Elevation of β-Amyloid Peptide 2–42 in Sporadic and Familial Alzheimer’s Disease and Its Generation in PS1 Knockout Cells*

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Urea-based β-amyloid (Aβ) SDS-polyacrylamide gel electrophoresis and immunoblots were used to analyze the generation of Aβ peptides in conditioned medium from primary mouse neurons and a neuroglioma cell line, as well as in human cerebrospinal fluid. A comparable and highly conserved pattern of Aβ peptides, namely, 1–40/42 and carboxyl-terminal-truncated 1–37, 1–38, and 1–39, was found. Besides Aβ1–42, we also observed a consistent elevation of amino-terminal-truncated Aβ2–42 in a detergent-soluble pool in brains of subjects with Alzheimer’s disease. Aβ2–42 was also specifically elevated in cerebrospinal fluid samples of Alzheimer’s disease patients. To decipher the contribution of potential different γ-secretases (presenilins (PSs)) in generating the amino-terminal- and carboxyl-terminal-truncated Aβ peptides, we overexpressed β-amyloid precursor protein (APP)-trafficking mutants in PS1+/+ and PS1−/− neurons. As compared with APP-WT (primary neurons from control or PS1-deficient mice infected with Semliki Forest virus), PS1−/− neurons and PS1+/+ neurons overexpressing APP-Dct (a slow-internalizing mutant) show a decrease of all secreted Aβ peptide species, as expected, because this mutant is processed mainly by γ-secretase. This drop is even more pronounced for the APP-KK construct (APP mutant carrying an endoplasmic reticulum retention motif). Surprisingly, Aβ2–42 is significantly less affected in PS1−/− neurons and in neurons transfected with the endocytosis-deficient APP-Dct construct. Our data confirm that PS1 is closely involved in the production of Aβ1–40/42 and the carboxyl-terminal-truncated Aβ1–37, Aβ1–38, and Aβ1–39, but the amino-terminal-truncated and carboxyl-terminal-elongated Aβ2–42 seems to be less affected by PS1 deficiency. Moreover, our results indicate that the latter Aβ species could be generated by a βAsp/Ala-secretase activity.

Proteolytic cleavages of the integral membrane protein β-amyloid precursor protein (APP)1 result in generation of β-amyloid (Aβ) peptides that accumulate to high levels in the brains of patients suffering from Alzheimer’s disease (AD) (1). In this regard, one recent notable achievement has been the identification of the β-secretase β-site amyloid-β-cleaving enzyme (BACE), a metalloproteinase of 50 kDa (2–5). Heterogeneity of β-secretase cleavage has been observed (6–9), suggesting the existence of alternative β-secretase activities. Another metalloproteinase, a disintegrin and metalloproteinase 10 (ADAM10), is a potential candidate for α-secretase, as are metalloproteinase disintegrin cysteine-rich protein 9 (MDC9) and tumor necrosis factor-α cleaving enzyme (TACE) (10–14).

Identification of γ-secretase is of crucial importance because this is the last step before amyloidogenesis and because γ-secretase-like activities are also involved in the processing of other proteins such as Notch (15). Even if the γ-secretase has not yet been formally identified, most of the existing in vivo and in vitro data based on presenilin (PS) knockout analysis, mutagenesis of specific amino acids, and drug targeting point to the narrow relationship between presenilins and an aspartyl γ-secretase activity (15–25). PSs are proteins with multiple transmembrane domains, which are essentially located in the ER (26). The precise cleavage sites of the γ-secretase and hence the generation of the shorter or longer Aβ peptide are widely believed to have important pathological consequences. Disease-linked mutations in PS1, PS2, and APP result in an increase in production of Aβ1–42, which is the major component of the β-amyloid plaques deposited in the brains of AD patients (27–30). Targeted deletion of the PS1 gene resulted in a greatly decreased production of Aβ peptides 1–42 as well as Aβ1–40 (16). The γ-secretase cleavage constitutes an obvious target for disease prevention. The relationship between generation of a wide range of Aβ species (with a 2- or 3-amino acid variation at both the amino- and carboxyl-terminal ends; see Fig. 1A) and the

