Defined neurofilament, τ , and β -amyloid precursor protein epitopes distinguish Alzheimer from non-Alzheimer senile plaques

(cytoskeletal proteins/A4 protein/neurodegenerative disease)

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ABSTRACT Eight antisera and one monoclonal antibody to synthetic peptides that corresponded to domains extending over the entire length of the β -amyloid precursor protein (β -APP), and an antiserum to the full-length 695-amino acid form of the β -APP, were raised to probe the composition of the core and corona of senile plaques (SPs). We localized distinct β -APP domains, including the β -amyloid protein or A4 region, within the SPs of 13 end-stage Alzheimer disease (AD) and 13 age-matched control samples of hippocampus and entorhinal cortex. The composition of SPs also was probed with antibodies to defined epitopes in tau (τ) as well as the large and mid-size neurofilament (NF) proteins. The most important observations were that β -APP domains outside the A4 region were largely restricted to SP coronas in the AD samples, together with τ and NF determinants, whereas the same epitopes were absent from A4-positive blood vessels and exceptionally rare in non-AD SPs. Indeed, samples from a subset of the non-AD cases contained a considerable number of A4-positive SPs totally devoid of any of the other β -APP, τ , and NF epitopes. These observations suggest that the deposition of the A4 protein in AD SPs results from the local processing of β -APPs in association with τ and NF protein fragments. It is unclear whether this association is fortuitous or linked by common mechanisms. However, differences between the complement of β -APP, τ , and NF protein epitopes in AD versus non-AD brains implicate a defect involving one or more steps in the posttranslational modification, degradation, or elimination of these proteins in AD brains, and this may account for the massive numbers of SPs that characterize AD.

Senile plaques (SPs) are constant abnormalities in Alzheimer disease (AD) brains, and it is speculated that SPs evolve from an early, primitive form into a later, mature form in association with neuritic abnormalities (1, 2). Although the major protein component of SPs consists of fibrillar deposits of 4- to 5-kDa peptides termed the β -amyloid or A4 protein (3–5), a number of other components, including cytoskeletal proteins (6–9), were also demonstrated in SPs. However, there is little information on the relative amounts of these components in SPs and their relationship to one another. Further, some A4 deposits—i.e., diffuse plaques—may not form amyloid fibrils or become associated with altered neurites (10–12).

Recent studies have shown that the A4 protein might originate from one or more of three precursor proteins with predicted lengths of 695, 751, and 770 amino acids, respectively (13–16). The predicted amino acid sequence of each of these β -amyloid precursor proteins (β -APPs)—i.e., β -APP₆₉₅, β -APP₇₅₁, and β -APP₇₇₀—are consistent with a membrane-

associated glycoprotein resembling a cell surface receptor protein (13–18), but homologies between the β -APPs and protease nexin II were noted recently (19). Although the mRNAs coding for each of the β -APPs exist in both neural and non-neural cells (1, 2, 13–21), the function of each of the β -APPs remains enigmatic. Further, the mechanisms causing the release of the A4 fragment from the precursor and its deposition in SPs have not been defined. The gene encoding the β -APP on chromosome 21 is not altered in AD, and there is no evidence for an increased β -APP gene dosage in AD (see refs. 1 and 2 for reviews). Thus, the excess deposition of β -amyloid fibrils in AD must be accounted for by other mechanisms, i.e., alterations in the transcriptional, translational, or posttranslational processing of the β -APPs.

In the present study, we developed 10 anti- β -APP antibodies for the immunohistochemical localization of different domains spanning the entire length of the β -APP to develop insights into the composition of the core and corona of SPs. In addition, the relationship of β -APP domains to tau (τ) and neurofilament (NF) proteins in SPs was investigated using additional antibodies raised to these proteins.

