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Antibodies to Non-beta Regions of the Beta-amyloid Precursor Protein Detect a Subset of Senile Plaques

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A central unresolved issue in Alzheimer's disease is the origin of the extracellular amyloid β protein (AβP) found in senile plaques and its relationship to the dystrophic neurites that intimately surround it. Here the presence and distribution within senile plaques of various epitopes of the β-amyloid precursor protein (APP) are compared with the distribution of AβP itself and markers for plaque neurites. Several principal findings emerge: 1) antibodies to regions of APP outside of AβP ('APP antibodies') recognize only a subgroup of senile plaques; 2) within these plaques, APP antibodies label discrete globular and granular structures morphologically resembling neurites; 3) virtually all of the plaques labeled by APP antibodies also contain neurites reactive with antibodies to tau; 4) double labeling with anti-tau and an APP antibody shows that the neuritelike profiles stained by the APP antibody are always closely associated with tau-positive neurites within the same plaque and that a minority of profiles appear to be labeled by both antibodies; and 5) antibodies to different regions throughout APP label the same profiles within plaques, suggesting the presence of the full-length precursor. The authors conclude that only a subgroup of senile plaques contain APP epitopes and that the immunostained structures are neurites. Because many AβP-containing plaques in neocortex, cerebellum, and striatum were found to be devoid of any APP labeling, as were vascular AβP deposits, it is unlikely that the extracellular AβP is principally derived from the APP found within dystrophic neurites. The immunodetection of apparently full-length APP, an axonally transported protein, in selected plaque neurites provides yet another protein marker of neuritic dystrophy, possibly indicative of an aberrant regenerative response. (Am J Pathol 1991, 138:373–384)

Spherical deposits of the amyloid β protein (AβP; also called A4) surrounded by dystrophic neuronal processes and glial cells are an invariant feature of Alzheimer's disease (AD). The origin of the AβP within these 'mature' neuritic plaques, and the steps involved in their formation, are subjects of considerable study. Emerging evidence from several laboratories has suggested that amorphous, largely nonfibrillar deposits of AβP may precede the appearance of altered neuritic and glial elements during the development of senile plaques.[1–11] It remains to be proven, however, that these 'diffuse' or 'pre-amyloid' plaques are indeed the forerunners of neuritic, amyloid core-containing plaques rather than a separate plaque type. The normal precursor polypeptide of AβP, the β-amyloid protein precursor (APP), is synthesized in neurons as well as many non-neuronal cells (see, for example, references 12 through 16). In neurons, APP has recently been shown to be transported down axons by fast axonal transport.[17] It has therefore been proposed that the extracellular AβP found in senile plaques is of neuronal origin[18,19] and, more specifically, that it derives from APP accumulating in the distal neurites of the plaques.[17,20] Other investigators have hypothesized that the AβP deposits might instead arise from local microglia,[21] astrocytes[22] or a vascular source.[23,24] The resolution of the relationship of AβP deposits to local cellular elements, particularly neurites, has important implications for the pathogenesis and, ultimately, the treatment of AD.

In this study, we have examined this relationship by simultaneously comparing the immunolabeling characteristics of senile plaques with antibodies to the AβP, to

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various regions of APP outside of AβP ('APP antibodies'), and to sensitive markers for plaque neurites. We found that while virtually all senile plaques are detected by AβP antibodies, only a subpopulation of plaques was recognized by APP antibodies, and the structures labeled by the latter resembled neurites. Large numbers of AβP-positive plaques in some brain regions, eg, dorsal caudatoputamen and cerebellar molecular cortex, showed no reaction with either APP antibodies or several markers for dystrophic neurites. AβP antibodies always labeled larger numbers of plaques in cerebral cortex than APP antibodies or neurite markers, an observation that is most apparent in those AD cases having few neocortical tangles and neuritic plaques. These results indicate that it is unlikely that the AβP deposited in plaques derives from APP present in morphologically detectable dystrophic neurites.

Materials and Methods

Patient Groups

All patients designated as AD carried that clinical diagnosis and fulfilled neuropathologic criteria for AD.[25] Brains of aged individuals without clinical or pathologic brain disease were also examined. Postmortem intervals ranged from 4 to 24 hours and were comparable in AD and control groups.

Tissue Processing

Six-micron sections of briefly formalin-fixed paraffin-embedded brain tissue were prepared. Samples of cerebral cortex, amygdala and hippocampus, caudate and putamen, and cerebellum from fresh brain obtained by autopsy were fixed for 1 hour in 10% neutral buffered formalin and then transferred to TRIS-buffered saline (150 mmol/l [millimolar] NaCl, 50 mmol/l TRIS HCl, pH 7.6) until paraffin embedding. Antigenic determinants for all of the antibodies used in this study were well preserved using this brief fixation period.

Antibodies/Markers

The antibodies used in this study are listed in Table 1. Two AβP antisera were used, one raised to AβP isolated from AD brain and the other raised to synthetic $A\beta P_{1-38}$. Four APP antibodies, spanning the APP from amino- to carboxyl-terminus were also examined, two of which were raised to synthetic peptides (αAPP_{45-62}; $\alpha APP_{676-695}$) and two that were raised to purified bacterial fusion proteins (7H5; $\alpha APP_{444-592}$). The monoclonal antibody, 7H5,[26] is specific for $APP_{751/770}$, having been raised to a bacterially synthesized protein corresponding to the region exhibiting Kunitz protease inhibitory (KPI) activity. It detects protein bands of about 120 to 140 kd on Western blots of membrane extracts of human 293 cells that have been transfected with APP_{751} cDNA[15,27] (Figure 1). These bands correspond to glycosylated full-length forms of APP_{751}[16,27] and comigrate with protein bands detected by the other APP antibodies used in this study (αAPP_{45-62}, $\alpha APP_{444-592}$, $\alpha APP_{676-695}$). The properties of antibody 7H5 will be characterized further elsewhere (Hyman, BT,Tanzi, R, Barbour, R, Schenk, D, submitted for publication). Tau, neurofilament, paired helical filament (PHF), and ubiquitin antibodies were used as neurite markers. The neurofilament monoclonal antibody SMI-34 was obtained from the Sternberger-Meyer Company (Jarrettsville, MD). The biotinylated lectin, *Ricinus*