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1 The abbreviations used are: APP, β-amyloid precursor protein; AD, Alzheimer’s disease; ER, endoplasmic reticulum; IR, infrared; MALDI, matrix-assisted laser desorption ionization; nAD, dementia of etiology other than AD; OND, other nondemented neuropsychiatric disease; PS, presenilin; PVDF, polyvinylidene difluoride; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; Aβ IGP-2D-PAGE, immobilized pH gradient electrophoresis focusing with Aβ SDS-PAGE/immunoblot as second analytical dimension; SFV, Semliki Forest virus; CSF, cerebrospinal fluid; Aβ, β-amyloid; BACE, β-site amyloid-β-cleaving enzyme; LB, Lewy body; PBS, phosphate-buffered saline; PBS-T, PBS containing Tween 20; RIPA, radioimmuneprecipitation buffer; mAb, monoclonal antibody; LBD, LB dementia; IPG, immobilized pH gradient.
existence of potential different γ-secretases and/or PS is not clear (31–33).

The location of the secretase activities within the cell has only been partially established. α-Cleavage occurs at the plasma membrane and also potentially occurs in intracellular post-Golgi compartments (13, 34–37). β-Secretase is active in acidic, nonlysosomal compartments (38). There is some evidence that the carboxyl termini of Aβ1–40 and Aβ1–42 are generated in different cellular compartments (39, 40).

Mass spectroscopy has indicated the existence of additional Aβ peptide species besides Aβ1–40 and Aβ1–42 are generated in different cellular compartments (39, 40).

We recently established one- and two-dimensional electrophoretic methods2 for high resolution expression profiling of APP metabolites (44, 45) that are based on the N,N,N'-bis-(2-hydroxyethyl)-glycine/Tris urea separation gels of Wiltfang et al. (47). The separation principle relies on conformational shifts of Aβ peptides in the presence of urea, which are specific for distinct Aβ peptide species (Fig. 1B). Interestingly, we observed a structure-function analogy in vivo and in vitro because the solubility of Aβ peptide species in vivo, e.g., increased aggregation of amino-terminal-truncated and carboxyl-terminal-elongated Aβ peptides, was closely and positively correlated with the electrophoretic mobility of Aβ peptide species in vitro. By using monoclonal antibody 1E8, which is specific for the first 2 amino acids of the Aβ peptide amino terminus (Fig. 1B), we realized a detection sensitivity in the subpicogram range.2 Here we combined immunoprecipitation (1E8) with the first 2 amino acids of the Aβ peptide amino terminus (Fig. 1A) with the second 2 amino acids of the Aβ peptide amino terminus (Fig. 1A), which allowed us to study the secretion of Aβ peptides into the supernatants of neuronal cell cultures and a neuroglioma cell line. In addition, we studied the APP metabolism profile in brain homogenates and the CSF of patients with AD, patients with non-AD dementias (nADs), and patients with various nondementive neuropsychiatric diseases (ONDs).

A comparable and highly conserved pattern of Aβ peptides, namely, Aβ1–40/Aβ1–42 and carboxyl-terminal-truncated Aβ1–37, Aβ1–38, and Aβ1–39, was found. Besides Aβ1–42, we also observed a striking and consistent elevation of amino-terminal-truncated Aβ2–42 in the brains of subjects with AD. Because Aβ2–42 was enriched in the detergent-soluble fraction of AD brain homogenates, it did not originate from β-amyloid plaque core. Aβ2–42 was also specifically elevated in CSF samples of AD patients. To decipher the contribution of potential different γ-secretases (presenilins) in generating the amino-terminal- and carboxyl-terminal-truncated Aβ peptides, we overexpressed APP-trafficking mutants in PS1+/+ and PS1−/− neurons. As compared with APP-WT (primary neurons from control or PS1-deficient mice infected with Semliki Forest virus), PS1−/− neurons and PS1+1+/+ neurons overexpressing APP-ΔC (a slow-internatizing mutant) or APP-KK (APP mutant carrying an ER retention motif) show a decrease of all secreted Aβ peptide species. Surprisingly, Aβ2–42 is significantly less affected in PS1- and endocytosis-deficient (APP-ΔCt) neurons.

