American Journal of Pathology, Vol. 134, No. 5, May 1989
Copyright © American Association of Pathologists

Rapid Communication

Monoclonal Antibodies to a Synthetic Peptide Homologous with the First 28 Amino Acids of Alzheimer's Disease β-Protein Recognize Amyloid and Diverse Glial and Neuronal Cell Types in the Central Nervous System

R. A. Stern, L. Otvos, Jr.,* J. Q. Trojanowski, and V. M.-Y. Lee

From the Department of Pathology and Laboratory Medicine (Neuropathology), The University of Pennsylvania School of Medicine, and The Wistar Institute, Philadelphia, Pennsylvania*

Studies were conducted to identify neural cells that synthesize and/or process cerebral amyloid using antisera and monoclonal antibodies (MAbs) raised to synthetic peptides based on the first 28 amino acids of the amyloid β-protein. Using rabbit and mouse antisera, and 7 MAbs, sections of neocortex, hippocampus, cerebellum, and spinal cord from Alzheimer's disease (AD), Down's syndrome (DS), and control cases were probed. The antibodies produced 3 distinct immunohistochemical patterns: 1) staining restricted to neuritic plaque and blood vessel amyloid only (antisera, 1 of 7 MAbs); 2) immunoreactivity confined to cytoplasmic granules in diverse neuronal, glial (astrocytes, ependyma) and other (leptomeningeal, perivascular, choroid plexus) cells (1 of 7 MAbs); 3) a summation of these 2 patterns (5 of 7 MAbs). Controls resembled the AD and DS cases, except for a paucity of immunoreactive plaques and blood vessels in the controls. Immunoreactivity was reduced or removed by the peptides used to produce these antibodies. Formalin- and Bouins-fixed tissues reacted weakly or not at all with these antibodies while microwave denatured tissues reacted very intensely with them. Specific staining was enhanced by treatment of the tissue sections with Triton X-100, $NaDodSO_4$, or trypsin. These studies significantly extend earlier studies that localized amyloid β-protein precursor mRNA to human brain cells, and they suggest that the β-protein, its precursor, and/or fragments thereof may exist in diverse neural cell types in AD, DS, and control brains. (Am J Pathol 1989, 134:973–978)

Alzheimer's disease (AD) is the most frequent cause of dementia in the elderly, but the etiology and pathogenesis of AD are poorly understood.[1] Brain pathology in AD is typified by abundant extracellular amyloid fibrils in neuritic plaques (NPs), and blood vessels, and by large numbers of neurofibrillary tangles.[2] Identical lesions occur to excess in the brains of Down's syndrome (DS) patients over 40 years old, but they are infrequent in brains of normal elderly subjects.[1,2]

NP and blood vessel amyloid are composed of nearly identical 4.2 kd peptides termed the A4 or β-protein,[3–8] but other proteins (eg, α-1-antichymotrypsin) may be components of brain amyloid.[9] Oligonucleotides based on the β-protein sequence were used to obtain human cDNA clones,[10–17] which localized to chromosome 21.[10–13] The predicted protein displayed features of a transmembrane protein with at least 2 possible glycosylation sites.[10] The carboxy terminal membrane-spanning domain included the β-protein.[10] Other clones with regions homologous to the Kunitz family of serine protease inhibitors, which may represent alternatively spliced

Supported by N.I.H. grants AG-06107, AG-06901, and BRSG grant 05415-27. Mr. R. A. Stern was the recipient of a "Medical Student Extended Study" Pathology Research Fellowship from the Department of Pathology and Laboratory Medicine and from an N.I.H. Short-Term Research Training Grant (5T35-HD 07217).

Accepted for publication March 8, 1989.

Address reprint requests to Dr. V. M.-Y. Lee, Division of Neuropathology, Department of Pathology and Laboratory Medicine, The University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Table 1. *Antibody Characterization*

Antibody	ELISA*	Staining pattern†	Enhancement‡
Rabbit antiserum	++	1	Formic acid§
Mouse antiserum	++	1	Formic acid§
AMY 33	++	1	Trypsin‖
AMY 56	++	3	$NaDodSO_4$‖
AMY 58	+	3	$NaDodSO_4$‖
AMY 62	++	3	Trypsin‖
AMY 67	+	2	Triton X-100‖
AMY 70	++	3	Triton X-100‖
AMY 74	++	3	Triton X-100‖

* Optical density readings of 0.300–0.500 (+) and >0.501 (++) using 0.5 μg of AMI^{1-28} peptide per well.

