Characterization of New Polyclonal Antibodies Specific for 40 and 42 Amino Acid–Long Amyloid β Peptides: Their Use to Examine the Cell Biology of Presenilins and the Immunohistochemistry of Sporadic Alzheimer's Disease and Cerebral Amyloid Angiopathy Cases

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ABSTRACT

Background: In Alzheimer's disease (AD), the main histological lesion is a proteinaceous deposit, the senile plaque, which is mainly composed of a peptide called Aβ. The aggregation process is thought to occur through enhanced concentration of Aβ40 or increased production of the more readily aggregating 42 amino acid–long Aβ42 species.

Materials and Methods: Specificity of the antibodies was assessed by dot blot, Western blot, ELISA, and immunoprecipitation procedures on synthetic and endogenous Aβ produced by secreted HK293 cells. Aβ and p3 production by wild-type and mutated presenilin 1–expressing cells transiently transfected with βAPP751 was monitored after metabolic labeling and immunoprecipitation procedures. Immunohistochemical analysis was performed on brains of sporadic and typical cerebrovascular amyloid angiopathy (CAA) cases.

Results: Dot and Western blot analyses indicate that IgG-purified fractions of antisera recognize native and denatured Aβs. FCA3340 and FCA3542 display full specificity for Aβ40 and Aβ42, respectively. Antibodies immunoprecipitate their respective synthetic Aβ species but also Aβs and their related p3 counterparts endogenously secreted by transfected human kidney 293 cells. This allowed us to show that mutations on presenilin 1 triggered similar increased ratios of Aβ42 and its p342 counterpart over total Aβ and p3. ELISA assays allow detection of about 25–50 pg/ml of Aβs and remain linear up to 750 to 1500 pg/ml without any cross-reactivity. FCA18 and FCA3542 label diffuse and mature plaques of a sporadic AD case whereas FCA3340 only reveals the mature lesions and particularly labels their central dense core. In a CAA case, FCA18 and FCA3340 reveal lepto-

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meningeal and cortical arterioles whereas FCA3542 only faintly labels such structures.

Conclusions: Polyclonal antibodies exclusively recognizing Aβ40 (FCA3340) or Aβ42 (FCA3542) were obtained. These demonstrated that FAD-linked presenilins similarly affect both p342 and Aβ42, suggesting that these mutations misroute the βAPP to a compartment where γ-secretase, but not α-secretase, cleavages are modified. Overall, these antibodies should prove useful for fundamental and diagnostic approaches, as suggested by their usefulness for biochemical, cell biological, and immunohistochemical techniques.

INTRODUCTION

One of the main histopathological lesions observed in the cortex of Alzheimer's disease-affected brains is the senile plaque (1). This proteinaceous deposit appears to be mainly composed of the amyloid β (Aβ) peptide (2,3) that is derived from a large polypeptidic precursor, the β amyloid precursor protein (βAPP) (4,5). Biochemical approaches have clearly established that the pathological process ultimately leading to the extracellular peptide aggregation is dependent upon the concentration and/or the nature of the Aβ species generated (6,7). Mutations responsible for the early-onset familial forms of Alzheimer's disease (AD) apparently trigger a disturbance of βAPP processing. Consistent with this view is the finding that a double mutation taking place adjacent to the N-terminus of the Aβ sequence (double Swedish mutation [8]) led to enhanced production of a 40 amino acid–long Aβ species (Aβ40; [9–11]). Another mutation located near the C-terminus of the Aβ sequence (12,13) appeared to increase the production of a longer 42 amino acid Aβ (14), the aggregation properties of which have been shown to be exacerbated (6,7). More recently, two genes have been identified on chromosomes 14 and 1 that account for most of the agressive early-onset familial forms of the disease (15–17). The gene products, presenilins 1 and 2, likely interfere with βAPP processing since pathological mutations in presenilins trigger increased Aβ42 over Aβ40 ratios (18–20).

Immunohistochemical studies have been conducted to establish the Aβ content of the diffuse, mature, and cerebrovascular neuropathological insults. The nature of the Aβ species composing the lesions is still somewhat controversial. This could be due to the nature of the immunological tools, emphasizing the need for the development of end-specific antibodies able to fully discriminate among the various Aβ species generated.

We have developed two polyclonal antibodies that specifically recognize Aβ40 (FCA3340) or Aβ42 (FCA3542) and another one able to serve as an aspecific probe for both Aβ species (FCA18). These novel antibodies are shown to be amenable to biochemical (ELISA, dot, and Western blots) and cell biological (immunoprecipitation of conditioned media) approaches. Furthermore, we clearly establish by immunohistochemistry the nature of the Aβ species observed in diffuse, mature, and vascular deposits of a sporadic AD case and those of a presenile AD case with a typical cerebrovascular amyloid angiopathy (CAA).

MATERIALS AND METHODS

Peptide Synthesis, Antigen Coupling, and Rabbit Immunization Procedures

All peptides were synthetized by classical solid phase (Boc strategy) with methyl benzhydrylamine resin by means of a semiautomatic apparatus (Neosystem NPS 4000) and were purified as described previously (21). The purity and amino acid composition were confirmed by amino acid analysis and electrospray mass spectrometry. The octapeptide corresponding to the common N-terminus of Aβ40 and 42 (displaying a cysteine residue at its C-terminus, DAEFRHDS-Cys) and octapeptides mimicking the specific C-terminal ends of Aβ40 and 42 (with an N-terminal cysteine, Cys-GLMVGGVV [Aβ40] and Cys-MVG-GVVIA [Aβ42]) were coupled as described previously (22) to either bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH, Calbiochem) that was previously conjugated to m-maleidobenzoic acid N-hydrosuccinimide (Sigma). KLH-coupled antigens (about 1 mg) were mixed with an equal volume of complete Freund adjuvant, then injected subcutaneously into New Zealand rabbits. Boosts (0.5 mg) were given 1 month later and at subsequent 3-week intervals. Immunopositive sera

were monitored by dot blot (see below) with BSA-coupled antigens as screening peptides.

Purification of IgG

The whole IgG fractions from various immune or preimmune sera were obtained after treatment with octanoic acid according to the procedure previously described (23).