MATERIALS AND METHODS

Generation of Rabbit Antisera to Different β -APP Domains. As summarized in Fig. 1, we synthesized 17 different peptides based on amino acid sequences deduced from β -APP₆₉₅, β -APP₇₅₁, and β -APP₇₇₀ cDNAs (13–16). Rabbits were immunized with peptides coupled to porcine thyroglobulin except for an unconjugated 42-amino acid peptide corresponding to the A4 domain. Pre- and postimmune sera were screened against the peptide immunogens by ELISA. It is noteworthy that Up96 and Up100 were raised to amino acid sequences specific only to β -APP₇₅₁ and β -APP₆₉₅, respectively, and that Up121, Up124, and Up125 were raised to peptides restricted to β -APP₇₇₀. Another rabbit antiserum (Up156 in Fig. 1) was raised to the entire unconjugated β -APP₆₉₅ purified from *Escherichia coli* cells (B.D.G. and D.E.L., unpublished work).

Generation of a Mouse Monoclonal Antibody (mAb) to the Extreme Carboxyl-Terminal Region of the β -APPs. A peptide corresponding to the last 20 amino acids at the carboxyl terminus of all three β -APP isoforms was synthesized (BPC in Fig. 1) and coupled with a 15-residue carrier peptide (31D, a rabies N-protein-derived peptide) that stimulates helper T lymphocytes when antigens conjugated to this peptide are presented to B lymphocytes (22). BALB/c mice were injected subcutaneously with 200 μ g of BPC-31D in 100 μ l of phosphate-buffered saline emulsified with an equal volume of Freund's complete adjuvant. Two booster injections with 150

Antibodies	AA residues			Fixatives			MW
	β -APP695	β -APP751	β -APP770	NBF	Bouin	EtOH	
NH2							
Up84	18-35	18-35	18-35	-	-	+	+
Up98	-	281-294	281-294	-	-	+	+
Up121	-	-	343-353	++	++	-	-
Up124	-	-	358-371	++	++	-	-
Up86	301-319	357-375	376-394	-	-	+	+
Up88	490-504	546-560	565-579	-	-	+	+
Up90	597-613	653-669	672-688	++	++	+	+
Up107	597-638	653-694	672-713	++	++	+	+
BPC	676-695	732-751	751-770	-	-	+	+
Up156	1-695	-	-	++	++	+	+
COOH							

μ g of this protein and Freund's incomplete adjuvant were given at 3-week intervals, and 4 days after a final intravenous booster, sciatic lymph nodes were harvested to produce hybridomas as described (23–28). All four hybridomas that produced antibodies to the BPC peptide were subcloned, and one mAb that reacted strongly with SPs in tissue sections was used here.

mAbs to τ and NF Proteins. The four anti- τ and anti-NF mAbs used here have been extensively characterized (23–29). Alz50 is a mAb that recognizes an AD polypeptide known as the A68 protein as well as τ (23, 29). T14 recognizes A68 and a τ epitope (within residues 83–120) that is independent of the phosphorylation state of these proteins (23, 28). RMO87 sees the multiphosphorylation repeat of the middle and high molecular weight NF subunits and also crossreacts with τ in a phosphate-dependent manner, while RMO3 is a phosphate-independent mAb specific for the mid-size NF subunit (24–26).

Gel Electrophoresis and Immunoblotting. Full-length β -APPs were purified from Sf9 (*Spodoptera frugiperda*) cells infected with baculovirus recombinants that contained the coding sequences for β -APP₆₉₅, β -APP₇₅₁, or β -APP₇₇₀ (B.D.G. and D.E.L., unpublished work; ref. 30). The recombinant proteins were run in NaDodSO₄/7.5% polyacrylamide gels, transferred to nitrocellulose replicas, and probed with various anti- β -APP antibodies as reported (23–28). In some experiments, *E. coli* cells that expressed the same β -APPs also were used.

