Amyloid β 1–42 deposits do not lead to Alzheimer's neuritic plaques in aged dogs

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In Alzheimer's disease, amyloid β ($A\beta$) is deposited in senile plaques and amyloid angiopathy. Longer $A\beta$ peptides, which extend to residue 42 ($A\beta$ 42), have been suggested to be critical for the seeding of amyloid. Aged dogs develop cerebral vessel amyloid and parenchymal preamyloid lesions. Preamyloid in humans is related to senile plaques, whereas in dogs such

progression is rare. We evaluated the composition of aged canine vessel amyloid and preamyloid both biochemically and immunohistochemically. The vessel amyloid extended mainly to residue 40 ($A\beta$ 40), while preamyloid contained a mixture of $A\beta$ 17–42 and $A\beta$ 42, with minimal $A\beta$ 40. Our results suggest other factors besides $A\beta$ 42 are important for neuritic plaque formation.

INTRODUCTION

Alzheimer's disease (AD) is neuropathologically defined by the deposition of amyloid in the form of senile plaques, congophilic angiopathy and neurofibrillary tangles (reviewed in [1]). The major constituent of both senile plaques and congophilic angiopathy is a 39–44 amino acid residue peptide, amyloid β ($A\beta$) [2–8]. $A\beta$ is found, at low concentrations, as a normal constituent of biological fluids, where it is called soluble $A\beta$ ($sA\beta$) [9–12]. $sA\beta$ is predominantly $A\beta$ 1–40, although shorter and longer sequences exist, including $A\beta$ 1–28 and $A\beta$ 1–42 [13]. Neuritic plaque amyloid was first sequenced in 1985 [3] and was found to extend mainly to $A\beta$ 42/43, while vascular amyloid was initially reported to extend to $A\beta$ 1–28 [2] and later to $A\beta$ 39/40 [5,14]. This heterogeneity at the C-terminus of $A\beta$ was attributed to differences in the local tissue processing. More recent reports using different techniques have also found $A\beta$ 42 in vascular amyloid deposits [15]. $A\beta$ in both types of amyloid deposits has a predominantly β -sheet structure; on the other hand, $sA\beta$ presumably is more random-coil and/or α -helical. An early stage in the development of senile plaques are 'preamyloid' or diffuse plaques [16–18]. This is based in part on studies in Down's syndrome (DS) individuals, where diffuse plaques occur at a very early age, preceding the development of senile plaques [19–21]. Preamyloid deposits are 'cotton-wool-like areas' that are immunoreactive with anti- $A\beta$ antibodies, have irregular borders and are associated with few dystrophic neurites [16–18]. Preamyloid deposits are not stained by Congo Red or Thioflavine S, and ultrastructurally are composed mainly of amorphous, non-fibrillar material. Aged individuals can have large numbers of diffuse plaques without any signs of neuronal dysfunction or dementia [22–24]. We hypothesize that senile plaque formation can be viewed as having at least two major steps, whereby $sA\beta$ first undergoes a conformational change and aggregates into preamyloid deposits and this is followed by a gradual process of compaction and fibrillization over many years. It is only in this form that significant toxicity has been documented with synthetic

$A\beta$ peptides *in vitro* [25–27]. An alternative hypothesis is that amyloid formation is a reactive process in AD [1]. Previous immunohistochemical studies using antibodies specific to either the C-terminus of $A\beta$ 40 or $A\beta$ 42 have suggested that $A\beta$ 42 predominated in preamyloid deposits [28–31]. This has led to the suggestion that $A\beta$ 42 is critical for the initiation and development of neuritic plaques [32].

We have chosen to evaluate the composition of preamyloid deposits in the aged canine model of $A\beta$, because dog $A\beta$ has the same sequence as human $A\beta$, and numerous previous studies have documented that aged dogs develop pathology very similar to human congophilic angiopathy and some familial AD forms such as Hereditary Cerebral Haemorrhage with Amyloidosis, Dutch type [33–36]. Aged dogs develop extensive vessel amyloid and parenchymal preamyloid deposits, however, Congo Red-positive parenchymal $A\beta$ plaques are very rare [35,36]. We have used both direct biochemical and complementary immunohistochemical methods with polyclonal antibodies specific to either $A\beta$ 40 or $A\beta$ 42 to clarify the composition of canine vascular amyloid and preamyloid. From this we can evaluate whether, like the human, the predominant $A\beta$ species in preamyloid is $A\beta$ 42. More importantly, we can clarify whether longer sequences of $A\beta$, such as $A\beta$ 42, are sufficient in themselves to lead to neuritic plaques, since dogs only rarely develop such lesions.