Table 1. *Antibodies Used in This Study*

Antibody	Immunogen	Species	Dilution
AβP Antibodies			
Antibody A[56,57]	HPLC-purified AD cortical amyloid	Rabbit	1:500
Antibody Y[52]	Synthetic $APP_{597-634}$ = $A\beta P_{1-38}$	Rabbit	1:750
APP Antibodies*			
αAPP_{45-62}[47,58]	Synthetic APP_{45-62}	Rabbit	1:200
7H5[26]	Bacterial fusion protein; specific for Kunitz protease inhibitory domain of $APP_{751/770}$	Mouse	1:200
$\alpha APP_{444-592}$[43]	Bacterial fusion protein ($APP_{444-592}$)	Rabbit	1:1000–1:2000
$\alpha APP_{676-695}$[15]	Synthetic $APP_{676-695}$	Rabbit	1:250
Neurite-labeling Antibodies			
5E2[59]	Human fetal tau	Mouse	1:20
SMI 34	Neurofilaments	Mouse	1:10,000
RT-97[60]	Neurofilaments	Mouse	1:250
DF-2[61]	Ubiquitin	Mouse	1:10
Antibody P[62]	SDS-isolated AD neurofibrillary tangles	Rabbit	1:250

APP, amyloid precursor protein; AD, Alzheimer's disease; HPLC, high-pressure liquid chromatography; SDS, sodium dodecyl sulfate.
* All APP residue numbering is that of the 695 amino acid form of APP except as noted.

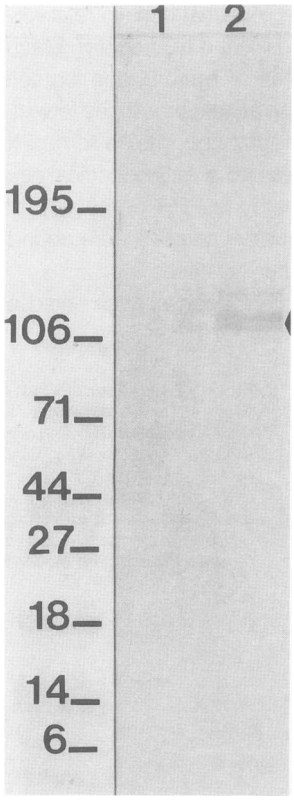


Figure 1. *The monoclonal APP antibody, 7H5, which is specific for $APP_{751/770}$, detects protein bands of ~120–140 kd on Western blots of membrane extracts of human 293 cells that have been transfected with APP_{751} cDNA.[15,27] Lanes: 1, nontransfected 293 cells; 2, APP_{751}-transfected 293 cells. 7H5 immunostains ~120–140-kd protein bands (arrowhead) corresponding to glycosylated forms of APP_{751} that are overexpressed in the APP_{751}-transfected cells. These protein bands comigrated with those labeled by the other APP antibodies used in this study. Molecular weight markers (kd) are shown on the left.*

communis agglutinin 1 (RCA-1) (Vector Laboratories, Burlingame, CA) was used as a marker for microglial cells,[28] as described previously.[4]

Plaque immunostaining by each of the antibodies to APP synthetic peptides (Table 1) was completely abolished by incubating the diluted antiserum with 20 μg of the synthetic peptide per 1 μl antiserum. Absorption of unrelated antisera with the same synthetic peptides (eg, absorption of glial fibrillary acidic protein (GFAP) antiserum with synthetic $APP_{676-695}$) produced no change in their immunostaining. Plaque and vascular amyloid labeling by antibody A (raised to the native, high-pressure liquid chromatography [HPLC]-purified AβP) was completely absorbed with isolated AD amyloid cores[56] in a ratio of 20 μg AD amyloid core protein to 1 μl antibody A. Identical absorptions of unrelated antisera with the isolated AD amyloid cores produced no discernible alteration in their immunoreactivity. Absorptions of $\alpha APP_{444-592}$, raised against a bacterially expressed protein, were accomplished using extracts of *Escherichia coli* cells expressing a 1.3-kb human cDNA encoding $APP_{296-695}$,[15] in a ratio of 500 μg total *E coli* protein to 1 μl $\alpha APP_{444-592}$. Absorption of unrelated antisera with the $APP_{296-695}$-containing *E coli* extract had no effect on their immunolabeling. All absorptions were performed overnight at 4°C.

Immunocytochemistry

TRIS-buffered saline was used as the standard buffer throughout. Endogenous peroxidase activity was blocked by incubating the sections with 0.3% H_2O_2 in methanol for 30 minutes. Primary antibody was applied overnight at 4°C. The avidin:biotinylated horseradish peroxidase complex system (Vector Laboratories, Burlingame, CA) was used to label the bound primary antibody and diaminobenzidine was used as the peroxidase substrate. For double-labeling studies, the avidin:biotinylated alkaline phosphatase complex system (Vector) was used to detect immunostaining by the second primary antibody, using the Vector Red reagent according to the supplier's directions. All sections were counterstained with hematoxylin. Control reactions included the following: 1) preabsorption of each antiserum with its specific antigen, which abolished the plaque immunolabeling of all αAPP antibodies; 2) sections in which primary antibody was omitted (uniformly negative); 3) use of preimmune sera (no specific labeling seen); 4) sections of AD amygdala included as positive controls for αAPP and αAβP senile plaque immunostaining in all experiments; 5) alternating application of first and second primary antibodies to confirm results in double-labeling experiments; 6) omitting either first or second primary antibody in double-labeling studies.