Taken together, our results indicate that the amino-terminal-truncated and carboxyl-terminal-elongated Aβ2–42 was generated within a post-ER secretory pathway, where most of the Aβ2–42 is generated by a βAsp40Asp-secretase activity, possibly as alternative BACE activity. The data confirm that PS1 is an essential γ-secretase protein/cofactor responsible for the production of Aβ1–40/Aβ1–42 and the carboxyl-terminal-truncated Aβ1–37, Aβ1–38, and Aβ1–39, but not for the amino-terminal-truncated and carboxyl-terminal-elongated Aβ2–42.

**MATERIALS AND METHODS**

**Patients**

We investigated a total of 104 patients by Aβ SDS-PAGE/immunoblot. The dementia group included 51 patients with probable AD (average age, 70.9 ± 9.8 years) and 12 patients with dementia of other etiology (nAD group; average age, 53.9 ± 20.1 years). Moreover, we investigated 41 patients with various ONDs (average age, 42.5 ± 15.5 years). The patient groups nAD and OND can be summarized as neuropsychiatriological diagnosis (n = 53). Neuropsychiatric diagnosis of AD was established by ICSD-10 criteria (54). Patients with probable AD had to satisfy DSM-IV criteria (55). Neuropsychiatriological diagnosis was performed using the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) diagnostic criteria based on semiquantitative analysis of neuropeptide anti-body (Bialoskowsky stain and β4 immunostain) as well as the Braak and Braak classification based on the distribution of neurofibrillary tangles and neuritic plaques (Bialoskowsky stain and β4 immunostain) (50, 51). Distribution and frequency of Lewy bodies (LBs) were evaluated according to the Consortium for the Evaluation of Dementia with LBs (52). The number of LBs was counted in the brain regions determined in the protocol (β-synuclein immunostain) and converted into scores of A (no LBs), B (1–4 LBs), and C (>5 LBs) for each area. Based on the total score, dementia with LB cases were divided into three subtypes: brainstem dominant, limbic, and neocortical. Diagnosis of Pick disease and dementia lacking distinctive histopathology of Pick disease according to Cooper et al. (53). Diagnosis of the tangle in the predominant form of senile dementia was made according to Jellinger and Bancher (54). Frozen brain tissue (frontal cortex and cerebellum) of the neuropsychiatically examined cases was used for immunoprecipitation with one- and two-dimensional Aβ SDS-PAGE/immunoblot.

**Neuronal Cell Culture and Neuroglioma Cell Line**

Generation of PS1 knockout mice has been described previously (16). For mouse neuronal primary cultures, 14-day-old embryos were taken from heterozygote (PS1+/−) or homozygote (PS1−/−) mice. Total brains were dissected and trypsinized. Cells were resuspended, plated on 95-cm² tissue culture dishes (Nunc) precoated with 1 mg/ml poly-[l]lysine (Sigma), and finally incubated in minimum Eagle’s medium (Life Technologies, Inc.) supplemented with 10% horse serum medium (Merck). After 4 h of culture, cells were incubated in normal medium (Life Technologies, Inc.) supplemented with the B27 complement mixture (Life Technologies, Inc.) for 24 h before the addition of 5 mg/ml cytosine arabinoside for inhibition of glial cell growth. A stably transfected H4 neuroglioma cell line expressing human mutant APP751–42 (Swedish double mutation, K670M/N671L) was generously provided by T. Dyrrs (Schering AG, Berlin, Germany). Cloning procedures, plasmid construction, transfection, and tissue culture were performed according to Urmont et al. (55).