MATERIALS AND METHODS

Canine brain tissue

Brain tissue from the following dogs was used: a male Brittany spaniel aged 18 years (dog 1), a male mixed terrier aged 16 years (dog 2), a golden retriever female aged 6–7 years (dog 3), a female boxer aged 12 years (dog 4), a male German shepherd aged 10 years (dog 5), a female cocker spaniel aged 12 years (dog 6), a male poodle aged 15 years (dog 7), a male beagle aged 17 years (dog 8), a male beagle aged 15 years (dog 9), a female beagle aged

Abbreviations used: AD, Alzheimer's disease; $A\beta$, amyloid β ; $A\beta$ c, $A\beta$ 17–42; $sA\beta$, soluble $A\beta$; LDMS, laser desorption mass spectroscopy; DS, Down's syndrome; RP-HPLC, reverse-phase HPLC; TFA, trifluoroacetic acid; PTH, phenylthiohydantoin; FCS, fetal calf serum; ECL, enhanced chemiluminescence.

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15 years (dog 10) and a female beagle aged 16 years (dog 11). Fresh frozen material was available from dogs 1–3 and dogs 8–11, while 4% paraformaldehyde-fixed paraffin-embedded material was available from dogs 1–7. Dog 1 and dogs 4–11 had extensive Congo Red-negative parenchymal A β immunoreactive deposits, as well as extensive congophilic angiopathy, while neither dog 2 nor 3 had A β immunoreactive lesions.

Leptomeningeal amyloid extraction

The following procedure [7] was used for each of the seven dogs where frozen material was available. A 3.0–6.0 g portion of vessels was carefully dissected and washed four times in 200 ml of 0.1 M Tris/HCl (pH 7.4)/2 mM EDTA at 4 °C with the following protease inhibitors: 200 μ g/ml PMSF, 1 μ g/ml leupeptin, 1 μ g/ml pepstatin and 1 μ g/ml aprotinin, and pelleted at 5000 *g* for 10 min at 4 °C. The material was then collected by filtration through 45 μ m mesh. This material was washed in 100 ml of 1% SDS/0.1 M Tris/HCl, pH 7.4, with protease inhibitors three times, and pelleted as above. The pellet was homogenized in 0.1 M Tris/HCl, pH 7.4, with 0.02% sodium azide, 2 mg/ml of collagenase (CLS-3; Sigma) and 10 μ g/ml DNase I (Worthington), incubated at 37 °C for 16 h, then washed three times in 0.1 M Tris/HCl, pH 7.4, and pelleted. The tissue was then suspended in 1.0 M sucrose and applied to a discontinuous sucrose gradient of equal volumes of 1.2 and 1.8 M sucrose, followed by centrifugation at 5000 *g* for 30 min at 10 °C. The pellet was completely dissolved in 100% glass-distilled formic acid over 1 h. The formic acid was partially evaporated under a stream of nitrogen.

The material was then applied to a C₄ reverse-phase (RP)-HPLC column (Vydac, 2.1 mm $\times$ 25 cm) on an Applied Biosystems 130A protein analyser. A gradient from 20% acetonitrile/0.1% trifluoroacetic acid (buffer A) to 80% acetonitrile at 200 μ l/min was applied over 60 min. Fractions were monitored at 220 nm. Each of the eluted fractions were assessed for the presence of A β peptides by SDS/PAGE and immunoblotting. A portion of the fractions was freeze-dried and solubilized in Laemmli sample buffer under reducing conditions. Samples were boiled and run on a 16% Tris/Tricine SDS/PAGE system [37]. The separated proteins were transferred to a nitrocellulose membrane by electroblotting. The membrane was blocked by 5% non-fat milk in 150 mM NaCl/20 mM Tris/0.05% Tween 20, pH 7.4 (TBST), and probed with monoclonal antibody 6E10, which is directed against residues 1–16 of A β [38]. After incubation with horseradish peroxidase-labelled sheep anti-(mouse immunoglobulin), antibody binding was visualized with an enhanced chemiluminescence detection system (ECL; Amersham).