Many of the immunocytochemical comparisons were performed using immediately adjacent 6-μm sections. Failure of a particular antibody (eg, APP antibodies; neurite markers) to label an individual plaque was regarded as meaningful if the same plaque was labeled by an AβP antibody in the adjacent 6-μm sections on either side.

Results

APP Antibodies Detect a Subgroup of Senile Plaques

Antibodies to AβP purified from AD brain (antibody A) or to synthetic AβP (antibody Y) immunostained essentially all senile plaques in briefly formalin-fixed sections of AD cerebral cortex and cerebellum, amygdala, caudate, and putamen, as judged by comparison to a highly sensitive modified Bielschowsky silver stain. In contrast, the antibodies to non-AβP APP epitopes (APP antibodies) rec-

ognized only a subpopulation of plaques (Figures 2 through 4). The APP antibodies recognized few, if any, plaques lacking dystrophic neurites detectable by tau, PHF, neurofilament (NF), or ubiquitin antibodies on adjacent or double-labeled sections. The proportion of plaques labeled by APP antibodies thus varied among AD cases and among different regions within the same AD brain. In general, APP-positive plaques were most numerous in the amygdala. Plaques were detected only by AβP antibodies and not by any of the APP antibodies in two brain regions that contain relatively pure populations of tau-, PHF- and NF-negative plaques, the dorsal caudatoputamen,[5] (Figure 5) and the cerebellar molecular cortex.[4] The AD brains with the fewest αAPP-positive plaques in cerebral neocortex also had few neocortical neuritic plaques and scant neocortical neurofibrillary tangles detectable by tau, PHF, and NF antibodies. Nonetheless AβP-positive plaques were abundant in neocortex of these latter AD cases.

Neocortex from aged nondemented and demented

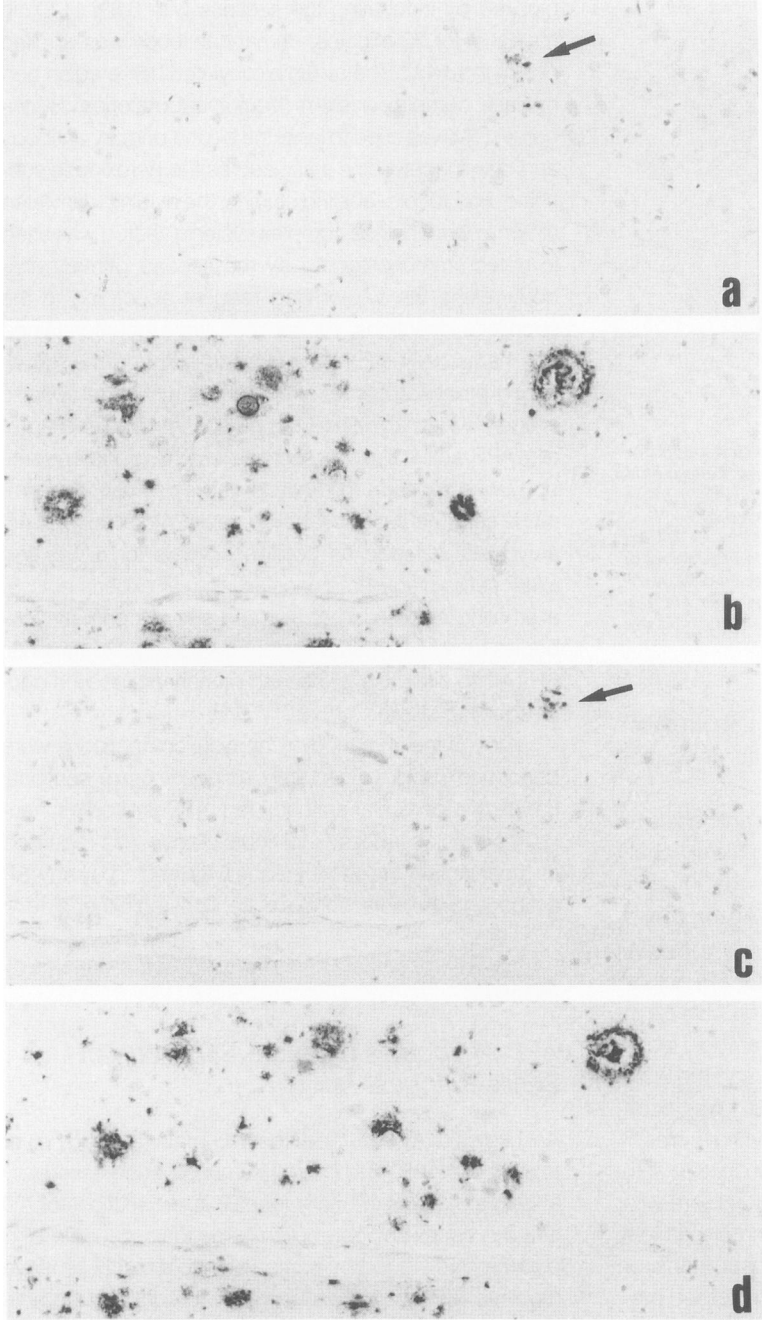


Figure 2. *APP antibodies immunolabel a subgroup of senile plaques.* **a** *to* **d** *are immediately adjacent 6-μm sections of AD parietal lobe cortex.* **a**: $\alpha APP_{444\text{-}592}$; **b**: *antibody Y* ($\alpha\text{-}A\beta P_{1\text{-}38}$); **c**: $\alpha APP_{676\text{-}695}$; **d**: *antibody Y. Only one plaque (arrow) is detected by the αAPP antibodies in this microscopic field containing numerous α-AβP-positive plaques (counterstained with hematoxylin ×50.)*