Dot Blot Analysis

BSA-coupled antigens (about 50 ng) or various Aβ species (resuspended in distilled water at a final concentration of 0.1 mg/ml) were dot-blotted (1–2 μg) onto nitrocellulose membranes (Hybond-C, Amersham, les Ulïs, France). Prior to the immunochemical reaction, membranes were treated in Tris buffer saline (TBS: 140 mM NaCl, 20 mM Tris-HCl, pH 7.5) containing 5% skim milk. Antibodies were then added for 4 hr at various dilutions (primary screening of immunsera) or overnight at 4°C at 1:500 (FCA18) and 1:100 dilutions (FCA3340 and FCA3542) in the above buffer. After rinsing (4 × 5 min) with TBS, nitrocellulose sheets were exposed for 1 hr at room temperature to peroxidase-conjugated secondary antibody (HRP-conjugated goat anti-rabbit from Immunotech or Promega, Marseille, France). Nitrocellulose was finally rinsed in TBS as above and immunoreactive complexes were revealed by enhanced chemiluminescence according to the recommendations of the manufacturer (kit from Amersham).

Western Blot Analysis

Electrophoretic separation of the various Aβ species was performed using a 16.5% SDS-polyacrylamide gel with the Tris-tricine procedure previously described (24). Briefly, dried samples and standards (low molecular weight proteins from 31 kDa to 2.5 kDa from Promega) were resuspended in 30 μl of loading buffer (125 mM Tris/HCl, pH 8.45, containing 2% SDS, 20% glycerol, and 5% β-mercaptoethanol). After heating for 5 min at 95°C, samples were electrophoresed at room temperature for 90 min at 150 V using an anode buffer at pH 8.9 and a cathode buffer containing 100 mM tricine, pH 8.45. Proteins were then transferred onto nitrocellulose membranes (Hybond C super, Amersham), then antibody hybridization and immunoreactivity analysis were performed as described above for dot blot analysis. In some early experiments, im-munological complexes were revealed with chloronaphtol as described previously (23).

Immunoprecipitation of Endogenous and Synthetic Aβ Species

Transfected HK293 cells overexpressing the Swedish mutated form of βAPP751 were obtained, cultured, and metabolically labeled as previously described (25). Conditioned media (10 ml) were collected, diluted in one-tenth volume of RIPA 10× buffer, incubated overnight with a 350-fold dilution of antibodies, then stirred for 4 hr in the presence of protein A-sepharose (100 mg/ml, 100 μl). Samples were centrifuged, washed three times with radioimmunoprecipitation assay (RIPA) 1×, resuspended with loading buffer, electrophoresed on a 16.5% Tris-tricine gel, and radioautographed as described elsewhere (26). Stable transfectants overexpressing wild-type, mutated, or delta Exon-9 presenilin 1 (ΔE9PS1) were transiently transfected with βAPP751, then metabolically labeled and analyzed for Aβ and p3 secretion as described above.

Immunoprecipitations of synthetic Aβ40 and Aβ42 (1 μg) were performed in 5 ml of RIPA 1× buffer with a 170-fold dilution of antibodies, then treated and analyzed as above.

ELISA Assay

The ELISA procedure is based on an analytical electrochemiluminescence method. Capture and secondary antibodies were labeled with either biotin or with the Origen TAG electrochemiluminescent label (ECL) according to the manufacturer recommendations (IGEN, Gaithersburg, USA). Labeled antibodies were separated from unincorporated label using PD-10 columns (Pharmacia) and concentrated to 0.5–1 mg/ml using Centricon-30 (Amicon) concentrators. Before use, the antibodies were diluted to 4 μg/ml with gelatin diluent buffer (IGEN), containing 0.5% tween 20. In addition, M-280 paramagnetic beads (IGEN) were diluted before use to 1.6 mg/ml in the same buffer. In each ELISA assay, 25 μl of Aβ samples were mixed at 20°C under constant stirring with 25 μl of paramagnetic beads, 25 μl of biotinylated antibody, and 25 μl of electrochemiluminescent-labeled antibody. After 2 hr, 200 μl of assay buffer (IGEN) was added and the samples were read using an IGEN Origen analyser. Standard curves were obtained

with known concentrations of synthetic Aβ40 or Aβ42 ranging between 25 and 1500 pg/ml.

Immunohistochemistry

The temporal neocortex of a patient affected by a sporadic form of Alzheimer's disease (AD case of the Charles Foix series [27] provided by Drs. C. Duyckaerts and J. J. Hauw, Hopital de la Pitié-Salpêtrière, Paris) and of a patient neuropathologically diagnosed as an AD case with typical cerebrovascular amyloid angiopathy (CAA) were formalin fixed. Paraffin-embedded specimens were cut (4–7 μm thick), dewaxed, dehydrated, and pretreated with formic acid (80%). Samples were rinsed in phosphate-buffered saline (PBS), then incubated for 10 min with 3% hydrogen peroxide to inhibit endogenous peroxidase. Non-specific sites were blocked by a 20–30 min exposure to 10% ovalbumin or BSA, then sections were incubated with various dilutions of FCA antibodies. Immunological complexes were revealed by sequential application of 1/200 biotinylated anti-rabbit IgG (Amersham), 1/400 streptavidin-peroxidase complex (Amersham), and 0.05% diaminobenzidine (Sigma) as described previously (27). Nuclei were counterstained by Harris hematoxylin.

Materials

Aβ40 peptide was purchased from Bale Biochimie or Neosystem, France. Aβ43 peptide was purchased from U.S. Peptides. Aβ42 was synthesized and purified, and the purity and the amino acid composition of Aβ42 were confirmed by electrospray mass spectrometry and amino acid analysis.