**Semliki Forest Virus (SFV) Constructs**

The cDNA coding for human APP695 and containing a Myc tag 3 amino acids after the signal sequence (inserted at the Kpn1 site) cloned in pSP65 was kindly provided by Drs. P. Tienari and K. Beyreuther (Zentrum für Molekulare Biologie der Universität Heidelberg, Heidelberg, Germany). This construct either (i) had the last 43 amino acids of the cytoplasmic tail deleted (from Tyr5 to Aom695) (APP-ct) as de-
scribed in Ref. 56 or (ii) was modified by site-directed mutagenesis (Stratagene) to add a di-lysine motif allowing its retention in the ER at position 692 and 693 (Q4 mutated to KK, APP-RK). To obtain SFVs containing those sorting mutants, all constructs were moved to pSFV-1 vector (provided by Dr. C. Dotti, EMBL) and linearized with SpeI. mRNAs were prepared by in vitro transcription using the SP6 polymerase and a mixture of mRNA from APP constructs and from pSFV-helper were cotransfected in BHK cells by electroporation to obtain recombinant SFV. BHK cells were grown in Dulbecco’s modified Eagle’s medium/F-12 (Life Technologies, Inc.) supplemented with 5% fetal calf serum (2-hydroxyethyl)-glycine, 1% (w/v) SDS, 15% (w/v) sucrose, and 0.004% nobilet, bound Aβ were finally solubilized in 25 times with DIP buffer and washed once with PBS/water (1:3). Samples were removed after 24 h of incubation and analyzed by immunoprecipitation with mAb 6E10 (Senetek, St. Louis, MO), we pooled the brain homogenates of eight AD patients (3 mg/ml protein content). 50 μl of the magnetic microparticles (2 μg mAb 6E10/3.36 × 10^7 beads) was added to 1 ml of homogenate. Except for two additional washes with 1× RIPA, immunoprecipitation was performed as described previously. For immunoprecipitation with mAb 6E10 (Senetek, St. Louis, MO), we pooled the brain homogenates of eight AD patients (3 mg/ml protein content). 50 μl of the magnetic microparticles (2 μg mAb 6E10/3.36 × 10^7 beads) was added to 1 ml of homogenate, and immunoprecipitation was performed as described previously.

**Preparation of Samples**

**CSF Samples**—After obtaining informed consent from patients and/or family members, CSF (3–10 ml) was drawn by lumbar puncture from patients and sampled in polypropylene vials. After centrifugation (1000 × g, 10 min, 4 °C), CSF samples were processed within 12 h, and aliquots of 150 μl were stored at −80 °C for subsequent one- and two-dimensional Aβ SDS-PAGE/immunoblot.

**Immunoprecipitation of Cell Culture Media**—Cell culture media were removed after 24 h of incubation and analyzed by immunoprecipitation for Aβ peptides. For immunoprecipitation, 400 μl of media were added to 100 μl of 5-fold concentrated RIPA detergent buffer (5 × RIPA = 2.5% Nonidet P-40, 1.25% sodium deoxycholate, 0.25% SDS, 750 mM NaCl, 250 mM HEPES, and 1 tablet of protease inhibitor mixture Complete™ Mini per 2 ml of 5 × RIPA, pH adjusted to 7.4 with NaOH) and 25 μl of magnetic microparticles (DynaBeads, Dynal, Germany) coated with monoclonal antibody 1E5 (1 μg of mAb 1E5/1.68 × 10^8 beads). The protease inhibitor mixture Complete™ Mini was obtained from Roche Molecular Biochemicals, and mAb 1E5 was obtained by T. Dyrrks. Samples were incubated under rotation for 15 h at 4 °C. Beads were washed four times with PBS/0.1% bovine serum albumin and one time with 10 mM Tris-HCl, pH 7.4. For Aβ SDS-PAGE/immunoblot, bound Aβ peptides were eluted by heating the sample to 95 °C for 5 min with 25 μl of sample buffer (42:1 Tris/HCl, pH 6.8, 2.5% SDS, 15% (w/v) sucrose, 0.004% (w/v) bromphenol blue). In case of Aβ IPG-2D-PAGE/immunoblot, Aβ peptides were eluted in the presence of 25 μl of 40% (v/v) formic acid by sonication for 10 min at 37 °C. Finally, the formic acid was removed by evaporation (SpeedVac, 40 °C).