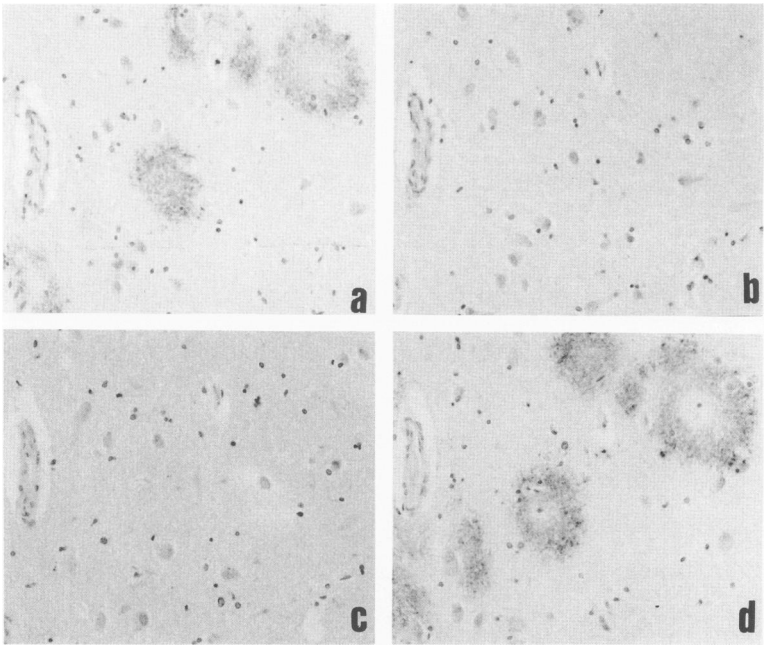


Figure 3. **a** *to* **d** *are adjacent sections of the* CA_1 *segment of AD hippocampus. Plaques immunostained by antibody Y in* **(a)** *and* **(d)** *are not recognized by* $\alpha APP_{444\text{-}592}$ **(b)** *or* $\alpha APP_{45\text{-}62}$ **(c)**. *Plaques labeled by the latter two antibodies were present elsewhere on the tissue sections (×66).*

control subjects, all of whom lacked neocortical plaques on modified Bielschowsky silver staining, also showed no plaque labeling by either APP or tau antibodies. Sections of amygdala from these aged controls showed rare APP-positive plaques in one case in which there were also plaques labeled by AβP and tau antibodies.

The monoclonal ubiquitin antibody, DF-2, recognized a larger number of plaques than did the tau, PHF, NF, or APP antibodies. AβP-positive cerebellar plaques, for example, were labeled only by the ubiquitin antibody, and not by the other examined neurite markers. Not all ubiquitin-positive plaques were recognized by the APP antibodies; thus APP antibodies detected a subgroup of neuritic plaques.

Within Plaques, APP Antibodies Label Structures Morphologically Resembling Neurites

The APP antibodies detected discrete globular and granular profiles having the appearance of dystrophic plaque neurites (Figure 6). Double labeling of sections of AD amygdala, hippocampus, and cerebral cortex with an af-

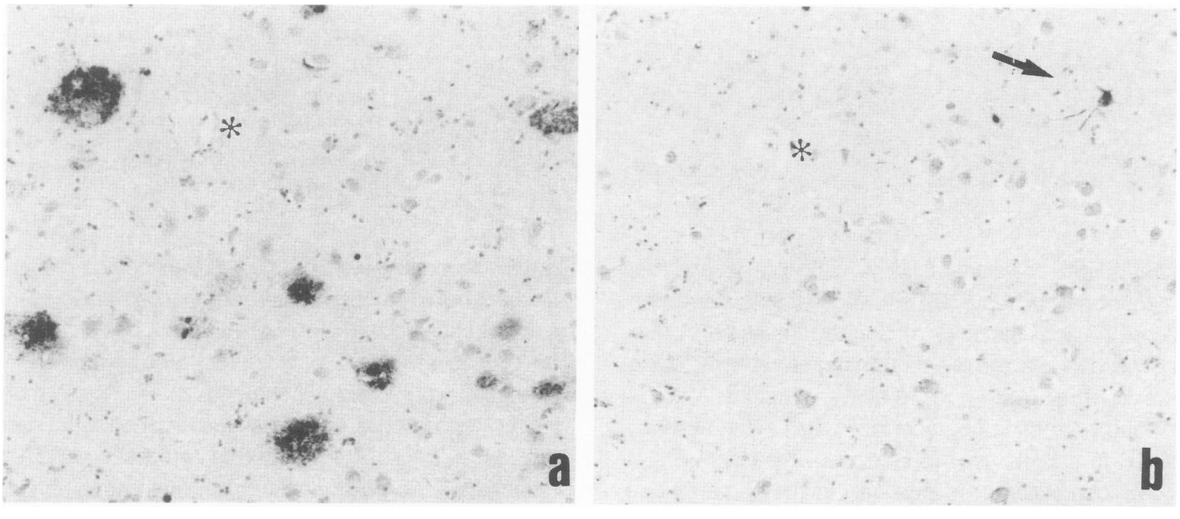


Figure 4. **a** *and* **b** *are adjacent sections of AD amygdala. None of the plaques immunostained by antibody Y in* **(a)** *is labeled by* $\alpha APP_{444\text{-}592}$ **(b)** *though an immunoreactive neuron (arrow) is seen. Asterisks mark a vessel present in both sections (×40).*