RESULTS

Specificity of FCA18, FCA3340, and FCA3542 Polyclonal Antibodies Toward Aβ-Related Sequences

FCA18 was theoretically designed to interact with the N-terminus of both Aβ40 and Aβ42 (see Materials and Methods). Western blot analysis illustrated in Figure 1A shows that the IgG-purified fraction of FCA18 interacts with the denatured forms of Aβ peptides, both Aβ40 and 42. Interestingly, FCA18 has an absolute requirement for the free aspartyl residue of Aβ. Thus, the intact N-terminal Aβ heptapeptide (corre-
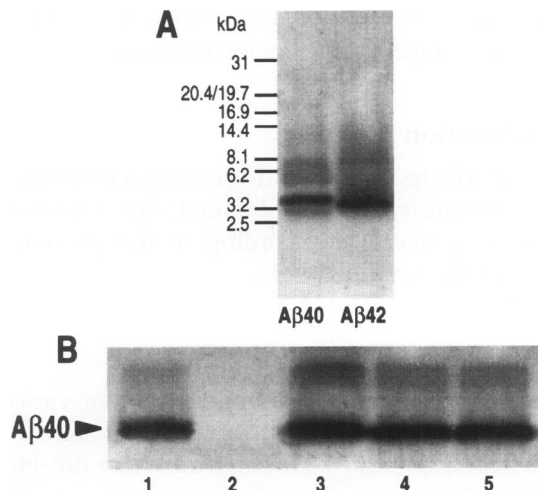

FIG. 1. Western blot analyses of FCA18 specificity

(A) One microgram of Aβ40 or Aβ42 was electrophoresed on a 16.5% Tris-tricine gel and transferred to nitrocellulose sheets followed by hybridization overnight at 4°C with a 1:500 dilution of the IgG-purified fraction of FCA18 (in TBS containing 3% bovine serum albumin [BSA]) as described in Materials and Methods. (B) One microgram of Aβ40 was electrophoresed and transferred to nitrocellulose sheets as in A which were incubated overnight at 4°C with a 1:500 dilution of the FCA18 IgG fraction preincubated (for 8 hr at 4°C) without (lane 1) or with 0.1 mM of synthetic peptide corresponding to Aβ1–7 (lane 2), acetyl Aβ1–7 (lane 3), DesAsp-Aβ1–7 (lane 4), or the C-terminal heptapeptide of APPβ (lane 5). In A and B, nitrocellulose sheets were then incubated (1 hr at room temperature) with goat-anti-rabbit HRP-conjugated-IgG (1:1000 dilution in TBS containing 1% BSA); then immunological complexes were revealed with chloronaphtol as described in Materials and Methods.

sponding to Aβ1-7) was able fully to compete for Aβ40 labeling by FCA18 (Fig. 1B, lane 2). In contrast, the corresponding N-terminally acetylated heptapeptide (Fig. 1B, lane 3), Des-Asp hexapeptide (Fig. 1B, lane 4), or an heptapeptide mimicking the C-terminal end of APPβ (Fig. 1B, lane 5) did not affect FCA18 labeling of Aβ40. In addition, FCA18 did not recognize full-length βAPP751 (not shown). Altogether, our data clearly indicate that FCA18 interacts only with the N-terminus part of Aβs.

Western blot analyses show that FCA3340 and FCA3542 specifically label denatured Aβ40 and Aβ42, respectively (Fig. 2). The specificity of FCA3340 was further documented by the ability of the octapeptide corresponding to Aβ40 C-terminus to displace Aβ40 labeling by
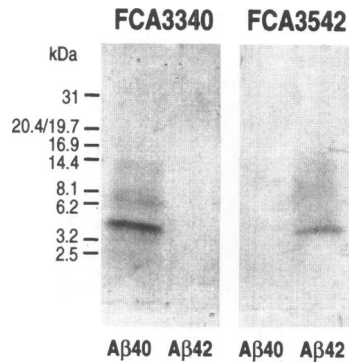
FIG. 2. Western blot analyses of FCA3340 and FCA3542 specificity

One microgram of Aβ40 or Aβ42 was electrophoresed, Western blotted, and incubated with a 1:100 dilution of FCA3340 or FCA3542; then complexes were revealed with chloronaphtol as described in Materials and Methods.
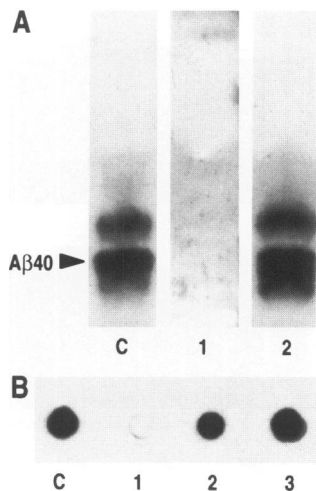

FIG. 3. FCA3340 specifically recognizes native and denatured Aβ40

(A) Two micrograms of Aβ40 was electrophoresed on a 16.5% Tris-tricine gel and Western blotted, and nitrocellulose sheets were incubated for 24 hr at 4°C with a 1:100 dilution of the IgG-purified fraction of FCA3340 (in TBS containing 5% skim milk) preincubated (for 24 hr at 4°C) without (lane C) or with 0.1 mM of synthetic octapeptides corresponding to Aβ33–40 (lane 1) or Aβ35–42 (lane 2); then immunological complexes were revealed by ECL as described in Materials and Methods. In B, 2 μg of Aβ were dot blotted, then hybridized for 18 hr at 4°C with a 1:100 dilution of FCA3340 preincubated for 24 hr at 4°C without (lane C) or with the synthetic peptides (0.1 mM) corresponding to Aβ33–40 (lane 1), Aβ35–42 (lane 2), or Aβ35–43 (lane 3). Immunoreactivities were revealed as above.
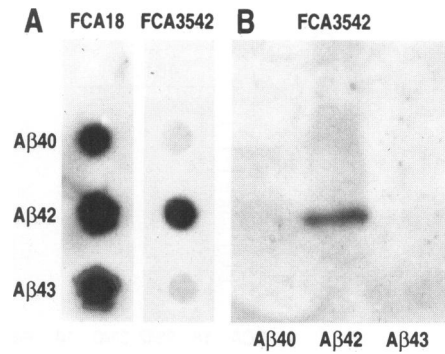

FIG. 4. FCA3542 specifically recognizes native and denatured Aβ42

(A) Two micrograms of Aβ40, Aβ42, and Aβ43 were dot blotted, hybridized for 18 hr at 4°C with a 1:500 dilution of FCA18 or a 1:100 dilution of FCA3542; then immunoreactive complexes were revealed by ECL as described in Materials and Methods. (B) Two micrograms of Aβ40, Aβ42, or Aβ43 were electrophoresed on a 16.5% Tris-tricine gel and Western blotted, and nitrocellulose sheets were hybridized for 24 hr at 4°C with a 1:100 dilution of the IgG-purified fraction of FCA3542 (in TBS containing 5% skim milk). Immunological complexes were revealed as above.

