42 preparations. Only the 74-kDa high mass oligomer band was evident, which was reduced significantly by treatment with nIgV 2E6, **, p < 0.005; *, p < 0.03. Inset, Aβ42 oligomers stained with a mixture of Aβ-binding monoclonal antibodies following treatment with nIgV MMF6 (lane 1) or nIgV 2E6 (lane 2). The 2.5-kDa product fragment is visible. C, superior inhibition of Aβ42 fibrillation by nIgV 2E6 compared with IgG1 59. Aβ42 fibrillation tested as in A was inhibited by nIgV 2E6 (1 μg/ml) but not the Aβ-binding ligG1 59 (1 or 30 μg/ml). Shown are ThT fluorescence data as a percentage of the values in the presence of control antibodies with the same scaffold structure as nIgV 2E6 and IgG1 59 (nIgV MMF6 and IgG1 SKT03, respectively), **, p < 0.005. Inset, IgV 2E6 at a concentration displaying readily detectable Aβ hydrolysis and anti-amyloid effects (4 μg/ml) did not bind the Aβ42 monomer or oligomers determined by immunoblotting (lane 4). Lane 5, control immunoblot after treatment of the same Aβ42 preparation with an equivalent control IgV MMF6 concentration. Lane 1, silver-stained trimer and tetramer bands in the Aβ42 preparation. Lanes 2 and 3, the Aβ42 oligomers stained with the Aβ-binding IgG1 59 and control nonimmune IgG1 SKT03, respectively. Error bars, S.D.

FIGURE 5. Reduced brain Aβ following intrabrain nIgV 2E6 injection. A, local plaque burden was reduced 7 days after nIgV 2E6 but not nIgV MMF6 or PBS injection into the right brain neocortex, determined by staining with anti-Aβ antibody (1 μg of nIgV; n = 7 SxFAD mice in the nIgV 2E6 group, n = 4 mice each in the nIgV MMF6 and PBS groups). The change in plaque burden following test treatments was computed as the percentage of plaque burden in the autologous untreated left hemisphere region. p values are indicated. B, images 1 and 2 show representative sections of the nIgV 2E6-injected right hemisphere neocortex and the corresponding tissue area in the control untreated left neocortex, respectively. Rectangles, area in which plaque burden was determined. Error bars, S.E.
the Aβ noncovalent binding epitope and scissile bonds, and (c) conformational transitions in Aβ. Small IgV concentrations were sufficient to hydrolyze particulate Aβ, but diffusional restrictions deaccelerate enzymatic digestion of particulate substrates compared with soluble substrates (50, 51). We acknowledge the potential of varying clearance rates for Aβ substrates compared with soluble substrates (50, 51). We restrict enzymatic digestion of particulate Aβ subtypes in mice treated with algalV MMF6-treated and algalV 2E6-treated mice, respectively. Horizontal bar, 0.25 mm. Arrow, a plaque. D, hippocampal Aβ plaque burden. Aβ removal in algalV 2E6-treated mice from B was confirmed in hippocampus (Hippo) sections (n = 3 mice/group; neocortex data from the same mice are included for reference), *p < 0.05 versus algalV MMF6. Error bars: in A, S.D.; in B and D, S.E.

| TABLE 2 | Aβ content of TgSWDI mouse brain extracts following treatment with algalV 2E6 and MMF6 |
|---------|-----------------------------------------------------------------------------------|
| Aβ40 and Aβ42 contents were measured by ELISA and are expressed per mg of brain tissue (mean ± S.D.). Particulate Aβ contents represent the difference between the observed total and soluble Aβ content. algalV effect size corresponds to the reduction of Aβ content in mice treated with algalV 2E6 expressed as a percentage of the Aβ content in mice treated with control algalV MMF6. p values are from Student’s t test, two-tailed. |
| Aβ species | algalV MMF6 | algalV 2E6 | algalV 2E6 effect size | p |
|------------|-------------|-------------|------------------------|----|
| Soluble Aβ40 | 15.5 ± 2.9 | 11.2 ± 2.6 | 21.3% | <0.04 |
| Soluble Aβ42 | 1.2 ± 0.2 | 0.8 ± 0.2 | 33.3% | <0.01 |
| Particulate Aβ40 | 3,453.5 ± 645.0 | 2,544.9 ± 672.0 | 26.3% | <0.03 |
| Particulate Aβ42 | 369.8 ± 68.2 | 240.1 ± 75.3 | 32.6% | <0.01 |

The Aβ conformational transition is helical (34) and acquires increasing β-sheet character with increasing aggregation of the mature Aβ40/42 peptides (52, 53). Such a conformational transition may explain poor utilization of the soluble APP substrate by the IgV.