All steps of the purification procedure were also followed by Congo Red staining. A β -containing fractions were subjected to N-terminal sequencing on a 477A protein sequencer and the resulting phenylthiohydantoin (PTH) derivatives identified using an on-line PTH-derivative analyser (Applied Biosystems). A β -containing fractions were also subjected to mass spectroscopy. Laser desorption mass spectroscopy (LDMS) was carried out on a VG/Fisons ToFSpec mass spectrometer that was operated in the +ve linear mode at an accelerating voltage of 25 kV and was equipped with a nitrogen laser (337 nm) and a 0.65 m linear-flight tube [39]. For each sample, two 1.5 μ l aliquots were added to the top of 1.0 μ l of α -cyano-4-hydroxycinnamic acid matrix solution that had been spotted on a new target. The laser beam was aimed at several different spots within each position. Over 30 shots were averaged for each spectrum, with over five spectra being acquired for each sample. The LDMS instrument was

calibrated with synthetic peptide A β 1–42 (*m/z* = 4514.1), gramicidin S (*m/z* = 1142.5) and insulin (*m/z* = 5734.5).

Preamyloid extraction

The subsequent procedure which was a modification of the protocol of Gowing et al. [40], was followed for dogs 1–3 and 8–11. Approx. 20 g of cerebral cortex was dissected free of any vessels with the use of a dissecting microscope. The material was homogenized in a Dounce homogenizer and a blender in 10 vol. of TB buffer (0.1 M Tris/HCl, pH 7.4, 1% SDS and 5 mM EDTA, with 0.02% sodium azide, 1 μ g/ml leupeptin, 1 μ g/ml pepstatin A, 100 μ g/ml PMSF and 1 μ g/ml aprotinin). To this buffer a 30% SDS solution was added to obtain a final concentration of 15% SDS. This solution was stirred for 36 h, followed by filtration through a series of 350 μ m, 150 μ m, 70 μ m and finally 30 μ m nylon meshes, in order to remove vessel contamination. The filtrate was resuspended in TB and centrifuged at 135000 *g* for 2 h at 20 °C. The resultant pellet was suspended in TB and centrifuged at 1000 *g* for 15 min. The resultant pellet was washed in 0.1 M Tris/HCl, pH 8.0, four times and digested by collagenase (CLS-3) (0.2 mg/ml; Worthington) and DNase I (10 μ g/ml; Worthington) in the presence of 2 mM CaCl₂ for 16 h at 37 °C. This material was then subjected to purification according to a protocol for neuritic plaques [7].

The supernatant from the 1000 *g* centrifugation was re-centrifuged for 1 h at 20 °C at 135000 *g*. The resultant pellet (P-135) was dissolved in 80% formic acid. To remove lipids, 1.5 ml of chloroform was added. After vigorous mixing the material was centrifuged at 1500 *g* for 15 min. The top layer was reduced in volume to 1 ml and applied either to a C4 RP-HPLC column (Vydac, 2.1 $\times$ 250 mm), as described for the leptomeningeal extraction or to a Pharmacia FPLC Superose 12 column (30 $\times$ 1 cm). The chromatography was performed in 80% formic acid at 0.4 ml/min and recorded at 280 nm. The material from the Superose 12 column eluting at a volume corresponding to less than 10 kDa was then applied to the C4 RP-HPLC column as above.

The 135000 *g* supernatant from the initial preparation was treated as previously described [40]. The resulting P-275 and P-500 pellets were applied to either a Superose 12 column or to the C4 RP-HPLC column as described for the P-135. All A β -containing fractions were subjected to amino acid sequencing and LDMS as detailed for the leptomeningeal extraction. All the steps of the purification procedure were followed for A β by fluorograms (ECL; Amersham) as described above.