FCA3340 (Fig. 3A lane 1), whereas the peptide mimicking the C-terminal sequence of Aβ42 was unable to affect the immunolabeling (Fig. 3A, lane 2). Dot blot analysis illustrates the ability of FCA3340 to recognize native Aβ40. This interaction was abolished by the C-terminal octapeptide of Aβ40 (Fig. 3B, lane 1) but not of Aβ42 (Fig. 3B, lane 2) or Aβ43 (Fig. 3B, lane 3). Complete specificity of FCA3542 for Aβ42 was also observed (Fig. 4). Thus, dot blot (Fig. 4A) and Western blot (Fig. 4B) analyses demonstrate that FCA3542 labeled both native and denatured Aβ42 but failed to detect Aβ40 and Aβ43.

Immunoprecipitation of Synthetic and Endogenous Aβ-Related Sequences by FCA18, FCA3340, and FCA3542

Immunoprecipitation of synthetic Aβ40 and 42 indicated that FCA18 precipitates both Aβ species whereas FCA3340 and FCA3542 only revealed Aβ40 and Aβ42, respectively (Fig. 5A). In order to examine whether FCA3340 could be used to precipitate the α- and γ-secretase-derived p340 fragment (4), we metabolically labeled human kidney 293 cells overexpressing the Swedish mutated βAPP751, since this mutation is thought to exacerbate the production of both
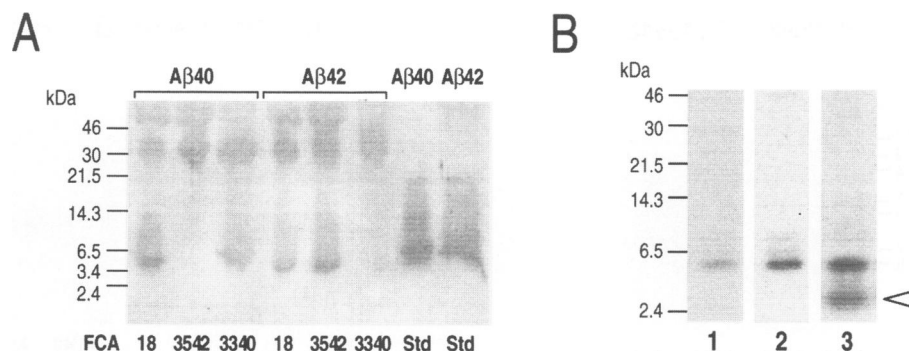
FIG. 5.   Immunoprecipitation of synthetic and endogenous Aβs by FCA antibodies

(A) Immunoprecipitations of synthetic Aβ40 and Aβ42 (1 μg) were performed in 5 ml of RIPA 1× buffer with a 170-fold dilution of the IgG-purified fraction of the indicated FCAs. Immunoprecipitates were washed, electrophoresed on a 16.5% Tris-tricine gel, Western blotted, and probed with a 1:500 dilution of FCA18. Immunoreactivities were revealed with chloronaphtol as described in Materials and Methods. (B) Transfected HK293 cells overexpressing the Swedish mutated form of βAPP were obtained, cultured, and metabolically labeled as described in Materials and Methods. Conditioned media (10 ml) were collected, diluted in a one-tenth volume of RIPA 10× buffer, incubated overnight with a 350-fold dilution of FCA18 (lanes 1), FCA3340 (lane 2), or FCA3542 (lane 3), then stirred for 4 hr in the presence of protein A-sepharose (100 mg/ml, 100 μl). Samples were centrifuged, washed three times with RIPA 1×, resuspended with loading buffer, electrophoresed on a 16.5% Tris-tricine gel, and radioautographed as described earlier (25). Arrow indicates the Aβ and p3 fragments.

Aβ species (see Introduction). FCA3340 not only immunoprecipitates endogenous Aβ40 but also allows the recovery of a low molecular weight product that did not interact with FCA18, indicating a lack of the N-terminus of Aβ (Fig. 5B). The fact that this 3-kDa product increased upon phorbol ester treatment of the cells (not shown), as expected for an α-secretase-derived product (28–31), strongly suggests that it could be genuine p340. FCA3542 interacted with Aβ42 and its p3 counterpart, the generation of which appears clearly lower than those of Aβ40 and p340 (Fig. 5B).

Immunoprecipitation of Aβ and Their p3 Counterparts Generated by Stable Transfectants Overexpressing Wild-Type and Mutated Presenilin 1

Figure 6 shows that HK293 cells overexpressing wild-type presenilin 1 (PS1) transiently transfected with wild-type βAPP751 secreted Aβ40 and Aβ42, the latter species accounting for about 10% of total Aβ recovered. Independent clones expressing mutated or ΔE9-PS1 secreted highly increased amounts of Aβ42, leading to augmented ratios of Aβ42 over total Aβ (Fig. 6A). Interestingly, the quantification of the various p3 counterparts indicated increased ratios of p342 over total p3 secreted (Fig. 6B) that fully paralleled those observed for Aβ.
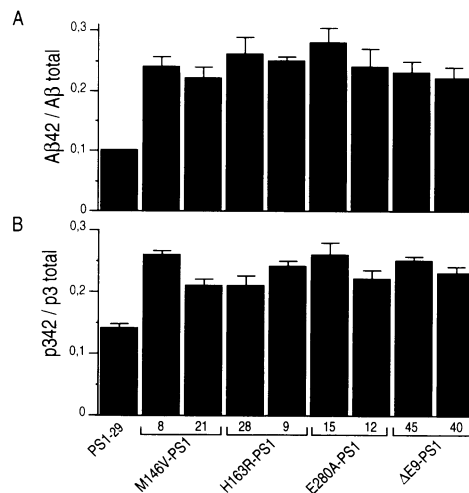