† Immunoreactivity was seen in NP and vascular amyloid only (1), in the cytoplasm of neural cells only (2), or in NP and vascular amyloid as well as neural cells (3).

‡ The enhancement procedure that most effectively improved staining on NBF-fixed§ or microwave-denatured‖ tissues with a given antibody as described in the text. No improvements were observed using Bouins-fixed tissues, and staining only improved in NBF-fixed tissues pretreated with formic acid and probed with antisera. The microwave-denatured tissues improved with a variety of treatments depending on the MAb used.

mRNA of the amyloid β-precursor protein (ABPP) gene, also were described.[15–17] Thus, multiple forms of the ABPP may exist. These and other observations[18–20] led to speculations that the ABPP is a neuronal plasma membrane molecule,[10,20] a protease inhibitor,[15–17] a peptide ligand,[18] or a heparin sulfate proteoglycan core protein.[19] Although ABPP mRNA were found in human and nonhuman nervous and non-nervous system tissues,[11–17,20–23] no information is available on the neural cells that synthesize and process the β-protein and/or the ABPP in human brain.[9,18,20,24–26] However, recent immunologic data in the rat suggested that neurons and astrocytes may be involved in these events.[20,27] Data presented here indicate that the same cells may play a similar role in the human nervous system.

Materials and Methods

Peptide Preparation

Five peptides homologous to three different regions in the ABPP were prepared. AMY I^{1-28} corresponds to the first 28 amino acids of the β-protein plus an additional N-terminal cysteine necessary for coupling to a carrier protein. These 28 amino acids (aa) correspond to aa 653-680 in the 751 aa long form ($ABPP^{751}$) of the ABPP.[17] AMY I^{1-12} and AMY I^{11-25} correspond to aa 1-12 and 11-25 of the β-protein respectively (aa 653-664 and 663-677 in $ABPP^{751}$). AMY II and III correspond to aa 53-72 and 309-327, respectively, of $ABPP^{751}$. AMY I^{1-28} was synthesized on PAM-resin using double couplings with BOC-amino acid preformed symmetrical anhydrides or pentafluorphenyl esters. AMY II, III, I^{1-12}, and I^{11-25} were synthesized using FMOC-amino acid symmetrical anhydrides.[28] AMY I^{1-28} was coupled to keyhole limpet hemocyanin (KLH) by incorporating a maleimide group into KLH and reacting this modified carrier protein with a 20 molar excess of the peptide.[28]

Generation And ELISA Screening Of Antibodies

Rabbit and mouse antisera and mouse MAbs were generated and screened by ELISA using 0.5 μg of AMY I^{1-28} per well as described.[28] Antibodies selected for study here are summarized in Table 1.

Immunohistochemistry

Antisera (diluted 1:200) and MAb supernatants (applied neat or in a 1:10 dilution) were used to stain 6-μ thick paraffin sections cut from brain tissue denatured by microwave energy, neutral buffered 10% formalin (NBF), or Bouins solution. The tissues came from three AD cases, two adult DS cases, and three controls without DS or AD (see Table 2). The diagnostic, fixation, and immunohistochemical procedures used here have been described.[28,29]

Pretreatment Of Tissue Sections

To expose potentially inaccessible epitopes,[30] we incubated tissue sections in formic acid, detergents, or enzymes before immunohistochemistry. NBF-fixed tissue was pretreated with 90.8% (stock) formic acid for 10 minutes, followed by a wash with 0.5 M TRIS HCl buffer (TB), pH 7.6. Formic acid treatment of Bouins-fixed tissue was identical to NBF-fixed tissue. A variety of similar formic acid pretreatments were used with the microwave denatured tissues. Other pretreatments were applied to the microwave denatured tissues including: trypsin (0.05 mg/ml TB with 0.134 g $CaCl_2$/ml of buffer at 37 C for 30 minutes followed by a 5-minute wash in TB and a 15-minute wash in 2% newborn calf serum in TB), $NaDodSO_4$ (0.1% $NaDodSO_4$ in TB for 30 minutes at 37 C followed by the same washes used with trypsin), and Triton X-100 (1% Triton X-100 in TB for 60 minutes at 37 C with washes identical to those used with trypsin).