Immunohistochemical studies

Paraffin sections (5 μ m) of dog fronto-parietal cortex were subjected to immunohistochemical staining by deparaffinization followed by treatment with 100% formic acid for 20 min. The slides were then blocked in PBS, 0.1% Tween 20 and 10% fetal calf serum (PBST-FCS). After washing sequential sections, the following primary antibodies were applied: BC40 (1:500), BC42 (1:500), 4G8 (1:200) and 6E10 (1:15000) in PBST-FCS. A sequential section was also subjected to Congo Red staining. 4G8 recognizes residues 17–24 of A β , while 6E10 recognizes residues 1–17 of A β [38]. BC40 specifically immunoreacts with the C-end of A β 40 and BC 42 specifically labels the C-end of A β 42 [31]. The secondary antibodies used were goat anti-rabbit IgG, biotin conjugated (1:800) (Sigma) and goat anti-mouse IgG, biotin conjugated (1:200) (Sigma), followed by streptavidin–horseradish peroxidase conjugate (1:300) (Amersham) in PBST-FCS. The slides were developed in

diaminobenzidine and H_2O_2 , with cobalt chloride hexahydrate (0.0005%) enhancement. $A\beta$ immunoreactive parenchymal lesions and $A\beta$ -positive vessels were counted in twenty 1 mm^2 areas, on sequential slides for each of the four primary antibodies. The number of immunoreactive cerebral vessels or preamyloid lesions for each of the 6E10, BC40 and BC42 antibody sections was matched to the adjacent 4G8 sections.

RESULTS

Purification of $A\beta$

Using the methods described, leptomenigeal and preamyloid $A\beta$ were purified from dog 1 and dogs 8–11. During the extraction procedure the material from the leptomenigeal vessels showed the characteristic apple-green birefringence of amyloid when viewed under polarized light after Congo Red staining. The dog preamyloid material was Congo Red negative, but was immunoreactive with anti- $A\beta$ antibodies (see insert Figure 1). Dog 1 and dogs 8–11 had numerous 4G8 immunoreactive parenchymal lesions, all of which were Congo Red negative.

The leptomenigeal pellet extracted from the sucrose gradient was highly enriched with $A\beta$ peptides. This was then purified on the C_4 RP-HPLC column. The $A\beta$ peptides eluted at 36–42% acetonitrile. After the purification for preamyloid, $A\beta$ peptides were found in pellets P-135 and P-275, with the greatest yield in P-135. Application of the partially purified $A\beta$ peptides directly onto the C_4 RP column gave a higher yield but with slightly more contaminants than when the material was first applied to the Superose 12 column. From the RP-HPLC, $A\beta$ peptides eluted at 37–43% acetonitrile, with an additional major peak eluting at 72% acetonitrile (Figure 1). All the $A\beta$ -containing fractions were subjected to N-terminal amino acid sequencing and MS.

Table 1 The peptides that were obtained from the canine leptomenigeal amyloid extraction as evidenced by N-terminal amino acid sequencing and MS are depicted

The observed (obs.) masses are shown in column 2, with the corresponding calculated (calc.) masses in column 3. * Indicates methionine oxidation: +16 mass units; † indicates formylation: +28 mass units.

$A\beta$ peptide	Obs. mass	Calc. mass	Yield (%)
1–40	4329.1	4329.8	52%
4–40	4013.8	4014.6	6%
1–42	4530.8	4530.1*	27%
4–42	4223.1	4226.8†	15%

Synthetic peptides $A\beta$ 1–40, $A\beta$ 1–42 and $A\beta$ 17–42 ($A\beta$ c) were also run in this HPLC system. It was found that $A\beta$ 1–40 eluted at 37–40% acetonitrile, $A\beta$ 1–42 at 39–43% acetonitrile and $A\beta$ c at 72% acetonitrile.