Case Material, Tissue Collection, and Immunohistochemistry. Thirteen AD and 13 control (non-AD) cases were studied. The diagnosis of AD was established using the criteria recommended by the National Institute on Aging (31), as described (23–26). The average age for the AD cases was 71.5 years (range 58–86), while that for the controls was 70.0 (range 55–87). The mean postmortem interval for the control and AD cases was 12.5 and 10.3 hr, respectively. Two of the AD cases had Down syndrome, 1 had Parkinson disease, and 1 had progressive supranuclear palsy. All tissue blocks used for both the initial screening of antibodies and for our epitope analysis were prepared as described (23–28), and 6- μ m-thick paraffin sections were cut for staining. In the initial screening of the anti- β -APP antibodies, we compared their performance in sections from blocks of AD neocortex fixed with NBF, Bouin's solution, or EtOH or by MW denaturation (26–28). Sections were probed with pre- and postimmune sera and the anti-BPC mAb by published immunoperoxidase procedures (23–28). The specificity of each antibody also was confirmed by absorption with its native antigen as detailed elsewhere

(27). Some sections also were pretreated with 90% formic acid for 5 min at room temperature as described (27, 32).

Antibodies that strongly stained SPs in neocortex were selected for the epitope analysis. EtOH- and MW-denatured tissues yielded the best staining results, and many antibodies to different β -APP domains did not stain NBF- or Bouin-fixed tissues (see *Results*). Thus, only AD and age-matched control cases fixed with EtOH or the MW procedure were examined in detail. The non-AD cases exhibited one or more SPs and/or tangles in conventional silver or dye stains. For quantitative epitope analysis, sections from the hippocampus with adjacent entorhinal cortex at the level of the lateral geniculate nucleus were probed extensively with antibodies specific for the A4 and other β -APP domains, including Up84 (1:6000 dilution), Up86 (1:2000), Up107 (1:2000), and anti-BPC mAb (undiluted or 1:10). Some sections were stained with Congo red or thioflavin S following immunostaining. Adjacent sections were also probed with RMO87, T14, Alz50, and RMO3.

Quantification of Immunostained SPs. The total number of immunoreactive SPs labeled by each antibody were counted in nearly serial sections cut from the same block. Counting was carried out twice at either $\times 100$ or $\times 200$ magnification in one section per antibody in each case and averaged. Since hippocampus and entorhinal cortex are severely atrophic in

FIG. 2. Immunoblots of Sf9 cell preparations containing β -APP₇₇₀ (lane 1), β -APP₇₅₁ (lane 2), or β -APP₆₉₅ (lane 3) with 80 μ g of total protein loaded in each lane. Bars at right indicate molecular mass standards (kDa).

end-stage AD, the SP counts in the non-AD cases almost always came from a larger area than in the AD cases.

RESULTS

ELISA. All 17 rabbit antisera and the anti-BPC mAb reacted with the synthetic peptides to which the antibodies were raised. Up107 reacted with synthetic peptides consisting of A4 residues 1–16 and 1–28 as well as with the 42-amino acid peptide to which Up107 was raised, but this antiserum did not bind synthetic peptides consisting of A4 residues 1–12 and 11–25. Up84, Up86, and anti-BPC mAb did not crossreact with the A4 synthetic peptide consisting of residues 1–28. The anti-BPC mAb did not react with the 31D carrier peptide. Up156 reacted with β -APP₆₉₅ purified from both Sf9 insect cells and *E. coli* cells infected with β -APP baculovirus recombinants, but not with any of the synthetic peptides described in Fig. 1.

Immunoblot Analysis. The anti-BPC mAb recognized a single 110-kDa protein in blots of purified β -APP₆₉₅ and 130- to 135-kDa bands in blots of β -APP₇₅₁ and β -APP₇₇₀ (Fig. 2). The rabbit antisera labeled the same bands in these preparations (data not shown). Up121 and Up124 stained only β -APP₇₇₀ when all three baculovirus-derived β -APPs were probed in the same Western blot (data not shown).