Results

Clones were considered to be positive if they produced an optical density equal to or greater than 0.10 at 450 nm by ELISA using AMY I^{1-28} (not KLH coupled). Positive clones were then screened against blank wells and wells

Table 2. *Cases Studied*

Case	Diagnosis	Age	Gender	Fixation	Postmortem interval (hours)
1	DS	67	Male	Microwave	15
2	DS	57	Female	Microwave	15
3	AD	82	Female	NBF, Bouins	7.5
4	AD	74	Male	Microwave	8
5	AD	66	Female	Microwave	15
6	Normal	69	Female	Microwave	13.5
7	Adult polyglucosan body disease	70	Female	Microwave	14
8	Idiopathic Parkinson's disease	66	Female	Microwave	15

One hemisphere from the first AD case (case #3) was fixed with NBF, the other with Bouins solution. The remainder of the case material was denatured by microwave treatment.

coated with either AMY II or AMY III, ie, peptides from unrelated regions of ABPP^{751}. The panel of MAbs selected for study here included those that produced optical densities with AMY I$^{1-28}$ greater than 200% higher than the optical densities obtained when the same MAbs were screened against blank wells or wells coated with the other 2 unrelated peptides (data not shown); 32 such clones were obtained from 2 separate fusions. Polyclonal antisera to AMY I$^{1-28}$ raised in rabbit and mouse behaved similarly. ELISA and immunohistochemical activity of the antibodies was absorbed out by serial incubation with AMY I$^{1-28}$ but not with AMY II. Two different peptides comprised of shorter AMY I$^{1-28}$ sequences, ie, AMY I$^{1-12}$ and AMY I$^{11-25}$, also were used for ELISA screening, and all of the MAbs and antisera failed to detect them.

All ELISA-positive MAbs and the antisera were screened by immunohistochemistry on microwave denatured DS frontal cortex (case 2) with abundant NP and vascular amyloid deposits, and seven MAbs that intensely stained these amyloid deposits and/or neural cells were selected for further study. Both rabbit and mouse antisera also stained NP and vascular amyloid. We then used these seven MAbs and the mouse antiserum to probe mid-frontal cortex, hippocampus, cerebellum, and spinal cord from five additional cases, and hippocampus from case 6 (see Table 1).

As summarized in Table 1, pretreatment of sections improved the staining ability of several of the antibodies, but different agents were found to be effective for different antibodies. Specifically, formic acid improved antiserum staining of amyloids in NBF-fixed tissue, but it did not improve staining by our MAbs, nor did it enhance staining in Bouins- and microwave-denatured tissues. Alternatively, trypsin treatment of microwave-denatured tissue dramatically improved AMY 33 and 62 staining and slightly improved antiserum staining, but it diminished NP amyloid, vascular amyloid, and neural cell staining by all other MAbs. In other studies of the microwave denatured tissues, 1% Triton-X 100 greatly increased the number of plaques and vessels stained by AMY 74, and moderately improved staining by AMY 58, 67, and 70, while 0.1% $NaDodSO_4$ treatment improved staining by AMY 56, 58, and 33. However, $NaDodSO_4$ treatment generally raised the levels of diffuse neuropil and white matter staining for all antibodies. Several generalizations about the staining properties of the MAbs are noteworthy: 1) all MAbs failed to stain Bouins- or NBF-fixed tissue whereas the mouse antiserum stained both; 2) each MAb stained only a subset of the NPs and amyloid-rich blood vessels detected by the mouse antiserum or thioflavin S in microwave fixed tissue; 3) for a given MAb the amount of NP and cerebrovascular amyloid staining relative to the mouse antiserum was consistent within each case from region to region, and 4) although each MAb stained NPs with variable intensity, the staining intensity was consistent across cases. In addition, striking differences were noted among these antibodies: 5 of 7 MAbs stained NP and vascular amyloid as well as granular profiles in the cytoplasm of several types of neural cells (Figure 1A, B, D–F), but the antisera and AMY 33 stained only NP and vascular amyloid (Figure 1C), and AMY 67 stained only granular profiles in neural cells. Although AMY 56, 58, 62, 67, 70, and 74 all stained the cytoplasm of some neurons and glia in all tissues examined, neurons in some cytoarchitechtonic areas were stained more intensely than others (eg, CA2 > CA1 of hippocampus in Figure 1A). However, no qualitative differences were observed between the AD, DS, and control cases using these antibodies. Representative data from these studies are shown in Figure 1.