Amino acid sequencing and MS analysis of $A\beta$

N-terminal Edman degradation sequencing of the leptomenigeal $A\beta$ yielded two sequences, starting at the Asp at residue 1 and the Phe at residue 4 of $A\beta$. The sequences obtained were: DAEF-RHDSGYEVHHQKLVFFAEDVGSNK and FRHDSGYEV-HHQKLVFFAEDV. LDMS yielded the masses shown in Table 1. The mass corresponding to the $A\beta$ 1–42 includes 16 units for methionine oxidation and the mass corresponding to $A\beta$ 4–42 includes 28 units for serine formylation. The percentage yield of

Figure 1 The elution profile of the extracted canine preamyloid, run on a C_4 RP-HPLC column

$A\beta$ peptides eluted at 37–43% acetonitrile with a second peak at 72% acetonitrile. Insert: a fluorogram (ECL; Amersham) developed using 4G8 (recognizes $A\beta$ 17–24) as a primary antibody. In lane 1, $A\beta$ peptides eluting at 39% acetonitrile were run and 4 and 8 kDa bands can be seen. In lane 2, $A\beta$ peptides eluting at 42% acetonitrile were run and only the 8 kDa dimer band of $A\beta$ can be seen. In lane 3, $A\beta$ peptides eluting at 72% acetonitrile were run. A 2.5 kDa band can be seen, corresponding to $A\beta$ c. In lane 4, 200 ng of synthetic $A\beta$ 1–42 was run. On the right side of the insert molecular-mass markers are shown in kDa.

Figure 2 Mass spectrum of A β peptides purified from canine preamyloid by RP-HPLC

Upper panel: dog 1 peptides eluted at 38–43% acetonitrile. Insert: observed (obs.) and calculated (calc.) molecular masses of A β peaks A–E with their predicted sequences. * Indicates methionine oxidation (+16 mass units). Lower panel: A β peptide eluted at 72% acetonitrile corresponding to A β c; observed and calculated mass is 2577.1.

each peptide was estimated from the signal intensity of the peptides obtained by MS and the yield from peptide sequences obtained by Edman degradation.

N-Terminal Edman degradation sequencing of the preamyloid A β , eluting at 37–43% acetonitrile, yielded four sequences that start at the Asp, Phe, Ser and Glu residues corresponding to residues 1, 4, 8 and 11 of A β respectively. The sequences obtained were: DAEFRHDSGYEVHHQKLVFFAEDVGV, FRHDSGY-EVHHQKLVFFAEDVGSNK, EVHHQKLVFFA and SGYE-VHHQK. LDMS yielded MS peaks of the fractions eluting from 37–43% acetonitrile (Figure 2A) that corresponded to A β 4–42, 1–42, 1–40, 11–42 and 8–42. The masses obtained for A β 4–42 at 4213.8, A β 1–42 at 4530.5 and A β 8–42 at 3658.9 indicate methionine oxidation.

N-Terminal Edman degradation sequencing of the preamyloid material eluting at 72% acetonitrile yielded a single sequence

corresponding to A β starting at residue 17: LVFFAEDVGSN. MS of this fraction yielded a single peak of mass 2577.1 (Figure 2B), which corresponds to the expected mass of A β c of 2577.1. The approximate relative yield of the A β peptides was estimated from the areas of the eluted peaks on RP-HPLC, the yields by N-terminal sequencing and by amino acid analysis. In dogs 8–11 the yields were: A β c, 45%; A β 4–42, 19%; A β 1–42, 17%; A β 11–42, 7%; A β 8–42, 4%; and A β 1–40, 9%. From each 20 g of cortical tissue about 90 μ g of A β c was obtained. However, in dog 1 the yield of A β c was lower, representing approx. 10% of the total A β peptides, suggesting that there is some case-to-case variation.

In the controls (dogs numbered 2 and 3) A β peptides were not obtained by amino acid sequencing or MS.

Immunohistochemistry

In each dog the number of diffuse plaques and congophilic vessels that were immunoreactive with 4G8 antibodies was compared with the number of these lesions that also immunoreacted with either BC40, BC42 or 6E10 (Figure 3). None of the parenchymal canine deposits of A β immunoreactivity were Congo Red positive, while the cerebral vessel deposits showed the characteristic tinctorial properties of amyloid. 4G8 recognizes A β 17–24; 6E10 recognizes A β 1–17 [38]; BC40 recognizes the C-end of A β 40 and BC42 recognizes the C-end of A β 42 [31]. 6E10 immunoreacted with slightly fewer preamyloid lesions than 4G8, and with less intense staining. BC42 immunoreacted with both 4G8 positive preamyloid lesions and cerebral vessels; however, the immunoreactivity in vessels was much less than with the other three antibodies. BC42 consistently immunolabelled a far greater percentage of the preamyloid lesions than BC40, while BC40 labelled more cerebral vessels, although the immunoreactivity was sometimes faint.