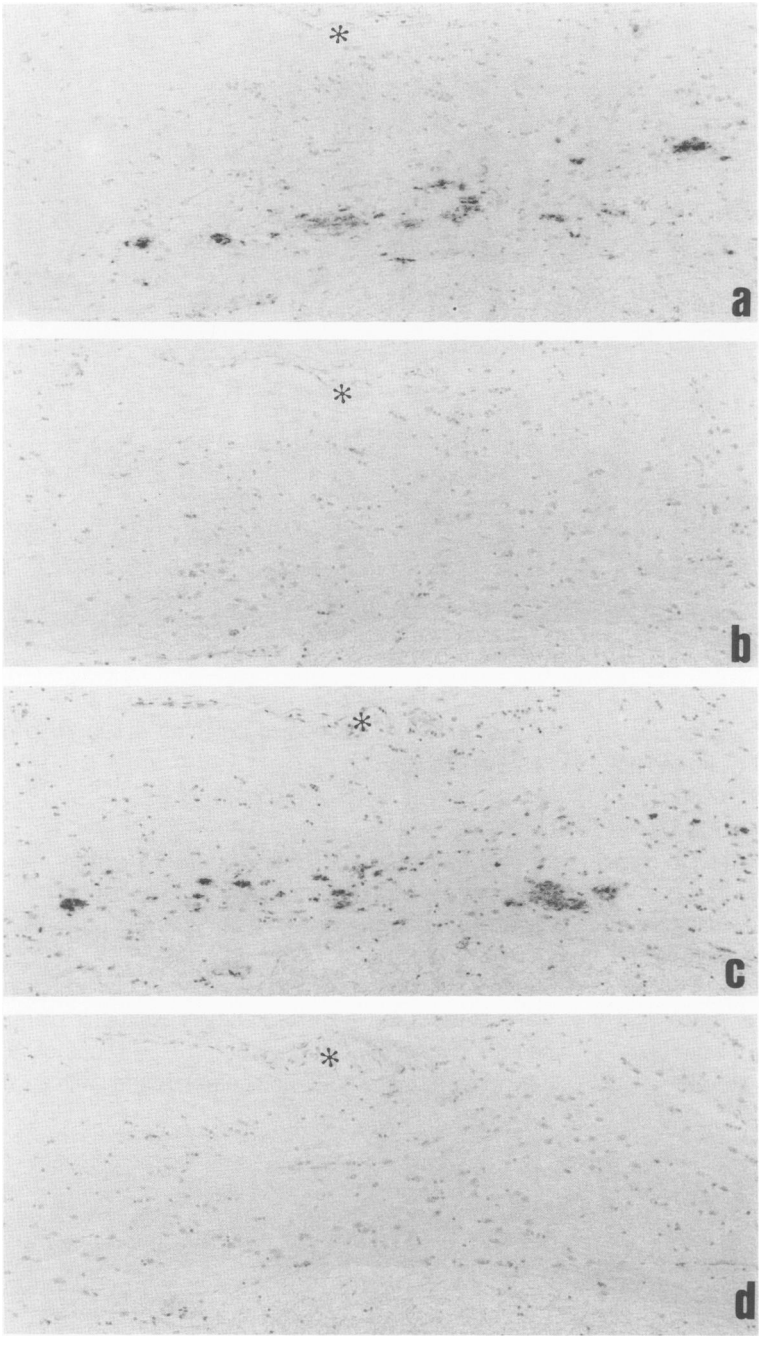


Figure 5. **a** *to* **d** *are adjacent sections of AD dorsal caudate. Plaques labeled by antibody Y in* **(a)** *and* **(c)** *are not recognized by the APP antibodies 7H5* **(b)** *or* $\alpha APP_{444\text{-}592}$ **(d)**. *Sections of amygdala from the same AD case immunostained at the same time provided positive controls for plaque labeling by the APP antibodies. The asterisks mark a vessel present in white matter in all four sections. No APP-labeled plaques were ever detected in the caudate/putamen or in cerebellar cortex (data not shown) (×33).*

finity-purified polyclonal antibody, $\alpha APP_{444-592}$, and a tau monoclonal antibody, 5E2, showed that, with rare exceptions, the two antibodies recognized the same plaques but usually detected different structures within them (Figure 7). The $\alpha APP_{444-592}$-reactive structures within a plaque were often not recognized by the tau antibody, although multiple neurites labeled by the latter were always located in close proximity in the same plaque. Some neuritelike structures appeared to be labeled by both the tau and $\alpha APP_{444-592}$ antibodies (Figure 7). The profiles immunostained by the APP antibodies did not resemble glial processes, and comparison of adjacent sections reacted with GFAP or APP antibodies showed no overlap of detected structures. Similarly the APP-labeled structures did not show the characteristic morphology of microglial cells. Examination of adjacent sections labeled with APP antibodies or the lectin RCA-1 (used as a microglial marker), however, showed that some APP-positive plaques also contained reactive microglial cells.