In the acquired immunity paradigm, specific antigen binding activity develops over a few weeks by immunogen-driven selection of sequence-diversified V_{L} and V_{H} domains. The paradigm does not explain specific Aβ(29–40) recognition by IgV 2E6. First, the IgV originated from non-aged humans without pathological amyloid accumulation. Second, antigen recognition developed by acquired immune processes occurs at the extensively mutated complementarity-determining regions (CDRs), whereas the IgV contains minimal CDR mutations (14). Third, V_{H} domain CDR3 and proper V_{L}-V_{H} domain pairing are specificity-defining factors in acquired immunity (54), but IgV 2E6 does not contain a V_{H} domain, and pairing of an Aβ hydrolytic V_{L} domain with a V_{H} domain suppressed the catalytic activity (14). Fourth, Aβ recognition without acquired immunity processes is not limited to catabodies; selective noncovalent binding of the C-terminal Aβ epitope by antibody fragments from “non-immune” humans was described by another group (55). Fifth, IgMs from non-aged healthy humans, the first antibodies produced by B cells, hydrolyzed Aβ specifically, whereas the IgGs produced by differentiated B cells were poorly catalytic (14). Similarly, IgGs induced by routine immunization with various antigens are poorly proteolytic, and immunization with transition state analogs induced only esterase IgGs that stabilized the transition state noncovalently, not catabodies capable of the complex peptide bond hydrolysis reaction (56). Together, these findings indicate specific Aβ hydrolysis as an innate immune property.

Microbial B-cell superantigens provide a precedent for specific Aβ recognition by subsets of innate catabodies (12) and conventional antibodies (57). In addition, the production of innate amyloid-directed catabodies is not limited to the Aβ target. Healthy humans synthesize catabodies specific for transthyretin amyloid, which is responsible for age-associated systemic amyloidosis (19). Humans produce no more than 100,000 non-antibody proteins, including enzymes and receptors expressing specificity for diverse biological ligands. In comparison, the combinational antibody repertoire derived from the germ line V, D, and J segments is far larger (~4 × 10^{9} V_{L}-V_{H} domain pairs derived by recombination of 152 V_{L} with 19 J genes and recombination of 273 V_{H} genes with 34 D and 13 J genes; germ line gene numbers from IgBLAST). It is reasonable,
therefore, to view the germ line V domains as a source of catabodies with varying selectivity for individual substrates.

If amyloid-specific catalysis is an innate immunity function in humans, this implies the existence of selective pressures guiding Darwinian evolution of the antibody germ line genes. Amyloidogenic proteins form harmful aggregates within minutes to days in the test tube, suggesting the existence of homeostatic mechanisms that control amyloid accumulation in vivo. The accumulation of misfolded proteins may begin decades prior to the appearance of disease symptoms (e.g. oligomeric and particulate Aβ in humans (58) and monkeys (59) of reproductive age). Early amyloidosis prior to reproduction will jeopardize survival of the species. This suggests a survival advantage gained from innate amyloid-clearing catabodies. Details of catabody evolutionary history have not been determined, but both Aβ and antibodies are ancient molecules. The human Aβ(29–40) epitope recognized by IgV Z6E is completely conserved in the cartilaginous fish *Narke japonica* (corresponding to residues 629–642 of amyloid precursor protein, GenBank accession number BAA24230.1). These fish appeared about 450 million years ago and represent the first extant organisms with antibody V genes bearing discernible sequence identity to the human V genes (60). Together, these arguments support our view of innate amyloid-hydrolyzing catabodies found in humans as functionally important mediators that may be useful for therapy of age-associated amyloidosis.