Immunohistochemistry. The results of the initial immunohistochemical screening are summarized in Fig. 1, and the following points are most noteworthy. (i) SPs were stained by nine antisera and the mAb, but only Up90, Up107, Up121, Up124, and Up156 labeled SPs in tissues fixed with NBF or Bouin's solution. (ii) Staining with Up90 and Up107 in NBF- or Bouin-fixed tissue sections was dramatically enhanced by formic acid pretreatment, but this had less effect on Up121, Up124, and Up156. (iii) SPs were labeled with Up84, Up86, Up88, Up90, Up98, Up107, Up156, and anti-BPC mAb in

FIG. 3. (A) Coronas of hippocampal SPs labeled with Up86. (B–E) Coronas of hippocampal SPs immunostained with anti-BPC mAb (B) or Up84 (D), respectively, followed by Congo red staining (C and E). (F) RMO3-positive coronas in hippocampal SPs (arrows). (G) T14-positive coronas in hippocampal SPs. (H and I) UP84-positive (H) extraneuronal tangle (arrow) in hippocampus that was Congo red-stained (I). (Bar in A = 25 μ m; B–G are at the same magnification and the bar in B = 10 μ m; H and I are at the same magnification and the bar in H = 20 μ m.)

EtOH- or MW-denatured tissue sections, but formic acid pretreatment did not have an enhancing effect. (iv) Up84, Up86, Up107, and anti-BPC mAb stained more intensely than Up88, Up90, Up98, and Up156. (v) Up107 labeled SPs with the same intensity in the EtOH- or MW-denatured tissue sections as in the formic acid-pretreated NBF- or Bouin-fixed sections. In view of these results, Up84, Up86, Up107, and anti-BPC were selected for detailed quantitative analysis in EtOH- and MW-denatured tissues (see below).

Up107 specifically stained SPs, and absorption of Up107 with the antigen used for immunization abolished this staining. Different types of immunoreactive SPs were demonstrated in both EtOH-fixed and MW-denatured tissue sections, and many of these conformed to the four types noted earlier in NBF-fixed, formic acid-pretreated tissues (11). However, because of their variable morphology, and because Up107 sometimes failed to react with Congo red- or thioflavin S-positive plaque cores in double-stained preparations, a separate enumeration of each of the four types of SPs was not carried out here. As reported elsewhere, diffuse infiltrates occupying large areas of the presubiculum (10), ribbonlike deposits in subpial regions (10, 11, 33), and vascular or perivascular deposits (27, 34) were also found using Up107. Intra-neuronal neurofibrillary tangles were not stained, but a small number (1–5 per section) of extracellular (“ghost”) tangles were immunoreactive with Up107 in four AD cases, as noted earlier (35).

Up84, Up86, and the anti-BPC mAb, which were raised to different domains of β -APP outside the A4 domain, labeled rodlike and punctate profiles in SP coronas as well as small structures in the neuropil of AD sections (Fig. 3 A–E). Little or no staining was seen in SPs and the neuropil of the non-AD sections (see below). Plaque cores identified in the center of AD SPs with Congo red were not labeled by these antibodies (Fig. 3E). The immunopositive profiles were eliminated by absorption of these antibodies with the corresponding immunogens. Blood vessel walls were never labeled by any of these anti- β -APP antibodies, even though vascular amyloid deposits were stained with Up107. Notably, Up84, Up86, and the anti-BPC mAb yielded spotty immunoreactive profiles in a small number (1–3 per section) of “ghost” tangles (Fig. 3 H and I). Up124, an antiserum specific to β -APP₇₇₀, labeled a subset of SPs (cores > coronas) following formic acid pretreatment only in NBF- or Bouin-fixed tissues. Up121 showed staining similar to that of Up124.

Up156, like Up84, Up86, and the anti-BPC mAb, stained SPs in EtOH-fixed and MW-denatured tissues. However, a subset of SP cores and coronas were weakly labeled by Up156 in NBF- or Bouin-fixed tissues following pretreatment with formic acid. Finally, the anti- τ and anti-NF antibodies never stained SP cores, but they did label profiles in the coronas of SPs (Fig. 3 F and G) similar to those produced by Up84, Up86, and the anti-BPC mAb.