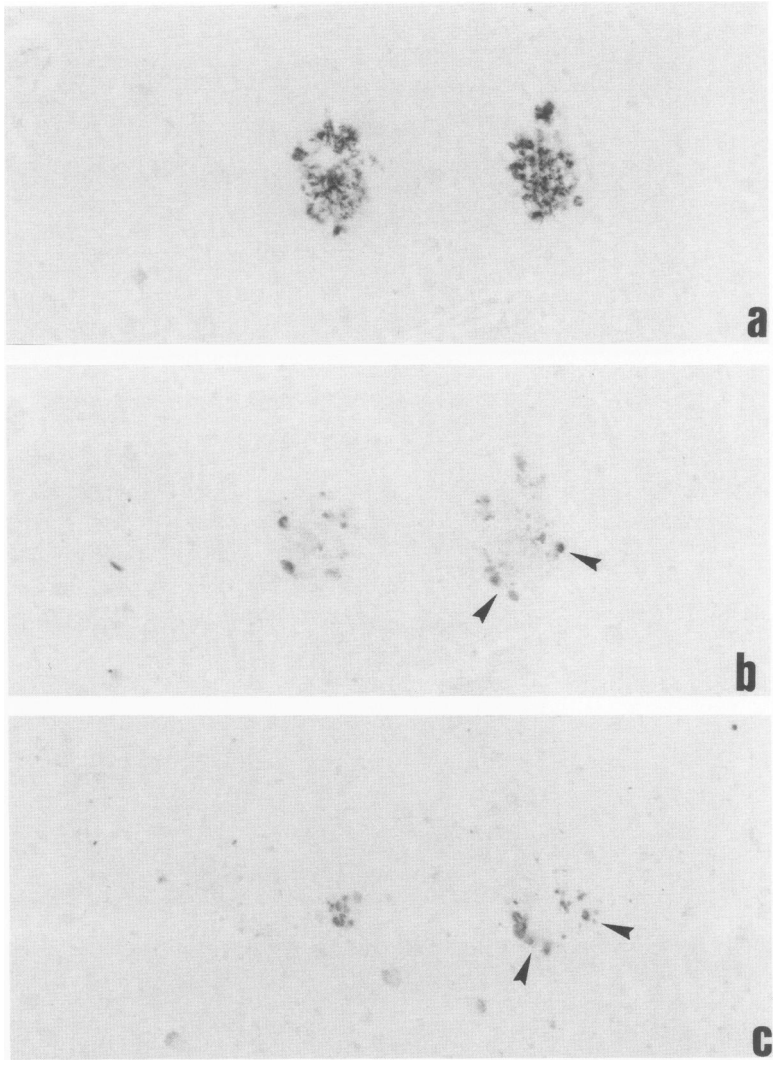


Figure 6. *Plaques that were immunostained by the APP antibodies also were labeled by markers for dystrophic neurites, on adjacent sections.* **a** *to* **c** *are adjacent sections of AD amygdala immunostained with antibody Y* **(a)**, $\alpha APP_{444\text{-}592}$ **(b)**, *and SMI 34* **(c)**. *The profiles immunostained by the APP antibodies morphologically resemble neurites; indeed some of the SMI-34-positive neurites also appear to be labeled by* $\alpha APP_{444\text{-}592}$ *(arrowheads) (×66).*

All of the Regional APP Antibodies Label the Same Profiles Within Plaques, Suggesting the Presence of the Full-length APP

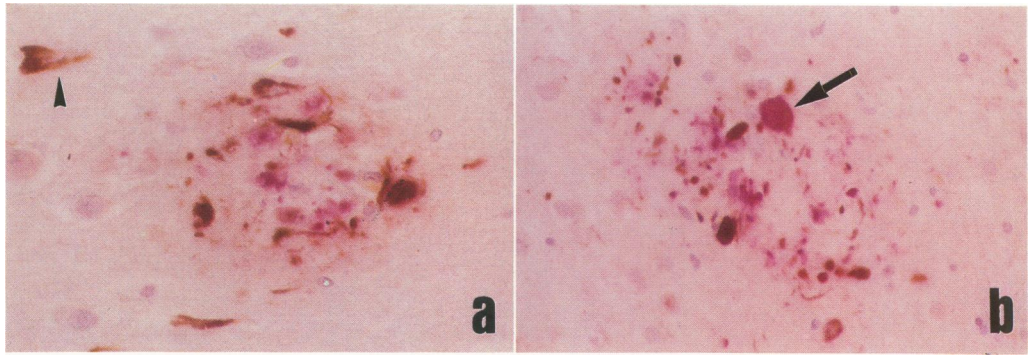
The APP antibodies we examined ($\alpha APP_{45\text{–}62}$, 7H5, $\alpha APP_{444\text{–}592}$, and $\alpha APP_{676\text{–}695}$), which span the precursor from its amino- to its carboxyl-terminus, recognized the same subpopulation of senile plaques and the same structures within plaques when immediately adjacent sections were examined (Figure 8). Double labeling with two APP antibodies showed apparent complete colocalization of the respective epitopes; that is, all immunoreactive structures within plaques appeared to have been labeled by both APP antibodies. There were no consistent differences among the APP antibodies in the intensity of plaque immunostaining.

None of the APP Antibodies Stain Cortical or Meningeal Vascular Amyloid Deposits or Compacted Amyloid Cores in Senile Plaques

Most plaques with dense amyloid cores showed αAPP-positive neuritelike profiles in the corona. The amyloid core itself, however, was uniformly negative with the APP antibodies (Figure 9). Neither normal nor amyloidotic blood vessels were labeled by the APP antibodies.