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REFERENCES

1. Haass, C., and Selkoe, D. J. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer’s amyloid β-peptide. *Nat. Rev. Mol. Cell Biol.* 8, 101–112
2. Attems, J., Jellinger, K., Thal, D. R., and Van Nostrand, W. (2011) Review: sporadic cerebral amyloid angiopathy. *Neuropathol. Appl. Neurobiol.* 37, 75–93
3. Jellinger, K. A. (2002) Alzheimer disease and cerebrovascular pathology: an update. *J. Neural Transm.* 109, 813–836
4. Bard, F., Cannon, C., Barbour, R., Burke, R. L., 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., Sofroniew, M. V., Vasquez, N., Weiss, K., Welch, B., Seubert, P., Schenk, D., and Yednock, T. (2000) Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat. Med.* 6, 916–919
5. Adolfsson, O., Pilggren, M., Toni, N., Varisco, Y., Buccarello, A. L., Antoniello, K., Lehnmann, S., Piorkowska, K., Gafner, Y., Atwal, J. K., Maloney, J., Chen, M., Gogineni, A., Weimer, R. M., Mortensen, D. L., Friesenhahn, M., Ho, C., Paul, R., Pfeifer, A., Muh, A., and Wats, R. J. (2012) An effector-reduced anti-β-amyloid (Aβ) antibody with unique Aβ binding properties promotes neuroprotection and glial engulfment of Aβ. *J. Neurosci.* 32, 9677–9689
6. Zago, W., Schreoter, S., Guido, T., Khan, K., Seubert, P., Yednoch, T., Schenk, D., Gregg, K. M., Games, D., Bard, F., and Kinney, G. G. (2013) Vascular alterations in PDAPP mice after anti-Aβ immunotherapy: implications for amyloid-related imaging abnormalities. *Alzheimers Dement.* 9, S105–S115
7. Sperling, S., Salloway, S., Brooks, D. J., Tampieri, D., Barakos, J., Fox, N. C., Raskind, M., Sabbagh, M., Honig, L. S., Porsteinsson, A. P., Lieberburg, I., Arrighi, H. M., Morris, K. A., Lu, Y., Liu, E., Gregg, K. M., Brashear, H. R., Kinney, G. G., Black, R., and Grundman, M. (2012) Amyloid-related imaging abnormalities in patients with Alzheimer’s disease treated with bapineuzumab: a retrospective analysis. *Lancet Neurol.* 11, 241–249
8. Salloway, S., Sperling, R., Fox, N. C., Blennow, K., Klund, W., Raskind, M., Sabbagh, M., Honig, L. S., Porsteinsson, A. P., Ferris, S., Reichert, M., Ketter, N., Nejadnik, B., Gruenzer, V., Miloslawsky, M., Wang, D., Lu, Y., Lull, J., Tudor, L. C., Liu, E., Gruneman, M., Yuen, E., Black, R., and Brashear, H. R. (2014) Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer’s disease. *N. Engl. J. Med.* 370, 322–333
9. Morkuniene, R., Zvirbliene, A., Dalgediene, I., Cizas, P., Jankeviciute, S., Balintyte, G., Jukubka, R., Jankunec, M., Valincius, G., and Borutaite, V. (2013) Antibodies bound to Ab oligomers potentiate the neurotoxicity of Aβ by activating microglia. *J. Neurochem.* 126, 604–615
10. Lei, C., Lin, S., Tao, W., Hao, Z., Liu, M., and Wu, B. (2013) Association between cerebral microbleeds and cognitive function: a systematic review. *J. Neurol. Neurosurg. Psychiatry* 84, 693–697
11. Piazza, F., Greenberg, S. M., Savoiardo, M., Gardinetti, M., Chiapparini, L., Raich, I., Nitrini, R., Sakaguchi, H., Brioni, M., Billo, G., Colombo, A., Lanzani, F., Piscosquito, G., Carrierio, M. R., Giaconne, G., Tagliavini, F., Ferrarese, C., and DiFrancesco, J. C. (2013) Anti-amyloid β autoantibodies in cerebral amyloid angiopathy-related inflammation: implications for
amloid-modifying therapies. Ann. Neurol. 73, 449–458
12. Paul, S., Planque, S. A., Nishiyama, Y., Hanson, C. V., and Massey, R. J. (2012) Nature and nurture of catalytic antibodies. Adv. Exp. Med. Biol. 750, 56–75