Comparison of A4 Protein, β -APP, τ , and NF Epitopes in AD and Non-AD SPs. The quantitative immunohistochemical data for entorhinal cortex are summarized in Table 1; similar data were obtained in hippocampus (not shown). Up107-positive SPs were observed in hippocampus and/or entorhinal cortex of 10 of the 13 controls, where poorly demarcated or diffuse SPs predominated. Notably, antibodies to β -APP domains outside the A4 region did not label SPs in the non-AD tissues except for a few SPs in entorhinal cortex of case 4. None of the mAbs specific for τ and/or NF proteins labeled SPs in control hippocampus or entorhinal cortex except for RMO3. In all of the AD cases, Up107 labeled numerous SPs in hippocampal and entorhinal cortex. Antibodies to β -APP domains outside the A4 region labeled a variety of morphological types of SPs in almost all of the AD cases, but these were generally less numerous than those

Table 1. Quantitative epitope analysis of SPs in control and AD entorhinal cortex

Case	No. of SPs labeled							
	Anti-A4 and anti- β -APP				Anti- τ and anti-NF mAbs			
	Up107	Up84	Up86	BPC	T14	Alz50	RMO87	RMO3
Cont.								
1	10	—	—	—	—	—	—	—
2	>100	—	—	—	—	—	—	—
3	>100	—	—	—	—	—	—	—
4	19	4	4	4	—	—	—	2
5	4	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	>100	—	—	—	—	—	—	—
9	32	—	—	—	—	—	—	—
10	5	—	—	—	—	—	—	—
11	2	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	>100	—	—	—	—	—	—	—
AD								
14	75	—	10	4	26	2	1	—
15	>100	2	30	68	>100	>100	13	2
16	>100	—	30	20	>100	>100	15	—
17	>100	54	36	45	1	—	25	22
18	>100	65	47	23	>100	55	>100	10
19	>100	44	>100	>100	>100	28	46	50
20	>100	40	45	40	>100	60	62	—
21	>100	42	32	32	>100	94	25	2
22	>100	80	27	50	>100	30	>100	35
23	>100	5	4	—	5	16	—	—
24	>100	33	81	47	26	16	30	4
25	>100	15	30	40	65	>100	6	3
26	>100	20	30	20	34	>100	18	9

The 13 control (Cont.) and 13 AD cases are numbered consecutively. The number of SPs labeled with a given antibody in a section of entorhinal cortex is shown.

labeled by Up107. The anti- τ and anti-NF mAbs also labeled a subset of the Up107-positive SPs.

DISCUSSION

The work described here yielded two important findings: (i) the localization of domains outside the amyloidogenic A4 peptide that extend from the carboxyl to the amino terminus of the β -APP within coronas of end-stage AD SPs; (ii) the demonstration of well-defined β -APP, τ , and NF determinants in the coronas of the SPs in end-stage AD that were almost completely absent from non-AD SPs and undetectable in A4-positive blood vessels. This work significantly extends previous studies (9, 12, 27, 33–40) of the molecular composition of AD and non-AD SPs by mapping the distribution of β -APP, τ , and NF protein epitopes to the core or corona of AD versus non-AD SPs in tissues fixed by one of four alternative methods. Notably, NBF-fixed tissues reacted poorly with many of these antibodies.