Notably, neurons were not the only class of cells stained by these antibodies, ie, in all areas examined, perivascular, astrocytic, ependymal, leptomeningeal, and choroid plexus cells were stained intensely. Staining of white matter was variable, but always present. Even among neurons, there were differences in immunoreactivity. For example, in neocortex, all layers stained, with the large neurons of layers III and V being especially immunoreactive. In hippocampus, the staining was intense in fields CA2-4, diminished in field CA1, and became robust again in subiculum. Neuron size did not seem to determine staining intensity; small neurons of the dentate gyrus were highly immunoreactive (Figure 1A), while Purkinje

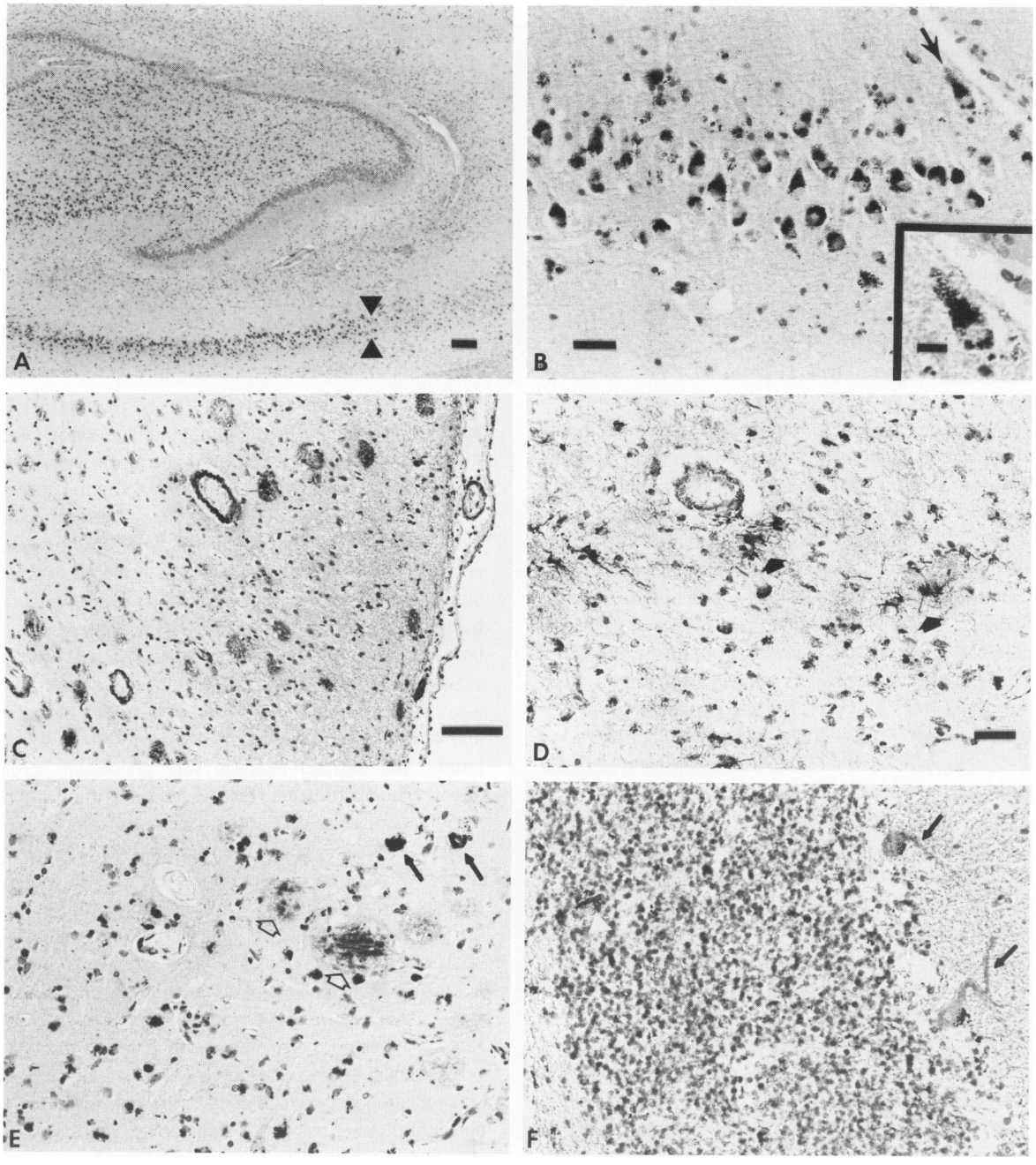

Figure 1. *Representative immunobistochemical data obtained with MAbs and antisera to the AMY I^{1-28} peptide in human tissues denatured by microwave energy. The regions illustrated and the antibodies used are: dentate gyrus and CA1-4 in hippocampus using AMY 62 (A); CA2 using AMY 74 (B); neocortex from middle frontal gyrus using the mouse antiserum (C); AMY 56 (D); and AMY 74 (E); cerebellar cortex using AMY 70 (F). The sections in panels D and E were pretreated with $NaDodSO_4$ and Triton X-100, respectively. The arrowheads in panel A demarcate the more intensely stained pyramidal neurons in the CA2 field (left) from the less intensely stained neurons in the CA1 field (right). The inset in panel B shows the granular staining of the neuron identified with the arrow at higher magnification. The arrows in panel D identify immunoreactive astrocytes. In panel E, the open arrows mark immunoreactive NPs while the closed arrows point to neurons with granular immunoreactivity. Granular immunoreactivity in Purkinje cell bodies and in a Golgi neuron are marked by the arrows and white arrowhead, respectively in F. All the sections were counterstained with hematoxylin. The bar in A = 200 μ, in B = 50 μ, in the inset in B = 10 μ, in C = 100 μ, in D = 50 μ; D to F are at the same magnification.*

cells displayed patchy granular immunoreactivity (Figure 1F). Fine processes of neurons reacted with these MAbs because stippled immunoreactivity was noted in the molecular layer of the dentate gyrus and cerebellum (Figure 1F) with some MAbs. In addition to cerebellum, other brain regions that remain free of amyloid accumulations displayed immunoreactivity. For example, in the spinal cord the large neurons of the grey matter and the ependy-