DISCUSSION

We choose to study the biochemical composition of preamyloid and vascular amyloid deposits in the aged dog, since these animals are an excellent model to determine the relationship of preamyloid lesions to senile plaques. Senile plaques are very rare in aged dogs [35,36], therefore, the presence or absence of particular A β peptides or other plaque constituents is a helpful indicator of their importance to senile plaque development. Our biochemical extraction and immunohistochemical studies using C-terminus A β -specific antibodies of aged canine leptomenigeal amyloid and preamyloid are in good agreement that A β 40 is the major peptide in the former and that A β 42 predominates in preamyloid deposits. Previous biochemical extractions of human vessel amyloid initially suggested that A β 39/40 is the major or only species [5,7], however, more recent studies using different methods have also shown the presence of A β 42 [15]. We confirm the presence of both A β 42 and A β 40 in our biochemical extraction of vessel amyloid, although A β 40 predominates. The latter is consistent with our and other immunohistochemical studies using polyclonal and monoclonal A β C-terminal-specific antibodies [29–31,41].

Only one study has biochemically extracted preamyloid from human AD patients' brains, isolating predominantly A β c and A β 1–42 [40]. Similar to the human material, both these peptides were present in the dog preamyloid. In four of the five dog brains used for biochemical extractions, the A β c peptide accounted for approx. 45% of the total A β peptide. The abundance of A β c in preamyloid deposits and its apparent absence in leptomenigeal amyloid would suggest that the lack of maturation and the relative, non-toxic nature of preamyloid, can, in part, be

Figure 3 Immunohistochemical studies of aged dog 1 frontal neocortex

(A), (B), (C) and (D) are sequential sections immunoreacted with 4G8, 6E10, BC40 and BC42 respectively. The large vessel at the bottom left of these panels (indicated by an asterisk) was immunoreacted with the first three of these antibodies, with only minimal immunoreactivity with BC42. The match between the parenchymal, preamyloid lesions immunoreacted with 4G8 (A) and BC42 (D) is almost one to one, whereas only one lesion shows immunoreaction with BC40 (C, see arrowhead).

attributed to the presence of this peptide. In support of this view our preliminary studies have found that although $A\beta$ synthetic peptides can form amyloid-like fibrils *in vitro*, it does so less well than either $A\beta$ 1–40 or $A\beta$ 1–42. However, the canine preamyloid lesions contained a substantial amount of longer $A\beta$ peptides, such as $A\beta$ 1–42, $A\beta$ 8–42 and $A\beta$ 11–42, all of which have been previously found in human senile plaques [7] and are known to be highly fibrillogenic *in vitro* [42,43]. In addition, a minor fraction of the biochemically extracted preamyloid was $A\beta$ 40 (less than 10%), in agreement with our and another immunohistochemically based study of AD tissue [31]. Other immunohistochemically based studies of AD and DS brains have not found $A\beta$ 40 in preamyloid, possibly reflecting case-to-case variation [28,41]. The $A\beta$ 40 which we found in preamyloid deposits may arise either as the result of limited proteolysis of already deposited $A\beta$ 42 or have a separate origin from the pool of s $A\beta$, where $A\beta$ 40 is the major $A\beta$ peptide.

Our studies indicate that canine preamyloid contains a significant proportion of $A\beta$ 42. Since canine preamyloid only rarely progresses to neuritic plaques, our results suggest that the presence of longer $A\beta$ peptides is not central for the seeding of amyloid *in vivo*. Therefore the lack of canine neuritic plaques must be related to other factors. It is possible that chaperone proteins, such as apolipoprotein E and α_1 -antichymotrypsin, as well as other factors, such as metal ions, are important in this process [44–47]. Neuritic-plaque formation is a multiple-step

process which proceeds over many years. In AD, as in many of the systemic amyloidoses [48], it is unlikely that the exact length of the amyloid-forming peptide is critical in the disease pathogenesis, and other genetic and environmental factors need to be considered.