FIG. 6.   Immunoprecipitation of Aβs and p3 from wild-type and mutated PS1-expressing HK293 cells

Stable transfectants overexpressing PS1 or the indicated mutated PS1 were transiently transfected with 2 μg of wild-type βAPP751. Two days after transfection, cells were metabolically labeled for 6 hr; then conditioned media were submitted to a two-step immunoprecipitation by FCA3340 and FCA3542 (350-fold dilution). Immunoprecipitated proteins were electrophoresed on a 16.5% Tris-tricine gel, radioautographed, and scanned as described in Materials and Methods. Panel A illustrates the ratio of Aβ42 over total Aβ (Aβ42 + Aβ40) and panel B indicates the ratio of p342 over total p3 (p342 + p340) observed with the indicated independent numbered clones. Values are the means ± S.E.M of three determinations.
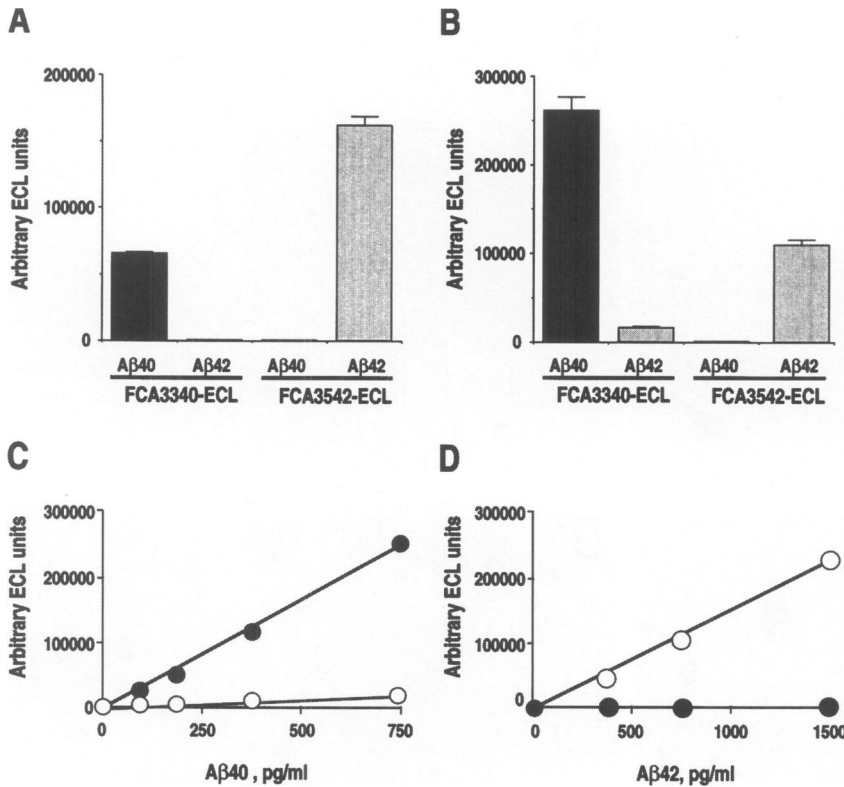
FIG. 7. ELISA assays

(A and B) 750 pg of Aβ40 or Aβ42 (in 25 μl) was mixed with paramagnetic beads (25 μl), electrochemiluminescent-labeled (ECL) FCA3340 or FCA3542 (25 μl, 1 μg/ml), and biotinylated-6E10 (A) or 4G8 (B) in the conditions described in Materials and Methods. After 2 hr, samples were read using an IGEN Origen analyser. Bars are the means ± SEM of independent determinations. Standard curves for Aβ40 (C) and Aβ42 (D) were obtained with various concentrations of synthetic Aβs (in 25 μl), ECL-FCA3340 (filled circles) or ECL-FCA3542 (open circles) (25 μl, 1 μg/ml), biotinylated 4G8 (25 μl) and paramagnetic beads (25 μl). Signals were monitored as described above.

ELISA Assays

The novel ELISA procedure described here employed, as capture primary antibody, 4G8 and 6E10 monoclonals and FCA3340 and FCA3542 as detecting antibodies. The two capture antibodies were biotinylated whereas the FCAs were labeled with the TAG electrochemiluminescent moiety (ECL). Figure 7 shows that biotinylated 6E10 (Fig. 7A) and 4G8 (Fig. 7B) in combination with either ECL-labeled FCA3340 or FCA3542 allow for specific detection of Aβ40 or Aβ42, respectively. We further examined the sensitivity of the ELISA by means of 4G8 as the primary capture antibody. Figure 7C shows that FCA3340 could allow the detection of 25 to 50 pg/ml of Aβ40. The standard curve was linear at concentrations of Aβ40 up to 750 pg/ml, without interference from Aβ42. Standard curves performed with Aβ42 (Fig. 7D) indicated that the FCA3542-derived signal remained linear up to 1500 pg/ml without cross-reactivity from Aβ40.

Immunohistochemistry of a Sporadic Alzheimer's Disease Case

The immunohistochemical labeling of the temporal neocortex of a patient affected by a spo-

radic form of Alzheimer's disease was examined using the IgG-purified fractions of FCA18, FCA3340, or FCA3542. As shown in Figure 8, FCA18 intensely detected a huge amount of diffuse and mature senile plaques (Fig. 8A, C, and D). Furthermore, Figure 8B indicates that FCA18 also labeled a vascular deposit. The treatment of the slice with the preimmune serum or omission of FCA18 antibodies led to a total absence of labeling (not shown). Treatment of the same specimen with FCA3340 revealed an intense labeling of mature plaques (Fig. 9A) but failed to detect diffuse plaques. Figure 9B indicates that only the central core of senile plaques is intensely labeled by FCA3340 whereas the peripheral halo of degenerating fibers was not stained. FCA3542 intensely labels both diffuse and mature lesions (Fig. 10A–D) and also stains the deposits located in the vascular wall of a cerebral vessel (Fig. 10D).