Occasional Neurons in AD Brain Are Immunolabeled by These APP Antibodies

Our APP antibodies did not detect the APP present in normal neuronal cell bodies in the briefly formalin-fixed,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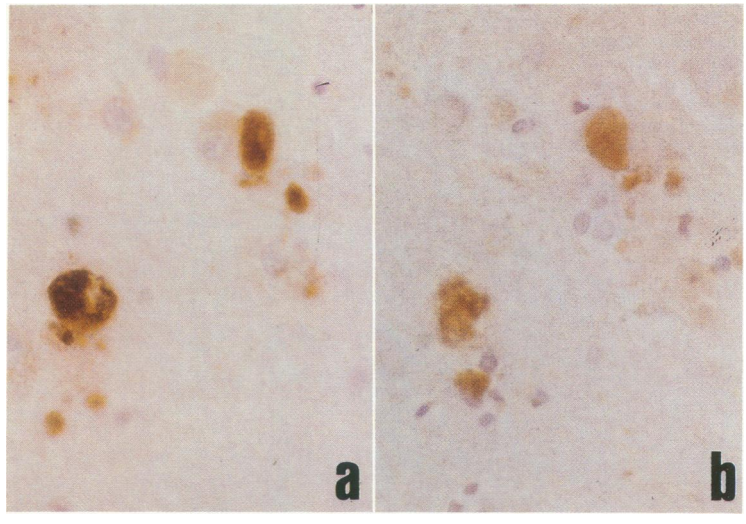
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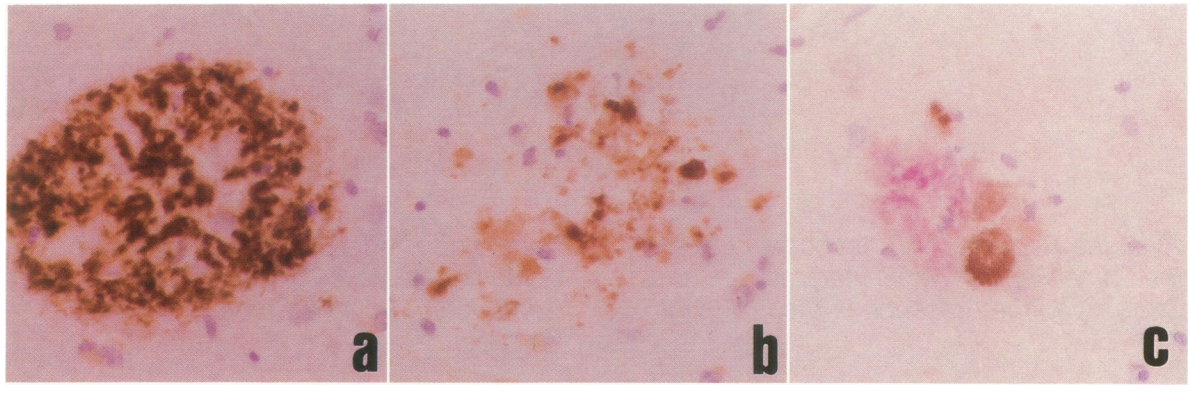
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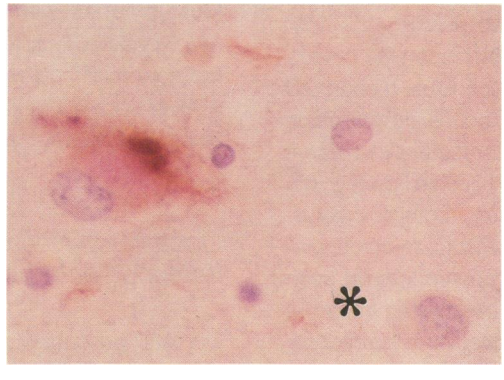
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Figure 7. *First row.* **a and b:** *Double-labeling with the tau antibody 5E2 (brown) and* $\alpha APP_{444\text{-}592}$ *(red) showed that most plaques contained epitopes for both antibodies. Within a plaque, profiles detected by the APP antibody (red) always were located in close proximity to tau-labeled neurites (brown). Occasional structures morphologically resembling neurites appeared to be immunostained by both antibodies (arrow). Neurofibrillary tangles (arrowhead) were labeled by the tau antibody (×80).*

Figure 8. *Second row. The same profiles in senile plaques were immunostained by antibodies to different regions of APP when adjacent sections were examined.* **a** *and* **b** *are adjacent sections of AD amygdala.* **a:** $\alpha APP_{676\text{-}695}$; **b:** $\alpha APP_{444\text{-}592}$ *(×100). Double labeling with two APP antibodies showed complete colocalization of labeled structures (data not shown).*

Figure 9. *Third row. Antibody Y* **(a)** *immunostained AβP-positive material in the core and corona of this classical senile plaque (×100).* $\alpha APP_{444\text{-}592}$ **(b)** *labels structures peripheral to the core and does not immunostain the central amyloid core itself (×100). Double labeling using antibody 7H5 (brown) and antibody Y (red)* **(c)** *also showed little, if any, overlap in immunostaining by the AβP and APP antibodies (×80).*

Figure 11. *Fourth row. A few neurons were labeled by both the tau antibody 5E2 (brown) and by* $\alpha APP_{444\text{-}592}$ *(red). The αAPP immunostaining is diffusely distributed throughout the cytoplasm, whereas the cytoplasmic tau immunoreactivity is focal. A neuron with unstained cytoplasm (asterisk) also is present (×132).*

postmortem brain sections that we employed. Cytoplasmic staining of occasional neurons in hippocampus and amygdala of AD brains was observed, however, with the APP antibodies (Figure 10). Such staining was also apparent in rare neurons of the amygdala in one aged nondemented control; scattered tau-positive neurofibrillary tangles were also present in this case. Most APP-labeled neurons did not appear to contain tangles. Double-staining experiments, however, showed that a few neuronal cell bodies were labeled by both an APP antibody and a tau antibody (Figure 11).

Discussion

Antibodies to regions of APP outside of AβP appear to recognize epitopes in dystrophic neurites in a subgroup of senile plaques in Alzheimer's disease. Those plaques that lacked neurites detectable by tau, PHF, or NF antibodies also lacked APP epitopes. The apparent intraneuronal staining by APP antibodies contrasts with that produced by antibodies to AβP, which is thought to be wholly extracellular. The APP antibodies we examined, which span the APP from amino- to carboxyl-terminus, excluding the AβP, all appeared to label the same neuritelike profiles, suggesting the presence of the full-length precursor in these structures. A neuritic[20,29–33] localization of certain APP epitopes in plaques has recently been proposed by others on the basis of light microscopic immunohistochemical results. We have expanded these observations with analyses of multiple adjacent and double-labeled brain sections using regional APP antibodies and sensitive neurite markers. Confirmation of the presumed neuritic localization will now require ultrastructural studies.