Since nearly all of the antibodies to domains outside the A4 region of β -APPs exclusively stained the corona of SPs in end-stage AD, the corona of SPs surrounding the A4-positive amyloid core may be the site where fragmentation of β -APPs occurs. This idea is supported by the finding that an antiserum against the whole β -APP₆₉₅ (Up156) produced a labeling pattern in SPs that was very similar to that produced by Up84, Up86, and the anti-BPC mAb in optimally fixed (i.e., EtOH- or MW-denatured) tissues. The absence of β -APP domains other than the A4 peptide in blood vessel walls of the AD cases is noteworthy because it suggests that the proteolytic processing of the β -APP into insoluble A4-rich amyloid filaments may occur directly within AD SPs, but not neces-

sarily in amyloid-laden blood vessels. The extreme paucity of immunoreactivity for the β -APP domains outside the A4 region in non-AD SPs and amyloid-laden blood vessels could be explained by one or more of the following factors: (i) a higher turnover or more rapid clearance of the non-A4 protein domains; (ii) the presence of low levels of these β -APP regions such that they are below the sensitivity of the method used here; (iii) synthesis and processing of these β -APP domains elsewhere with subsequent transport of the A4 fragment alone to blood vessels or sites where SPs are formed in non-AD brains.

The second striking difference between AD and non-AD SPs, the infrequent demonstration of τ and NF epitopes in the coronas of non-AD SPs, suggests that the deposition of the A4 peptide in non-AD brains occurs not only in the relative absence of precursor domains but also without substantial amounts of τ - and/or NF-positive neuritic pathology. Nevertheless, this dissociation between A4 deposits and τ - or NF-positive neuritic pathology was not confined to control brains. Indeed, none of the blood vessel amyloid deposits stained by Up107 in the AD cases were associated with τ or NF immunoreactivity. Thus, with respect to the formation of SPs, we speculate that the deposition of the A4 peptide in the neuropil of control brains may be an age-related process. In contrast, the formation of SP coronas from τ - and NF-rich dystrophic neurites, as well as non-A4 β -APP domains, may be more disease-specific. By extension, one is led to speculate further that blood vessel amyloid deposits, which are not restricted to AD, may not contribute to, or be causally linked with, the formation of SPs in AD. It will be important to pursue these ideas further in order to establish whether or not the findings noted here reflect metabolic abnormalities specific to AD that account for neuron dysfunction and the massive loss of neurons in this disease.