mal cells of the central canal showed intense staining (data not shown). Furthermore, spinal cord white matter stained like white matter of other regions, ie, glial cytoplasmic immunoreactivity was often noted.

Discussion

The antibodies described here are the first to recognize both amyloid and intracellular antigens in the human brain, although antibodies raised to synthetic peptides based on the ABPP were recently shown to label the cytoplasm of normal brain cells in the rat.[20,27] Our data are likely to reflect the expression of the ABPP and/or the β-protein in amyloid deposits and brain cells of AD, DS, and control brains for the following reasons: 1) the ELISA data showed that the antibodies specifically recognized the AMY I^{1-28} peptide (which is capable of forming amyloidlike fibrils *in vitro*[31]), but not other ABPP peptides (AMY II, III) or the short AMY I^{1-12} and AMY I^{11-25} sequences; 2) antibody reactivity was completely removed by absorption with AMY I^{1-28}, but not with the other peptides; 3) 5 of 7 MAbs labeled typical amyloid deposits as well as diverse neural cells in tissue sections; 4) *in situ* hybridization studies using an ABPP cDNA probe demonstrated ABPP mRNA in populations of neurons in hippocampus and neocortex of both normal and diseased individuals[21-23] similar to those that were labeled by 6 of 7 of our MAbs; 5) Northern blot analyses demonstrated ABPP mRNA in a wide variety of brain regions, including the cerebellum,[11,13,15,16] where we observed immunoreactivity with our MAbs; 6) antibodies raised to synthetic peptides based on aa sequences from other ABPP domains were recently shown to label rat brain neuronal and glial cells[20,27] almost exactly like our antibodies. Although glial, perivascular, and leptomeningeal cells have not been reported to contain ABPP mRNA,[18,21] this may reflect low ABPP mRNA levels in these cells, differences in the sensitivity of *in situ* hybridization *vs.* immunohistochemistry, or the endocytosis of the β-protein or other ABPP derivatives in cell types that do not synthesize the ABPP. Because different tissue denaturation methods and tissue pretreatment protocols differentially enhanced staining by our antibodies, this may reflect conformational changes in the same or similar β-protein derivatives induced by these procedures. These distinct conformations could arise during processing (both normal and aberrant) of the ABPP. Indeed, recent aa sequence data support the concept that β-protein deposits in NPs and blood vessels are not completely identical.[6]