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REFERENCES

- 1 Wisniewski, T., Ghiso, J. and Frangione, B. (1994) *Neurobiol. Aging* **15**, 143–152
- 2 Glenner, G. G. and Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **122**, 1131–1135
- 3 Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. and Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245–4249
- 4 Selkoe, D. J., Abraham, C. R., Podlisny, M. B. and Duffy, L. K. (1986) *J. Neurochem.* **146**, 1820–1834
- 5 Prelli, F., Castaño, E. M., Glenner, G. G. and Frangione, B. (1988) *J. Neurochem.* **51**, 648–651
- 6 Roher, A. E., Lowenson, J. D., Clarke, S., Wolkow, C., Wang, R., Cotter, R. J., Reardon, I. M., Zurcher-Neely, H. A., Henrikson, R. L., Ball, M. J. and Greenberg, B. D. (1993) *J. Biol. Chem.* **268**, 3072–3083

- 7 Miller, D. L., Papayannopoulos, I. A., Styles, J., Bobin, S. A., Lin, Y. Y., Biemann, K. and Iqbal, K. (1993) *Arch. Biochem. Biophys.* **301**, 41–52
- 8 Wisniewski, T., Lalowski, M., Levy, E., Marques, M. and Frangione, B. (1994) *Ann. Neurol.* **246**, 245–246
- 9 Haass, C., Schlossmacher, M. G., Hung, A. Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B. L., Lieberburg, I., Koo, E. H., Schenk, D., Teplow, D. B. and Selkoe, D. J. (1992) *Nature (London)* **359**, 322–326
- 10 Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Whaley, J., Swindlehurst, C., McCormack, R., Wolfert, R., Selkoe, D., Lieberburg, I. and Schenk, D. (1992) *Nature (London)* **359**, 325–327
- 11 Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X.-D., McKay, D. M., Tintner, R., Frangione, B. and Younkin, S. G. (1992) *Science*, **258**, 126–129
- 12 Busciglio, J., Gabuzda, D. H., Matsudaira, P. and Yankner, B. A. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2092–2096
- 13 Vigo-Pelfrey, C., Lee, D., Keim, P., Lieberburg, I. and Schenk, D. B. (1993) *J. Neurochem.* **61**, 1965–1968
- 14 Joachim, C. L., Duffy, L. K., Morris, J. H. and Selkoe, D. J. (1988) *Brain Res.* **474**, 100–111
- 15 Roher, A. E., Lowenson, J. D., Clarke, S., Woods, A. S., Cotter, R. J., Gowing, E. and Ball, M. J. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10836–10840
- 16 Tagliavini, F., Giaccone, G., Frangione, B. and Bugiani, O. (1988) *Neurosci. Lett.* **93**, 191–196
- 17 Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M. and Ihara, Y. (1988) *Acta Neuropathol.* **76**, 541–549
- 18 Ikeda, K., Haga, C., Kosaka, K. and Oyanagi, S. (1989) *Acta Neuropathol.* **78**, 137–142
- 19 Giaccone, G., Tagliavini, F., Linoli, G., Bouras, C., Frigerio, L., Frangione, B. and Bugiani, O. (1989) *Neurosci. Lett.* **97**, 232–238
- 20 Mann, D. M. (1989) *Neurobiol. Aging* **10**, 397–399
- 21 Motte, J. and Williams, R. S. (1989) *Acta Neuropathol.* **77**, 535–546
- 22 Crystal, H. A., Dickson, D. W., Sliwinski, M. J., Lipton, R. B., Grober, E., Marks-Nelson, H. and Antis, P. (1993) *Ann. Neurol.* **34**, 566–573
- 23 Davies, L., Wolska, B., Hilbich, C., Multhaup, G., Martins, R., Simms, G., Beyreuther, K. and Masters, C. L. (1988) *Neurology* **38**, 1688–1693