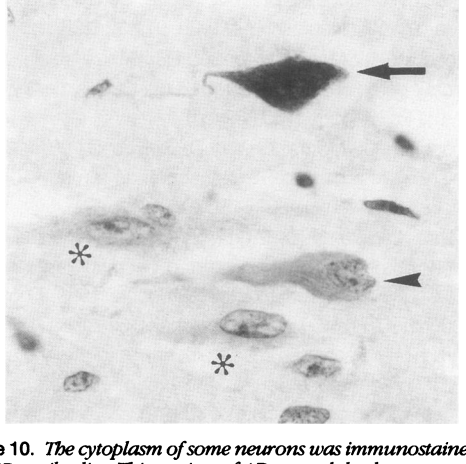

Figure 10. *The cytoplasm of some neurons was immunostained by the APP antibodies. This section of AD amygdala shows a neuron labeled by* $\alpha APP_{444\text{-}592}$ *(arrow). A tangle-bearing neuron(arrowhead) and two tangle-free neurons (asterisks) are unstained (counterstained with hematoxylin, ×132).*

If the APP epitopes are actually present within dystrophic plaque neurites, as these findings suggest, then the detection of such APP immunoreactivity may indicate a relatively nonspecific neuritic change. A wide variety of neuronal proteins have previously been detected immunocytochemically in the dystrophic neurites of senile plaques in humans and primates, including structural proteins (the microtubule-associated proteins, tau and MAP 2; the neurofilament heavy and medium subunit proteins; tubulin; neuropeptides[34–37]; enzymes (acetylcholinesterase,[38,39] choline acetyltransferase,[40] casein kinase II[41]); and other proteins (synaptophysin,[20] chromogranin A[42]). The presence of epitopes for such a diverse group of neuronal proteins within the dystrophic plaque neurites may be interpreted as evidence of an apparently aberrant attempt at neuritic regeneration; as such, many neuronal proteins, including the APP, might be altered in expression, amount, or conformation and thus immunocytochemically detectable within abnormal neurites in AD brain. In the peripheral nervous system, the APP is known to be axonally transported by means of the fast anterograde component.[17] β-Amyloid precursor protein synthesized in central nervous system neurons may, as postulated,[17] be delivered to the dystrophic neurites in senile plaques.

Alternatively the presence of APP epitopes in plaque neurites may indicate a more specific response. It is now known that the secreted form of APP containing the Kunitz protease inhibitor domain is the same molecule as

a previously described secreted protease inhibitor, protease nexin 2 (PN-2).[43,44] The protease nexins (PN), once secreted, are believed to bind to the catalytic sites of certain serine proteases, thereby inactivating them. The PN–protease complexes are then postulated to bind to the cells that initially secreted the PN, be taken up, and degraded. This mechanism presumably allows cells to regulate the activity of proteases at or near the cell surface.[45,46] The APP, if present within plaque neurites as our findings suggest, may possibly then be secreted into the extracellular space after truncation of its carboxyl-terminus,[16,27,47] and function as an inhibitor of serine proteases that may be associated with senile plaques. A number of proteases and protease inhibitors are known to be present in senile plaques, including the serine protease inhibitor, α-1 antichymotrypsin,[48] serum amyloid P component,[49] which is postulated to act as an elastase inhibitor,[50] and activated complement factors, certain of which may function as serine proteases.[51]

Regardless of whether the accumulation of APP in dystrophic neurites is a specific or relatively nonspecific phenomenon, the question of whether it serves as the precursor for the AβP in plaques must be considered. Masters et al[18] postulated that the AβP deposited in plaques derives from precursor molecules that are present in neurons, although other investigators support a possible hematogenous source for plaque and microvascular AβP,[23,24,52] or hypothesize that it arises from microglia[21] or astrocytes.[22] In our studies, it was striking that many cortical and subcortical plaques in AD brains were not detected by the APP antibodies. Specifically plaques lacking tau-, PHF-, or NF-positive neurites were not labeled by the APP antibodies, suggesting that APP epitopes appeared only when dystrophic neurites detectable by these markers had developed. In cerebral neocortex, AβP antibodies always recognized more plaques than did the APP antibodies or the neurite markers, αtau, αPHF, or αNF. Furthermore many AβP-containing plaques that were not associated with detectable APP accumulation were found in the caudatoputamen and cerebellar molecular cortex. Thus, it appears that AβP can be deposited in brain tissue in the absence of either tau-, PHF-, or NF-positive neuritic reaction or local APP accumulation, an observation that has been noted by others.[53] It is also notable that extracellular AβP deposits in meningeal and cortical vessels are not labeled by the APP antibodies. These findings are not easy to reconcile with the proposition that APP in neurites is the sole or principal source of plaque and vascular AβP.

Occasional neurons in AD brain were immunolabeled by the APP antibodies in a granular cytoplasmic pattern, sometimes with extension into proximal cell processes. β-Amyloid precursor protein is known to be synthesized in neurons[12] but, in contrast to the pathologic neurites containing APP epitopes, it is not readily immunocytochemically detectable in normal neuronal cell bodies or processes in postmortem human brain tissue.[26,30,54,55] Most of the APP-labeled neurons we did see did not appear to contain neurofibrillary tangles, but their presence in tangle-rich regions of AD brains (amygdala, hippocampus) and the failure to detect them in all but one control brain suggests that the immunoreactive APP molecules are associated with a pathologic change in certain neuronal perikarya rather than representative of the normal cellular distribution of APP. This conclusion is supported by the fact that normal neuronal processes in AD or control brain are similarly not immunolabeled by our APP antibodies in the postmortem brain sections employed. The APP has been shown to be concentrated in neuronal lysosomes, and abnormally dense immunostaining of atrophied neurons has been observed in AD hippocampus.[55]

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