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- Selkoe, D. J. (1989) *Neurobiol. Aging* **10**, 387-395.
- Price, D. L., Koo, E. H. & Unterbeck, A. (1989) *BioEssays* **10**, 69-74.
- Glennner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120**, 885-890.
- Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L. & Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4245-4249.
- Selkoe, D. J., Abraham, C. R., Podlisny, M. B. & Duffy, L. K. (1986) *J. Neurochem.* **46**, 1820-1834.
- Kosik, K. S., Joachim, C. L. & Selkoe, D. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4044-4048.
- Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H. M. & Binder, L. I. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4913-4917.
- Dickson, D. W., Farlo, J., Davies, P., Crystal, H., Fuld, P. & Yen, S.-H. (1988) *Am. J. Pathol.* **132**, 86-101.
- Barcikowska, M., Wisniewski, H. M., Bancher, C. & Grundke-Iqbal, I. (1989) *Acta Neuropathol.* **78**, 225-231.
- Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M. & Ihara, Y. (1988) *Acta Neuropathol.* **76**, 541-549.
- Ikeda, S.-I., Allsop, D. & Glennner, G. G. (1989) *Lab. Invest.* **60**, 113-122.
- Joachim, C. L., Morris, J. H. & Selkoe, D. J. (1989) *Am. J. Pathol.* **135**, 309-319.
- Kang, G., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. & Mueller-Hill, B. (1987) *Nature (London)* **325**, 733-736.
- Ponte, P., Gonzales-DeWitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. & Cordell, B. (1988) *Nature (London)* **331**, 525-527.
- Tanzi, R. E., McClatchey, A. I., Lamperti, E. D., Villa-Komaroff, L., Gusella, J. F. & Neve, R. L. (1988) *Nature (London)* **331**, 528-530.
- Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. & Ito, H. (1988) *Nature (London)* **331**, 530-532.
- Dyrks, T., Weidemann, A., Multhaup, G., Salbaum, J. M., Lemaire, H.-G., Kang, J., Mueller-Hill, B., Masters, C. L. & Beyreuther, K. (1988) *EMBO J.* **7**, 949-957.
- Shivers, B. D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K. & Seeburg, P. H. (1988) *EMBO J.* **7**, 1365-1370.
- Oltersdorf, T., Fritz, L. C., Schenk, D. B., Lieberburg, I., Johnson-Wood, K. L., Beattie, E. C., Ward, P. J., Blacher, R. W., Dovey, H. F. & Sinha, S. (1989) *Nature (London)* **341**, 144-147.
- Bahmanyar, S., Higgins, G. A., Goldgaber, D., Lewis, D. A., Morrison, J. H., Wilson, M. C., Shankar, S. K. & Gajdusek, D. C. (1987) *Science* **237**, 77-80.
- Neve, R. L., Finch, E. A., Dawes, L. R. (1988) *Neuron* **1**, 669-677.
- Otvos, L., Jr., Dietzschold, B., Hollosi, M., Gore, M., Kajtar, J., Niu, H. & Koprowski, H. (1989) *Peptide Res.* **2**, 167-170.
- Kosik, K. S., Orecchio, L. D., Binder, L., Trojanowski, J. Q., Lee, V. M.-Y. & Lee, G. (1988) *Neuron* **1**, 817-825.
- Lee, V. M.-Y., Otvos, L., Jr., Carden, M. J., Hollosi, M., Dietzschold, B. & Lazzarini, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1998-2002.
- Lee, V. M.-Y., Otvos, L., Jr., Schmidt, M. L. & Trojanowski, J. Q. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7384-7388.
- Schmidt, M. L., Lee, V. M.-Y., Hurtig, H. & Trojanowski, J. Q. (1988) *Lab. Invest.* **59**, 460-466.
- Stern, R. A., Otvos, L., Jr., Trojanowski, J. Q. & Lee, V. M.-Y. (1989) *Am. J. Pathol.* **134**, 973-978.
- Trojanowski, J. Q., Schuck, T., Schmidt, M. L. & Lee, V. M.-Y. (1989) *J. Histochem. Cytochem.* **37**, 209-215.
- Wolozin, B. L., Pruchnicki, A., Dickson, D. W. & Davies, P. (1986) *Science* **232**, 648-651.
- Summers, M. D. & Smith, G. E. (1988) *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* (Tex. Agric. Exp. Stn., College Station), Bull. 1555.
- Khachaturian, Z. S. (1985) *Arch. Neurol.* **42**, 1097-1105.
- Kitamoto, T., Ogomori, K., Tateishi, J. & Prusiner, S. B. (1987) *Lab. Invest.* **57**, 230-236.
- Bancher, C., Grundke-Iqbal, I., Iqbal, K., Kim, K. S. & Wisniewski, H. M. (1989) *Neurobiol. Aging* **10**, 125-132.
- Ogomori, K., Kitamoto, T., Tateishi, J., Sato, Y., Suetsugu, M. & Abe, M. (1989) *Am. J. Pathol.* **134**, 243-251.
- Hyman, B. T., Van Hoesen, G. W., Beyreuther, K. & Masters, C. L. (1989) *Neurosci. Lett.* **101**, 352-355.
- Shin, R.-Y., Ogomori, K., Kitamoto, T. & Tateishi, J. (1989) *Am. J. Pathol.* **134**, 1365-1371.
- Perry, G., Lipphardt, S., Kancherla, M., Gambetti, P., Maggiora, L., Lobl, T., Mulvihill, P., Mijares, M., Sharma, S., Cornette, J. & Greenberg, B. (1988) *Lancet* **ii**, 746.
- Tate-Ostrow, B., Majocha, R. E. & Marotta, C. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 745-749.
- Selkoe, D. J., Podlisny, M. B., Joachim, C. L., Vickers, E. A., Lee, G., Fritz, L. C. & Oltersdorf, T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7341-7345.
- Ishii, T., Kametani, F., Haga, S. & Sato, M. (1989) *Neuropathol. Appl. Neurobiol.* **15**, 135-147.