13. Shuster, A. M., Golobolov, G. V., Kvashuk, O. A., Bogomolova, A. E., Smirnov, I. V., and Gabibov, A. G. (1992) DNA hydrolyzing autoantibodies. Science 256, 665–667
14. Taguchi, H., Planque, S., Sapparapu, G., Boivin, S., Hara, M., Nishiyama, Y., and Paul, S. (2008) Exceptional amloid β peptide hydrolyzing activity of nonphysiological immunoglobulin variable domain scaffolds. J. Biol. Chem. 283, 36724–36733
15. Fukuchi, K., Tahara, K., Kim, H. D., Maxwell, I. A., Lewis, T. L., Accvittii-Loper, M. A., Kim, H., Ponnazhagan, S., and Lalonde, R. (2006) Anti-β single-chain antibody delivery via adeno-associated virus for treatment of Alzheimer’s disease. Neurobiol. Dis. 23, 502–511
16. Sapparapu, G., Planque, S. A., Nishiyama, Y., Foung, S. K., and Paul, S. (2009) Antigen-specific proteolysis by hybrid antibodies containing promiscuous proteolytic light chains paired with an antigen-binding heavy chain. J. Biol. Chem. 284, 24622–24633
17. Zhang, J., Liu, X., Bell, A., To, R., Baral, T. N., Azizi, A., Li, J., Cass, B., and Durocher, Y. (2009) Transient expression and purification of chimeric heavy chain antibodies. Protein Expr. Purif. 65, 77–82
18. Nishiyama, Y., Taguchi, H., Hara, M., Planque, S. A., Mitsuda, Y., and Paul, S. (2014) Metal-dependent amloid β-degrading catalytic antibody construct. J. Biotechnol. 180, 17–22
19. Planque, S. A., Nishiyama, Y., Hara, M., Sonoda, S., Murphy, S. K., Watanabe, K., Mitsuda, Y., Brown, E. L., Massey, R. J., Primmer, S. R., O’Nuallain, B., and Paul, S. (2014) Physiological IgM class catalytic antibodies selective for transthyretin amloid. J. Biol. Chem. 289, 13243–13258
20. Nishiyama, Y., Karle, S., Mitsuda, Y., Taguchi, H., Planque, S., Salas, M., Hanson, C., and Paul, S. (2006) Towards irreversible HIV inactivation: stable gp120 binding by nucleophilic antibodies. J. Mol. Recognit. 19, 423–431
21. Dahlgren, K. N., Manelli, A. M., Stine, W. B., Jr., Baker, L. K., Krafft, G. A., and LaDu, M. J. (2002) Oligomeric and fibrillar species of amloid-β peptides differentially affect neuronal viability. J. Biol. Chem. 277, 32046–32053
22. Paul, S., Planque, S., Zhou, Y. X., Taguchi, H., Bhatia, G., Karle, S., Hanson, C., and Nishiyama, Y. (2003) Specific HIV gp120-cleaving antibodies induced by covalently reactive analog of gp120. J. Biol. Chem. 278, 20429–20435
23. Oakley, H., Cole, S. L., Logan, S., Maus, E., Shao, P., Craft, J., Guillotzet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L., Berry, R., and Vassar, R. (2006) Intraneuronal amloid-β aggregates, neurodegeneration, and eneurotoxicity from a penetrating injury model. Proc. Natl. Acad. Sci. U.S.A. 103, 505–515
24. Davis, J., Xu, F., Deane, R., Romanov, G., Previti, M. L., Zeiger, K., Zlokovic, B. V., and Van Nostrand, W. E. (2004) Early-onset and robust cerebrovascular accumulation of amloid β-protein in transgenic mice expressing low levels of a vasculotropic Dutch/low mutan form of amloid β-protein precursor. J. Biol. Chem. 279, 20296–20306
25. Miao, J., Xu, F., Davis, J., Otte-Höller, I., Verbeek, M. M., and Van Nostrand, W. E. (2005) Cerebral microvascular amloid β protein deposition induces vascular degeneration and neuroinflammation in transgenic mice expressing human vasculotropinc mutant amloid β precursor protein. Am. J. Pathol. 167, 505–515
26. Li, L., Sun, M., Gao, Q. S., and Paul, S. (1996) Low level formation of potent catalytic IgG fragments mediated by disulfide bond instability. Mol. Immunol. 33, 593–600
27. Sumimoto, M., Shen, R., and Nanus, D. M. (2005) Involvement of neutral endopeptidase in neoplastic progression. Biochim. Biophys. Acta 1751, 52–59