Immunohistochemistry of a Presenile Alzheimer's Disease Case with Typical Cerebral Amyloid Angiopathy (CAA)

A presenile case of Alzheimer's disease with typical CAA was examined. FCA18 reveals an in-
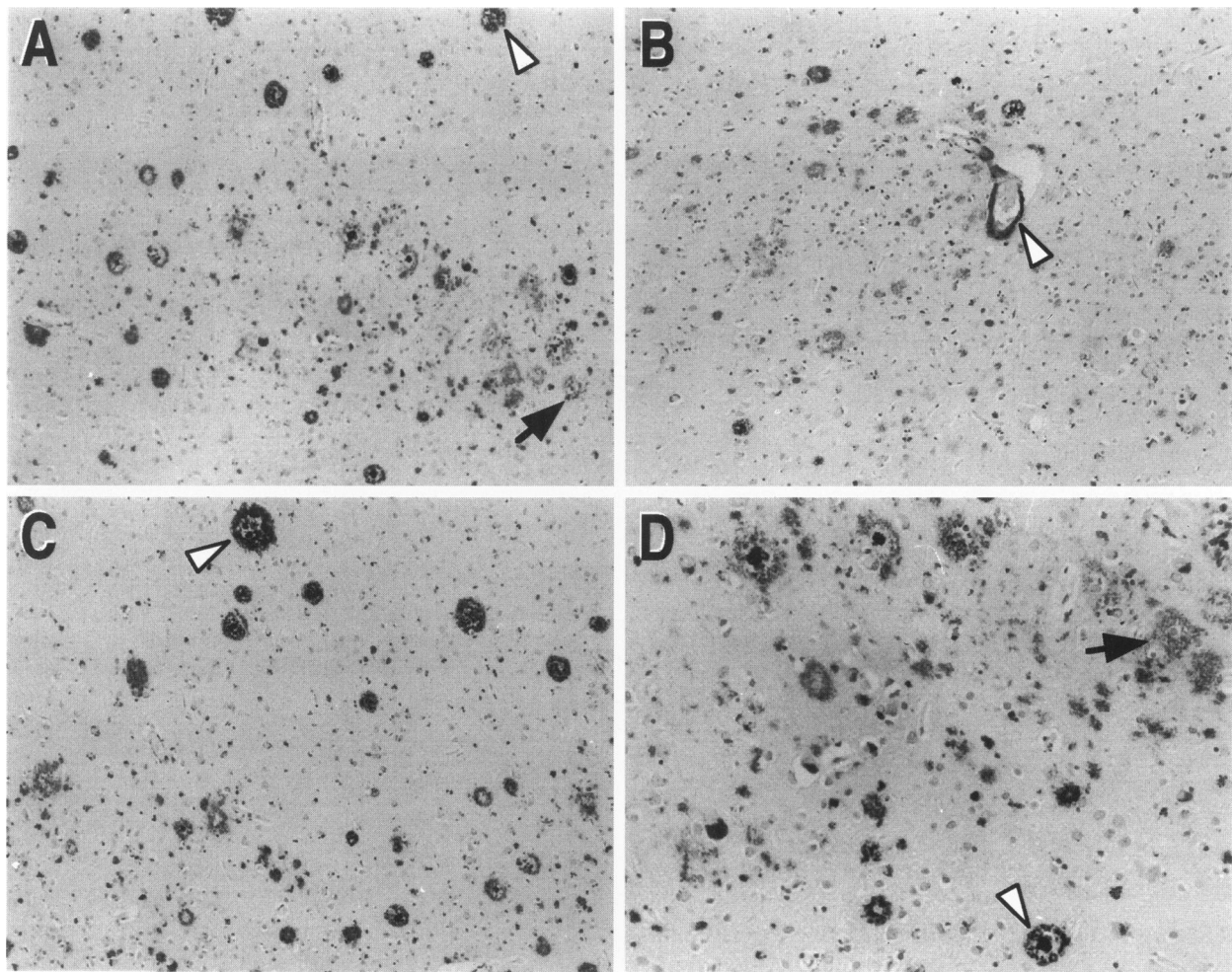
FIG. 8.   Immunohistochemical analysis of the temporal neocortex of a sporadic case of AD by FCA18 IgG-purified fraction

The temporal neocortex of a patient with sporadic Alzheimer's disease was examined by immunohistochemistry using a 500-fold dilution of the IgG-purified fraction of FCA18. Immunological complexes were revealed by the biotin-streptavidin-peroxidase procedure described in Materials and Methods. White arrowheads in A, C, and D indicate mature senile plaques while black arrows (A, D) indicate diffuse plaques. Note in B (arrowhead) the labeling of a cortical arteriole. Magnification: A–C, ×100; D, ×200.

tense labeling of both leptomeningeal and cortical arterioles (Fig. 11A). FCA3340 also labels the wall of cortical arterioles, but it also reveals numerous senile plaques (Fig. 11B). By contrast, FCA3542 only faintly stains the vascular lesions whereas numerous immature and mature plaques are still detectable (Fig. 11C).

DISCUSSION

The mechanisms underlying the production and deposition of Aβ in the brain of Alzheimer's disease–affected patients are likely of central phys-

iopathological importance. Genetic and cell biological approaches combined with histochemical studies should bring their own clues to reconstruct the puzzle of the molecular events altered in Alzheimer's disease. These distinct approaches necessitate the development of useful tools that could serve as molecular probes. Of particular interest are those probes capable of discriminating between the various "physiological" and potentially "pathogenic" species of Aβ.