Our observations in human brain, and those recently described in the rat,[20,27] are consistent with the view that the ABPP is produced by diverse neuronal and glial cell types in brain. It is notable that the granular immunoreactivity we observed in human brain samples resembles that seen in the rat,[20,27] and that proteoglycans, a class of molecules to which the ABPP is postulated to belong,[19] are concentrated in cytoplasmic granules.[32] However, the precise nature of the ABPP remains to be determined, and it is unclear which of the diverse cell types stained by the antibodies described here synthesize the β-protein precursor and which cells process it into amyloid deposits. To fully understand the pathogenesis of amyloid deposition in AD and DS it is necessary to obtain more complete information on the metabolism of the ABPP and its derivatives, such as the β-protein, as well as on the cells involved in processing the β-protein into amyloid fibrils.

References

1. Katzman R, Lasker B, Bernstein N: Advances in the diagnosis of dementia: Accuracy of diagnosis and consequences of misdiagnosis of disorders causing dementia, Aging and the Brain. Edited by RD Terry, New York, Raven Press 1988, pp 17–62
2. Selkoe DJ: Protein chemistry of neurofibrillary tangles and amyloid plaques in Alzheimer disease: Relation to the cytoskeleton, Aging and the Brain. Edited by RD Terry. New York, Raven Press 1988, pp 165–181
3. Glenner GG, Wong CW: Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Rsch Comm 1984, 120:885–890
4. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RL, Beyreuther, K: Neuronal origin of cerebral amyloid: Neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J 1985, 4:2757–2763
5. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuher K: Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 1985, 82:4245–4249
6. Prelli F, Castano E, Glenner GG, Frangione B: Differences between vascular and plaque core amyloid in Alzheimer's disease. J Neurochem 1988, 51:648–651
7. Selkoe DJ, Abraham CR, Podlisny MB, Duffy LK: Isolation of low-molecular weight proteins from amyloid plaque fibers in Alzheimer's disease. J Neurochem 1986, 46:1820–1834
8. Castano EM, Frangione B: Human amyloidosis, Alzheimer disease and related disorders. Lab Invest 1988, 58:122–132
9. Abraham CR, Selkoe DJ, Potter H: A serine protease inhibitor, α-1-anti-chymotrypsin, is a component of amyloid deposits in Alzheimer's disease brain. Cell 1988, 52:487–501
10. Kang J, Lemaire H-G, Unterbeck A, Salbaum JM, Masters CL, Grzeschik K-H, Multhaup G, Beyreuther K, Mueller-Hill B: The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987, 325:733–736
11. Tanzi RE, Gusella JF, Watkins PC, Bruns GA, St George-Hyslop P, Van Keuren ML, Patterson D, Pagan S, Kurnit DM, Neve RL: Amyloid β protein gene: cDNA, mRNA distribution,