28. Radisky, E. S., Lee, J. M., Lu, C. J., and Koshland, D. E., Jr. (2006) Insights into the serine protease mechanism from atomic resolution structures of trypsin reaction intermediates. Proc. Natl. Acad. Sci. U.S.A. 103, 6835–6840
29. Randall, A. D., Witton, J., Booth, C., Hynes-Allen, A., and Brown, J. T. (2010) The functional neuropathology of the amloid precursor protein (APP) processing pathway. Neuropharmacology 59, 243–267
30. Kasturirangan, S., Boddapati, S., and Siersk, M. R. (2010) Engineered proteolytic nanobodies reduce Aβ burden and ameliorate Aβ-induced cytoxicity. Biochemistry 49, 4501–4508
31. Efthimiopoulos, S., Vassilacopoulou, D., Ripellino, J. A., Tezapisidis, N., and Robakis, N. K. (1996) Cholinergic agonists stimulate secretion of soluble full-length amloid precursor protein in neuroendocrine cells. Proc. Natl. Acad. Sci. U.S.A. 93, 8046–8050
32. Kitazume, S., Yoshihisa, A., Yamaki, T., Oikawa, M., Tachida, Y., Ogawa, K., Imamaki, R., Hagiwara, Y., Kinoshita, N., Takeishi, Y., Furukawa, K., Tomita, N., Arai, H., Iwata, N., Saio, T., Yamamoto, N., and Taniguchi, N. (2012) Soluble amloid precursor protein 770 is released from inflamed endothelial cells and activated platelets: a novel biomarker for acute coronary syndrome. J. Biol. Chem. 287, 40817–40825
33. Barrett, P. J., Song, Y., Van Horn, W. D., Hustedt, E. J., Schafer, J. M., Hadziselimovic, A., Beel, A. J., and Sanders, C. R. (2012) The amloid precursor protein has a flexible transmembrane domain and binds cholesteral. Science 336, 1168–1171
34. Mukherjee, A., Song, E., Kihiko-Ehmnn, M., Goodman, J. P., Jr., Pyrek, J. S., Estus, S., and Hersh, L. B. (2000) Insulysin hydrolizes amloid β peptides to products that are neither neurotoxic nor deposit on amloid plaques. J. Neurosci. 20, 8745–8749
35. Banks, W. A., Farr, S. A., Robinson, S. M., Nonaka, N., and Morley, J. E. (2002) Passage of amloid β protein antibody across the blood-brain barrier in a mouse model of Alzheimer’s disease. Peptides 23, 2223–2226
36. Banks, W. A., Farr, S. A., Morley, J. E., Wolf, K. M., Geylis, V., and Steinritz, M. (2007) Anti-amloid β protein antibody passage across the blood-brain barrier in the SAMP8 mouse model of Alzheimer’s disease: an age-related selective uptake with reversal of learning impairment. Exp. Neurol. 206, 248–256
37. Salloway, S., Sperling, R., Gilman, S., Fox, N. C., Blennow, K., Raskind, M., Sabbagh, M., Honig, L. S., Doody, R., van Dyck, C. H., Mulnard, R., Barakos, J., Gregg, K. M., Liu, E., Lieberburg, I., Schenk, D., Black, R., Grundman, M., and Bainzumab 201 Clinical Trial, I. (2009) A phase 2 multiple ascending dose trial of Bainzumab in mild to moderate Alzheimer disease. Neurology 73, 2061–2070
38. Vasilevko, V., Xu, F., Previti, M. L., Van Nostrand, W. E., and Cribs, D. H. (2007) Experimental investigation of antibody-mediated clearance mechanisms of amloid β in CNS of TgSwDI transgenic mice. J. Neurosci. 27, 13376–13383
39. Doody, R. S., Thomas, R. G., Farlow, M., Iwatsubo, T., Vellas, B., Joffe, S., Kieburtz, K., Raman, R., Sun, X., Aisen, P. S., Siemers, E., Liu-Seifert, H., and Mohs, R. (2014) Phase 3 trials of solanezumab for mild-to-moderate Alzheimer’s disease. N. Engl. J. Med. 370, 311–321
40. Kou, J., Yang, J., Lim, J. E., Pattanayak, A., Song, M., Planque, S., Paul, S., and Fukuchi, K. (2015) Catalytic immunoglobulin gene delivery in a mouse model of Alzheimer’s disease: prophylactic and therapeutic applications. Mol. Neurobiol. 51, 43–56