**Western Blotting, Immunostaining, and Quantification**—Western blotting, immunostaining, and quantification were performed as described by Wiltfang et al. Aβ peptides were transferred for 30 min at 1 mA/cm^2 and room temperature under semidry conditions ( Hoefer Semi-
was pinnhole-perforated at three sites. This guaranteed exact realignment of the Aβ peptide species after the stack had been dismantled. Before electroblotting, the stack was left for 5 min to allow cathodic contact blotting. For subsequent IR-MALDI mass analysis, one of the two anodic PVDF membranes was incubated in an aqueous matrix solution (0.3 M succinic acid) for 20 min at room temperature directly after the Western blot, while the membrane was still wet. This was followed by slow drying at room temperature. The other anodic PVDF membrane was washed for 3 × 5 min with double distilled H2O, dried, and used for subsequent gas-phase sequencing. Meanwhile, the cathodic PVDF membrane was immunostained (mAb 1E8) as described above but developed with diaminobenzidine (peroxidase substrate kit; Vector Laboratories) instead of enhanced chemiluminescence. This membrane served as a template to excise the areas of the anodic PVDF membrane corresponding to Aβ1–42 and the unknown Aβ peptide.

IR-MALDI mass analysis (wavelength, 2.94 μm; spot, 100 μm; pulse, 90 ns) was performed directly from the pieces of matrix-embedded PVDF membrane (58). For gas-phase sequencing of electroblotted Aβ peptides, spots were excised from the PVDF membrane and applied to a Procise cLC protein sequencer (Applied Biosystems). For amino-terminal sequencing, standard protocols were used according to the manufacturer’s instructions.

Synthetic Aβ Peptides

Synthetic Aβ peptides 1–38, 1–40, and 1–42 were obtained from Bachem (Bubendorf, Schweiz). Other synthetic Aβ peptides were synthesized automatically using Fmoc (N-(9-fluorenylmethoxycarbonyl) chemistry according to Janek et al. (59).

Statistics

Groups were characterized by mean values and S.D. Group differences were tested for significance (p < 0.05, two-sided level) by the Mann-Whitney U test (adjusted for small sample size) and, in case of dichotomous variables, by χ2 analysis according to Fisher’s exact test. Computations were performed using the statistical software package Statistica for Windows, Version 5.1 F.

RESULTS

High Resolution Analysis of Aβ Peptides—Whereas Aβ peptides migrate as a single species by conventional Aβ SDS-PAGE, the urea-based separation gels resolve a complex but highly conserved Aβ peptide pattern. By Aβ SDS-PAGE/immunoblot, we were able to demonstrate that in addition to Aβ peptides 1–40 and 1–42, the carboxyl-terminal-truncated spe-
cies 1–37, 1–38, and 1–39 are regularly found in human CSF (Fig. 1B). This Aβ peptide quintet was observed in all human CSF samples (n > 500) investigated thus far (Fig. 2A, lane 4).

An Additional Amino-terminal-truncated Aβ Species in Secretions and Patients with Alzheimer's Disease—Surprisingly, in neuroglioma H4 cells transfected with human APP carrying the Swedish double mutation and in primary mouse neurons virus-transfected with human APP, a minor Aβ peptide species became prominent in addition to the Aβ peptide quintet 1–37, 1–38, 1–39, 1–40, and 1–42 (Fig. 2A, lanes 2 and 3) migrating below (anodically) Aβ1–42. Interestingly, in a subset of AD patients, a reduced CSF concentration of Aβ1–42 was paralleled by a detectable level of the additional Aβ peptide species (Fig. 2A, lane 6), whereas other AD patients presented only reduced CSF levels of Aβ1–42 (Fig. 2A, lane 5). In contrast, in the CSF of nAD or OND patients, Aβ1–42 was usually not decreased, and the additional Aβ peptide species was not detectable (Fig. 2A, lane 4). Moreover, the additional Aβ peptide species was highly abundant in the detergent (RIPA)-soluble fraction of brain homogenates from the frontal lobe of patients with Alzheimer’s disease (Fig. 2B, lane 2*).