- 24 Delaere, P., Duyckaerts, C., Beyreuther, K., Masters, C., Peitte, F. and Hauw, J. J. (1990) *Neurosci. Lett.* **116**, 87–93
- 25 Kosik, K. and Coleman, P. (1992) *Neurobiol. Aging* **13**, 535–627
- 26 Simmons, L. K., May, P. C., Tomaselli, K. J., Rydel, R. E., Fuson, K. S., Brigham, E. F., Wright, S., Lieberburg, I., Becker, G. W. and Brems, D. N. (1994) *Mol. Pharmacol.* **45**, 373–379
- 27 Ueda, K., Fukui, Y. and Kageyama, H. (1994) *Brain Res.* **639**, 240–244
- 28 Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N. and Ihara, Y. (1994) *Neuron* **13**, 45–53
- 29 Mak, K., Yang, F., Vinters, H. V., Frautschy, S. A. and Cole, G. M. (1994) *Brain Res.* **667**, 138–142
- 30 Murphy, G. M., Forno, L. S., Higgins, L., Scardina, J. M., Eng, L. F. and Cordell, B. (1994) *Am. J. Pathol.* **144**, 1082–1088
- 31 Yamaguchi, H., Sugihara, S., Ishiguro, K., Takashima, A. and Hirai, S. (1995) *Amyloid* **2**, 7–16
- 32 Younkin, S. G. (1995) *Ann. Neurol.* **37**, 287–288
- 33 Wisniewski, T. and Frangione, B. (1992) *Mol. Neurobiol.* **1**, 75–86
- 34 Johnson, E. M., Chaney, M. O., Norris, F. H., Pascual, R. and Little, S. P. (1991) *Mol. Brain Res.* **10**, 299–305
- 35 Wisniewski, H. M., Wegiel, J., Morys, J., Baucher, C., Soltysiak, Z. and Kim, K. S. (1990) in *Alzheimer's Disease. Epidemiology, Neuropathology, Neurochemistry and Clinics* (Maurer, K., Rieder, D. and Beckman, H., eds.), pp. 151–168, Springer-Verlag, Berlin
- 36 Cummings, B. J., Su, J. H., Cotman, C. W., White, R. and Russell, M. J. (1993) *Neurobiol. Aging* **14**, 547–560
- 37 Schagger, H. and Jagow, G. (1987) *Anal. Biochem.* **166**, 368–379
- 38 Kim, K. S., Miller, D. L., Sapienza, V. J., Chen, C. M. J., Bai, C., Grundke-Iqbal, I., Currie, J. and Wisniewski, H. M. (1988) *Neurosci. Res. Commun.* **2**, 121–130
- 39 Williams, K. R. and Stone, K. L. (1995) in *Techniques in Protein Chemistry VI* (Crabbe, J., ed.), vol. 6, pp. 143–152, Academic Press, San Diego
- 40 Gowing, E., Roher, A. E., Woods, A. S., Cotter, R. J., Chaney, M., Little, S. P. and Ball, M. J. (1994) *J. Biol. Chem.* **269**, 10987–10990
- 41 Iwatsubo, T., Mann, D. M. A., Odaka, A., Suzuki, N. and Ihara, Y. (1995) *Ann. Neurol.* **37**, 294–299
- 42 Barrow, C. J., Yasuda, A., Kenny, I. T. M. and Zagorski, M. G. (1992) *Mol. Biol.* **225**, 1075–1093
- 43 Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C. and Glabe, C. (1992) *J. Biol. Chem.* **267**, 546–554
- 44 Bush, A. I., Pettingell, W. H., Multhaup, G., Paradis, M., Vonsattel, J. P., Gusella, J. F., Beyreuther, K., Masters, C. L. and Tanzi, R. E. (1994) *Science* **265**, 1464–1467
- 45 Ma, J., Yee, A., Brewer, H. B., Das, S. and Potter, H. (1994) *Nature (London)* **372**, 92–94
- 46 Wisniewski, T. and Frangione, B. (1992) *Neurosci. Lett.* **135**, 235–238
- 47 Wisniewski, T., Lalowski, M., Golabek, A., Vogel, T. and Frangione, B. (1995) *Lancet* **345**, 956–958
- 48 Castaño, E. M. and Frangione, B. (1991) *Brain Pathol.* **1**, 263–271