41. Colcher, D., Pavlinkova, G., Beresford, G., Booth, B. J., Choudhury, A., and Batra, S. K. (1998) Pharmacokinetics and biodistribution of genetically-
engineered antibodies. Q. J. Nucl. Med. 42, 225–241
46. Robert, R., and Wark, K. L. (2012) Engineered antibody approaches for Alzheimer’s disease immunotherapy. Arch. Biochem. Biophys. 526, 132–138
47. Berger, V., Richter, F., Zettlitz, K., Unverdorben, F., Scheurich, P., Herrmann, A., Pfizenmaier, K., and Kontermann, R. E. (2013) An anti-TNFFR1 scFv-HSA fusion protein as selective antagonist of TNF action. Protein Eng. Des. Sel. 26, 581–587
48. Spencer, B. J., and Verma, I. M. (2007) Targeted delivery of proteins across the blood-brain barrier. Proc. Natl. Acad. Sci. U.S.A. 104, 7594–7599
49. Thomas, F. C., Taskar, K., Rudraraju, V., Goda, S., Thorsheim, H. R., Gasch, J. A., Mittapalli, R. K., Palmieri, D., Steeg, P. S., Lockman, P. R., and Smith, Q. R. (2009) Uptake of ANG1005, a novel paclitaxel derivative, through the blood-brain barrier into brain and experimental brain metastases of breast cancer. Pharm. Res. 26, 2486–2494
50. Schurr, J. M., and McLaren, A. D. (1966) Enzyme action: comparison on soluble and insoluble substrate. Science 152, 1064–1066
51. Cury-Bagger, N., Elmerdahl, J., Praestgaard, E., Borch, K., and Westh, P. (2013) A steady-state theory for processive cellulases. FEBS J. 280, 3952–3961
52. Barrow, C. J., Yasuda, A., Kenny, P. T., and Zagorski, M. G. (1992) Solution conformations and aggregational properties of synthetic amyloid β-peptides of Alzheimer’s disease: analysis of circular dichroism spectra. J. Mol. Biol. 225, 1075–1093
53. Petkova, A. T., Ishii, Y., Balbach, J. I., Antzutkin, O. N., Leapman, R. D., Delaglio, F., and Tycko, R. (2002) A structural model for Alzheimer’s β-amyloid fibrils based on experimental constraints from solid state NMR. Proc. Natl. Acad. Sci. U.S.A. 99, 16742–16747
54. Kuroda, D., Shirai, H., Jacobson, M. P., and Nakamura, H. (2012) Computer-aided antibody design. Protein Eng. Des. Sel. 25, 507–521
55. Yoshihara, T., Takiguchi, S., Kyuno, A., Tanaka, K., Kuba, S., Hashiguchi, S., Ito, Y., Hashimoto, T., Iwatsubo, T., Tsuyma, S., Nakashima, T., and Sugimura, K. (2008) Immunoreactivity of phage library-derived human single-chain antibodies to amyloid β conformers in vitro. J. Biochem. 143, 475–486
56. Pollack, S. J., Hsiun, P., and Schultz, P. G. (1989) Stereospecific hydrolysis of alkyl esters by antibodies. J. Am. Chem. Soc. 111, 5961–5962
57. Goodyear, C. S., and Silverman, G. J. (2005) B cell superantigens: a microbe’s answer to innate-like B cells and natural antibodies. Springer Semin. Immunopathol. 26, 463–484
58. Lesnê, S. E., Sherman, M. A., Grant, M., Kuskowski, M., Schneider, J. A., Bennett, D. A., and Ashe, K. H. (2013) Brain amyloid-β oligomers in aging and Alzheimer’s disease. Brain 136, 1383–1398
59. Kalinin, S., Willard, S. L., Shively, C. A., Kaplan, J. R., Register, T. C., Jorgensen, M. J., Polak, P. E., Rubinstein, L., and Feinstein, D. L. (2013) Development of amyloid burden in African Green monkeys. Neurobiol. Aging 34, 2361–2369
60. Greenberg, A. S., Avila, D., Hughes, M., Hughes, A., McKinney, E. C., and Flajnik, M. F. (1995) A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature 374, 168–173
61. Tucker, H. M., Kihiko-Ehmann, M., and Estus, S. (2002) Urokinase-type plasminogen activator inhibits amyloid-β neurotoxicity and fibrillogenesis via plasminogen. J. Neurosci. Res. 70, 249–255