Identification of the New Aβ Peptide as Aβ2–42—RIPA-soluble Aβ peptides from the frontal lobe of a patient with sporadic AD were immunoprecipitated (1E8), separated by Aβ SDS-PAGE, and blotted onto PVDF membranes as described previously. IR-MALDI mass analysis was performed directly from the pieces of matrix-embedded PVDF membrane and yielded a molecular weight of 4523.3 ± 7 for the PVDF area corresponding to the Rf value of synthetic Aβ1–42 (Mᵣ 4514.14). The PVDF area containing the unknown Aβ peptide yielded a major peak with a Mᵣ of 4405.6 ± 10 and a minor peak (<10%) with a Mᵣ of 4334.5 ± 12, corresponding most probably to Aβ2–42 (Mᵣ 4399.01) and 3-42 (Mᵣ 4327.93), respectively.

Gas-phase sequencing of the PVDF membrane area corresponding to the Rf of synthetic Aβ1–42 yielded a major sequence of DAEFRHDSGY, corresponding to Aβ1–x, and a minor sequence (<10%) of AEFR, corresponding to Aβ2–x. The PVDF area corresponding to the Rf value of the unknown Aβ peptide migrating anodically of Aβ1–42 gave the sequence AEFRHDSGY, corresponding to Aβ2–x.

Taken together, IR-MALDI mass analysis and gas-phase sequencing identified the unknown Aβ peptide migrating anodically of Aβ1–42 as Aβ2–42. According to IR-MALDI mass analysis, a small amount of Aβ3–42 was immunoprecipitated with Aβ2–42, which was not detected by gas-phase sequencing. The minor amount of Aβ2–x comigrating with Aβ1–42 most probably corresponded to a contamination with Aβ2–42.

This data prompted us to synthesize the Aβ peptides 2/3–40 and 2/3–42 and the pyroglutamate derivatives 3p-40/42 for subsequent comigration experiments.

Two-dimensional electrophoretic separation of the latter Aβ peptides showed that synthetic Aβ peptides 2–40/42 share a shift of their isoelectric points of one pH unit (5.37 ⇒ 6.37) during IEF due to the missing amino-terminal aspartate. The same shift is observed for Aβ peptides 3–40/42 because alanine at position 2 is not charged. Aβ2–40 and Aβ2–42 can be separated within the second analytical dimension due to differential shifts in comigration in the presence of urea (Fig. 3A). Aβ peptides 3–40 (data not shown) and 3–42 were barely detected by mAb 1E8 (Fig. 4B; cross reactivity < 5%). Synthetic pyroglutamate derivatives Aβ3p-40/42 did not comigrate with Aβ2/3–42 (Fig. 4C) and were not detected by mAb 1E8 (data not shown; cross reactivity < 5%).

Accordingly, Aβ IPG-2D-PAGE/immunoblot (mAb 1E8) allowed us to identify the additional two-dimensional spot pres-
ent in the RIPA-soluble fraction of brain homogenates (Fig. 3C), CSF samples (Fig. 3B), and supernatants from primary mouse neurons (Fig. 3D) as Aβ2–42.

Comparison of the Relative Abundance of Additional Amino-terminal-truncated Aβ Peptide Species in AD Brain Homogenates—We immunoprecipitated (mAb 6E10) a pool of the detergent-soluble fraction from the frontal lobes of AD patients (n = 8). Immunoprecipitates were analyzed by Aβ SDS-PAGE/immunoblot using either mAb 6E10 (Fig. 4, A and C) or 1E8 (Fig. 4B). For comparison, the Western immunoblots contained a dilution series of synthetic Aβ2–42 and Aβ3–42 (Fig. 4, A and B) or Aβ3p-40 and Aβ3p-42 (Fig. 4C).

Analysis of the synthetic Aβ peptides revealed that mAb 1E8 barely detected Aβ peptides that were amino-terminal by more than 1 amino acid (cross reactivity < 5%). Interestingly, the high specificity of mAb 1E8 for the Aβ peptide amino terminus was restricted to our Aβ SDS-PAGE/immunoblot because Aβ2–42 and Aβ3–42 were immunoprecipitated with approximately the same yield by mAb 1E8 (data not shown). Synthetic Aβ2/3–42 showed an identical migration pattern and migrated faster than Aβ1–42 (Fig. 4, A and B). The highly amyloidogenic Aβ peptides 3p-40 and 3p-42 migrated even faster than Aβ2/3–42 but were not further separated by Aβ SDS-PAGE/immunoblot (Fig. 4C).