and genetic linkage near the Alzheimer locus. Science 1987, 235:880–884
12. Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC: Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 1987, 235:877–880
13. Robakis NK, Ramakrishna N, Wolfe G, Wisniewski HM: Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. Proc Natl Acad Sci USA 1987, 84:4190–4194
14. Zain SB, Salim M, Chou W-G, Sajdel-Sulkowska EM, Majocha RE, Marotta CA: Molecular cloning of amyloid cDNA derived from mRNA of the Alzheimer disease brain: Coding and noncoding regions of the fetal precursor mRNA are expressed in the cortex. Proc Natl Acad Sci USA 1988, 85: 929–933
15. Ponte P, Gonzalez-DeWhitt P, Schilling J, Miller J, Hsu D, Greenberg B, Davis WW, Lieberburg I, Fuller F, Cordell B: A new A4 amyloid mRNA contains a domain homologous to serine proteinase inhibitors. Nature 1988, 331:525–527
16. Tanzi RE, McClatchey AI, Lamperti ED, Villa-Komaroff L, Gusella JF, Neve RL: Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. Nature 1988, 331:528–530
17. Kitaguchi N, Takahashi Y, Tokushima Y, Shiojiri S, Ito H: Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. Nature 1988, 331:530–532
18. Allsop D, Wong CW, Ikeda S-I, Landon M, Kidd M, Glenner GG: Immunohistochemical evidence for the derivation of a peptide ligand from the amyloid β-protein precursor of Alzheimer disease. Proc Natl Acad Sci USA 1988, 85:2790–2794
19. Schubert D, Schroeder R, LaCorbiere M, Saitoh T, Cole G: Amyloid β protein precursor is possibly a heparan sulfate proteoglycan core protein. Science 1988, 241:223–241
20. Shivers BK, Hilbich C, Multhaup G, Salbaum M, Beyreuther K, Seeburg PH: Alzheimer's disease amyloidogenic glycoprotein: Expression pattern in rat brain suggests a role in cell contact. EMBO J 1988, 7:1365–1370
21. Bahmanyar S, Higgins GA, Goldgaber D, Lewis DA, Morrison JH, Wilson MC, Shankar SK, Gajdusek DC: Localization of Amyloid β protein messenger RNA in brains from patients with Alzheimer's disease. Science 1987, 237:78–80
22. Lewis DA, Higgins GA, Young WG, Goldgaber D, Gajdusek DC, Wilson MC, Morrison JH: Distribution of precursor amyloid-β-protein messenger RNA in human cerebral cortex: Relationship to neurofibrillary tangles and neuritic plaques. Proc Natl Acad Sci USA 1988, 85:1691–1695
23. Higgins GA, Lewis DA, Bahmanyar S, Goldgaber D, Gajdusek DC, Young WG, Morrison JH, Wilson MC: Differential regulation of amyloid-β-protein mRNA expression within hippocampal neuronal subpopulations in Alzheimer disease. Proc Natl Acad Sci USA 1988, 85:1297–1301
24. Dickson DW, Farlo J, Davies P, Crystal H, Fuld P, Yen S-H: Alzheimer's disease: A double-labeling immunohistochemical study of senile plaques. Am J Pathol 1988, 132:86–101
25. Majocha RE, Benes FM, Reifel JL, Rodenrys AM, Marotta CA: Laminar-specific distribution and infrastructural detail of amyloid in the Alzheimer disease cortex visualized by computer-enhanced imaging of epitopes recognized by monoclonal antibodies. Proc Natl Acad Sci USA 1988, 85:6182–6186
26. Selkoe DJ, Podlisny MB, Joachim CL, Vickers EA, Lee G, Fritz LC, Oltersdorf T: β-amyloid precursor protein of Alzheimer disease occurs as 110- to 135-kilodalton membrane associated proteins in neural and nonneural tissues. Proc Natl Acad Sci USA 1988, 85:7341–7345
27. Card JP, Meade RP, Davis LG: Immunocytochemical localization of the precursor protein for β-amyloid in the rat central nervous system. Neuron 1988, 1:835–846
28. Lee VM-Y, Otvos L, Carden MJ, Hollosi M, Dietzschold B, Lazzarini RA: Identification of the major multi-phosphorylation site in mammalian neurofilaments. Proc Natl Acad Sci USA 1988, 85:1998–2002
29. Trojanowski JQ, Schuck T, Schmidt ML, Lee VM-Y: The distribution of tau in the normal human nervous system. J Histochem Cytochem 1989, 37:209–215
30. Kitamoto T, Ogomori K, Tateishi J, Prusiner SB: Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. Lab Invest 1987, 57:230–236
31. Kirschner DA, Abraham C, Selkoe DJ: Synthetic peptide homologous to β protein from Alzheimer disease forms amyloid-like fibrils in vitro. Proc Natl Acad Sci USA 1986, 83:503–507
32. Fransson LA: Structure and function of cell-associated proteoglycans. Trends Biochem Sci 1987, 12:406–411

Acknowledgment

The authors thank Mr. E. Clark, Ms. L. Lee, Ms. C. Page, Ms. T. Schuck, and Mr. M. Wagner for technical assistance. Dr. R. Gur, Dr. G. P. Pietra, Dr. L. B. Rorke, Mr. P. DiVirgilio and Mr. J. DiRienzi aided in the collection of tissues. Ms. H. Comstock of the Philadelphia Alzheimer's Disease And Related Disorder Association, Inc., Dr. M. Kurtz and associates at the Elwyn Institutes, and the families of the patients studied here made our work possible through their generous efforts to foster research.