We have obtained an antiserum (FCA18) that can be used as a ubiquitous tool for recognizing all Aβ species. The characterization of the epitope recognized by FCA18 indicates that it
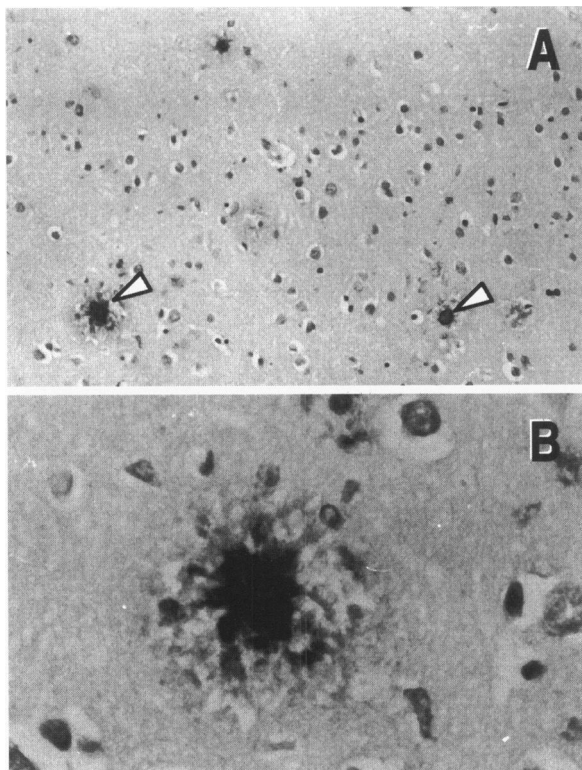
FIG. 9. Immunohistochemical analysis of the temporal neocortex of a sporadic case of AD by FCA3340 IgG-purified fraction

The temporal neocortex (sporadic AD case) was incubated with a 250-fold dilution of the IgG-purified fraction of FCA3340 and immunoreactivity was revealed by the biotin-streptavidin-peroxidase method. Arrows in A label the central dense core of mature plaques without revealing diffuse lesions as also shown in B. Magnification: A, ×100; B, ×400.

included the first aspartyl residue of Aβ. This residue is not recognized when it is blocked or engaged in a peptidyl bond, as shown by the absence of FCA18 recognition of acetylated aspartyl residue (see Results) or full-length βAPP (not shown). FCA18 immunoprecipitates an intracellular 12-kDa product characterized as the β-secretase-derived p12 fragment (25), indicating that FCA18 can interact with the N-terminus of all Aβ and Aβ-containing products. Furthermore, FCA18 was used to immunoprecipitate Aβ secreted from human kidney HK293 stably transfected cells overexpressing the wild-type and Swedish mutated forms of βAPP (26).

We have now developed two other polyclonal antibodies, FCA3340 and FCA3542, that display clearcut specificity toward Aβ40 and

Aβ42, respectively. These antibodies recognize both native and denatured forms of Aβ and can be used in dot and Western blot approaches. This allowed us to establish the content of the various Aβ species in micropunched tissues from sporadic AD and Down syndrome brains as well as in *microcebus* lemurian brains (manuscripts in preparation). The two antibodies also appear potentially useful for cell biological approaches since they immunoprecipitate their respective Aβ species as well as their p3-related fragments. Particularly interesting was the observation that the pathogenic mutations or deletion on the presenilin 1 not only affected the recovery of Aβ42 but also, to a very similar extent, that of its p342 counterpart. This suggests that the occurrence of such mutations likely trigger phenotypic alterations of the γ- but not the α-secretase cleavages.

The ELISA assay described here allows the detection of 25–50 pg/ml of Aβ40 and Aβ42. It should be noted that these levels of sensitivity permit the quantification of the various Aβs in the biological fluids of patients affected by the disease.

There are divergent views concerning the nature of Aβ in parenchymal deposits. Mori and co-workers reported on a major Aβ40 species (32), while others showed a major contribution of Aβ42 (33,34). Our IgG-purified fractions of FCAs appeared particularly adapted to immunohistochemistry studies, as underlined by the specific labeling of amyloid deposits as well as the very low aspecific background staining. Clearly, the nature of the Aβ species present in the neocortex of a sporadic AD case varies according to the type of lesion. Diffuse plaques were not labeled by FCA3340 but were detected by FCA18 and FCA3542, indicating that Aβ42 was likely the prominent form observed in early lesions. This agrees well with other studies of AD and Down's syndrome brains (35,36), as well as of other mammalian brains (37,38), documenting the fact that Aβ42 formation likely preceeds Aβ40 deposition. All antibodies labeled the mature plaques, but FCA3340 stained only their central core as previously reported (39). It is important to note that the study of Iwatsubo and co-workers was carried out with Aβ42-directed antibodies which also recognize Aβ43 (36). In this context, it is interesting that FCA3542 does not recognize Aβ43 (see Results), ruling out the possible contribution of this species to the labeling of lesions.