Pooled AD brain homogenates immunoprecipitated and analyzed by mAb 6E10 (Fig. 4, A and C) revealed a pattern similar to mAb 1E8 for Aβ peptides 1–38, 1–40, 1–42, and 2–42 (Fig. 4B). However, when detection was performed by mAb 6E10, an additional band was observed. This band comigrated with synthetic Aβ3p-40/42 (Fig. 4, A and C).

According to Fig. 4D, the enhanced chemiluminescence signal was additive for Aβ2/3–42 if both peptides were run as a mix (duplicate; 500 pg each) but differentiated by quantification of one blot membrane by mAb 6E10 and the other by mAb 1E8. This differential Western immunoblot technique allowed us to estimate the amount of Aβ3–42 comigrating with Aβ2–
24. It is noteworthy that the avidity of mAb 6E10 for Aβ–42 during Aβ SDS-PAGE/immunoblot is 2.8-fold lower than that for Aβ–42. Again, we did not observe a significant difference with regard to the avidity of mAb 6E10 for Aβ–3–42 if the antibody was used during immunoprecipitation (results not shown).

The relative amounts of Aβ peptide species in the detergent-soluble pool of homogenates from the frontal lobe of AD patients are summarized in Fig. 4. Relative to Aβ1–42 (100%; data not shown), the next prominent Aβ peptide is Aβ–42 (11%), followed by Aβ1–40 (5%), Aβ3–40/42 (3%), Aβ1–38 (2%), and Aβ–38 (1%). Relative to Aβ–42, the amount of Aβ–38 was estimated as ~12%. This corresponds to the amount of Aβ–38 comigrating with Aβ–42, which we determined by Aβ SDS-PAGE and IR-MALDI time-of-flight when we analyzed the immunoprecipitate (mAb 1E8) from the brain homogenate of a single AD patient (see above).

Aβ2–42 Is Specifically Elevated in Brain and CSF Samples of AD Patients—After Aβ1–42, Aβ2–42 was the next most prominent species in the RIPA-soluble fraction for the whole set of AD brain homogenates investigated (n = 9), and both Aβ peptides were grossly elevated as compared with Aβ1–38 and Aβ1–40. Interestingly, a patient with familial AD due to PS1 mutation T115C did show the same Aβ peptide pattern as described for the sporadic AD cases. In line with the comparatively low β-amyloid plaque density of the cerebellum in AD, the cerebellar concentration of Aβ1–42 and the additional Aβ peptide species was more than 10-fold lower than that present in the frontal lobe (Fig. 2A, lane 2; Table I). This difference between frontal lobe and cerebellum was most pronounced for Aβ2–42. The additional Aβ peptide species and Aβ1–40, Aβ1–38, and Aβ1–40 were also present in the RIPA-soluble fraction of brain homogenates from patients with frontotemporal dementia lacking distinct histopathology (n = 2), Pick’s disease (n = 3), tangle predominant form of senile dementia (n = 1), or nondemented controls (n = 4), but to a much lower extent (Fig. 2B, lanes 3 and 4). Interestingly, patients with Lewy body dementia (LBD) and pronounced β-amyloid plaque load (LBD CERAD C; n = 2) also showed the striking elevation of Aβ2–42 and the Aβ peptide pattern of AD patients, whereas a LBD patient without a concomitant β-amyloid pathology (LBD CERAD A; n = 1) did not show these changes. The concentrations of Aβ peptide species 2–42, 1–42, 1–40, and 1–38 in the brain homogenates from AD patients (frontal lobe and cerebellum), patients with other dementias (frontal lobe), and controls (frontal lobe) are summarized in Table I.