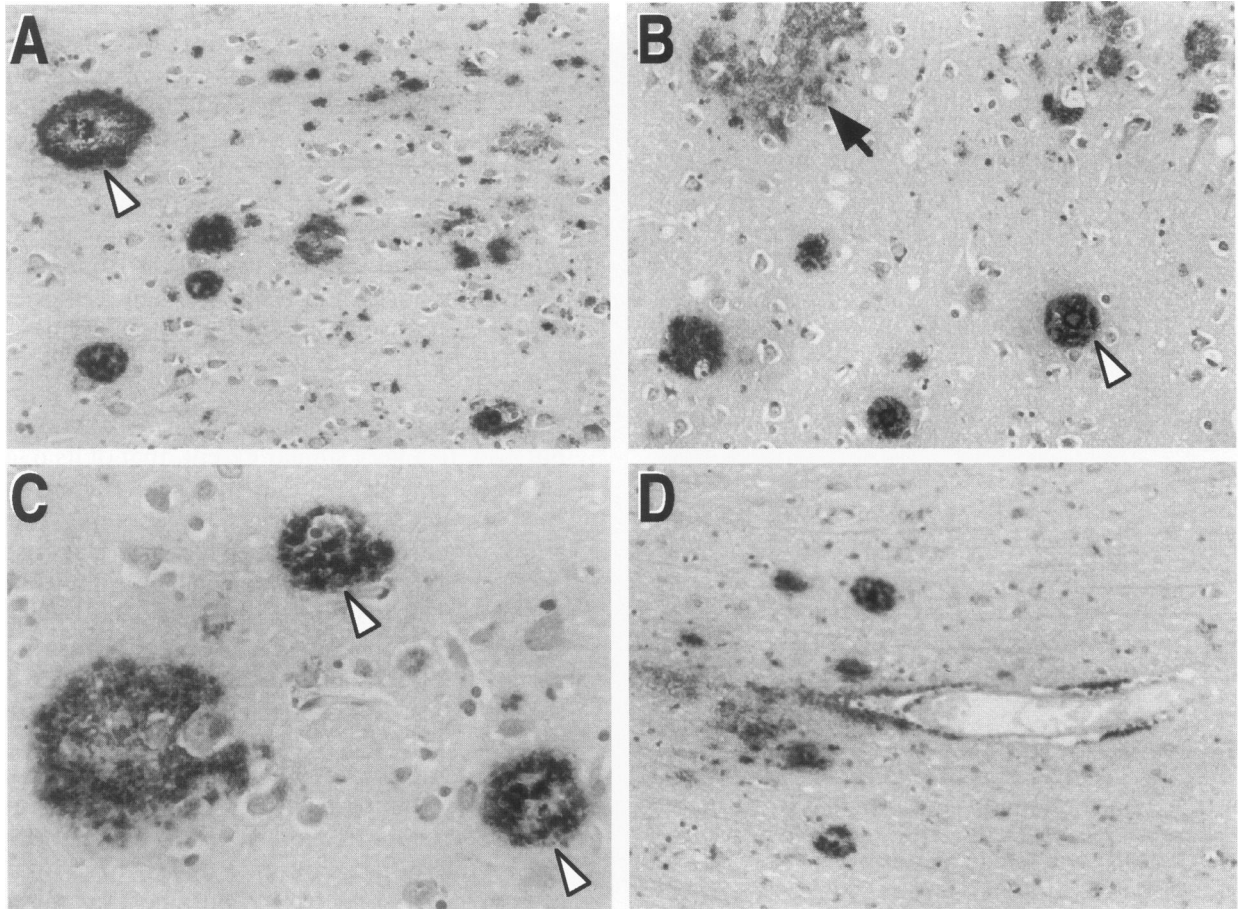Discordant observations also exist concerning the nature of the vascular deposits, since

FIG. 10.   Immunohistochemical analysis of the temporal neocortex of a sporadic case of AD by FCA 3542 IgG-purified fraction

The temporal neocortex (sporadic AD case) was incubated with a 250-fold dilution of the IgG-purified fraction of FCA3542, then immunoreactivity was revealed by the biotin-streptavidin-peroxidase method. White arrowheads in A, B, and C indicate mature plaques while black arrow in B indicates an immature plaque. Note in D the labeling of the vascular wall of a cortical arteriole. Magnification: A, B, and D, ×200; C, ×400.

Roher et al. (40) reported on virtually equal amounts of $A\beta40$ and $A\beta42$, which is in disagreement with other studies (41,42). Our examination of a typical CAA case indicates an intense labeling of leptomeningeal vessels and cortical arterioles by FCA18 and FCA3340, whereas a very weak staining was observed with FCA3542. The pattern observed in this CAA case suggests a major contribution of $A\beta40$ in leptomeningeal and cortical vessels, which is in agreement with a previous study showing that the major $A\beta$ species identified in cerebrovascular lesions ended at valine 40 residue (33).

In conclusion, our study describes novel end-specific $A\beta$ antibodies that can be used in biochemical, cell biological, and immunochemical approaches to identify and/or quantify various $A\beta$ species. These tools should be of great help in investigating the $A\beta$-related dysfunctions that take place in Alzheimer's disease neuropathology.
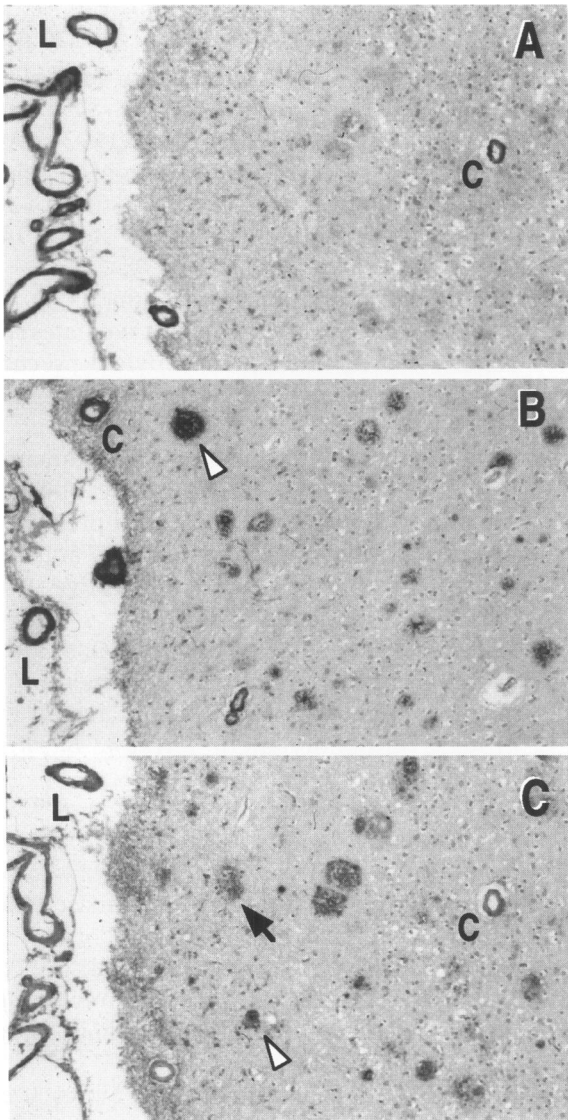
ACKNOWLEDGMENTS

FIG. 11. Immunohistochemical analysis of a presenile AD case with typical cerebral amyloid angiopathy by the IgG-purified fractions of FCAs

The cortex of a neuropathologically diagnosed presenile AD case with CAA was incubated with the IgG-purified fractions of FCA18 (A; 1:500 dilution), FCA3340 (B, 1:50 dilution), or FCA3542 (C, 1:50 dilution), and immunoreactivity was revealed by the biotin-streptavidin-peroxidase method. L, leptomeningeal arterioles; C, cortical arterioles; black arrow indicates immature plaque, and white arrowheads indicate mature lesions. Magnification: A–C, ×50.

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