Moreover, Aβ2–42 was detected in the CSF of a subset of patients with AD (n = 18 of 51; 35%). In contrast, Aβ2–42 was detected significantly less frequently in nAD and OND patients (Fig. 2A, lane 4). Only 2 of the 12 nAD patients (17%) and 4 of the 41 OND patients (10%) had detectable CSF levels of Aβ2–42 (AD versus nAD/OND, p = 0.005). Among the two nAD patients with detectable CSF levels of Aβ2–42, one 71-year-old female was diagnosed with probable LBD, and a 61-year-old female was classified as having unspecified dementia (International Classification of Diseases 10 F03) due to atypical symptomatology. According to our neuropathological data, the former patient may represent a LBD CERAD C case. Interestingly, three of the four OND patients with Aβ2–42-positive CSF presented with cognitive impairment as part of their psychiatric symptomatology.

The subgroup of AD patients with Aβ2–42-positive CSF samples was characterized by significantly lower CSF concentrations of Aβ1–37 (p < 0.05) as compared with the AD patients without detectable CSF concentrations of Aβ2–42 (Fig. 5A). This drop in Aβ1–37 was even more pronounced if Aβ1–37 was
expressed as percentage of total Aβ peptides (percentage of Aβ1–37, p < 0.01) and was paralleled by a significant increase in the percentage of Aβ1–39 (p < 0.025; Fig. 5B).

Amino- and Carboxyl-terminal-truncated Aβ Species in Presenilin-1-deficient Neurons and APP-trafficking Mutants—We wanted to investigate to what extent the generation of aminoterminal- and carboxyl-terminal-truncated Aβ peptides was dependent on presenilin activity and intracellular trafficking. For this purpose, we infected primary mouse neurons from control animals and presenilin-1-deficient mice with Semliki Forest Virus-expressing human APP. In addition, we expressed human APP, which is retained in the ER due to an ER retention motif (APP-KK) and another APP variant lacking the cytoplasmic domain essential for reinternalization (APP-Δct).

According to Fig. 6, the expression level of holo-APP was comparable in both PS-1-deficient and control neurons as well as in the two trafficking mutants. The infected cells overexpressed holo-APP severalfold as compared with the endogenous level (Fig. 6).

In addition to Aβ1–40 and Aβ1–42, substantial amounts of the carboxyl-terminal-truncated Aβ peptides 1–37, 1–38, and 1–39 and a minor amount of the aminoterminal-truncated Aβ2–42 are secreted into supernatants of PS wild-type supernatants (Fig. 7). As described previously (16), secretion of Aβ peptides was drastically reduced in PS1-deficient neurons. However, the PS1-dependent reduction was different for the single Aβ peptide species studied. Interestingly, the carboxyl-terminal-truncated Aβ peptides were decreased to at least the extent of Aβ1–40 and Aβ1–42, in contrast to the much less pronounced reduction of Aβ2–42 (Fig. 7).

According to Fig. 8, A and B, compares the effect of trafficking mutants on Aβ peptide secretion for PS-deficient neurons and controls. According to Fig. 8A, which depicts the slow-internalizing mutant (APP-Δct) versus wild-type APP in PS1+/+ neurons, we observed an APP-Δct-dependent reduction in the range of 69 ± 12.6% (Aβ1–40) to 79 ± 10.1% (Aβ1–38) for all Aβ peptide species except Aβ2–42, which was reduced in the order of only 25 ± 29.1%. The APP-Δct-dependent reduction of Aβ2–42 was differential effect for the carboxyl-terminal-truncated Aβ peptides and the amino-terminal-truncated Aβ2–42 (Fig. 7).

The concentrations of secreted Aβ peptides in APP wild-type transfected PS1+/+ neurons were as follows (mean ± S.D.; four independent experiments): 137.6 ± 69.9 pg/ml, Aβ1–37; 202.9 ± 73.9 pg/ml, Aβ1–38; 159.4 ± 91.8 pg/ml, Aβ1–39; 1133.3 ± 302.0 pg/ml, Aβ1–40; 211.9 ± 95.0 pg/ml, Aβ1–42; and 35.2 ± 12.7 pg/ml, Aβ2–42. Interestingly, this Aβ peptide pattern does closely resemble the profile we observed in human CSF (data not shown).

In the following text, Aβ peptide results will be expressed as ratios (Figs. 8 and 9) or percentage of change (see the body of text explaining Figs. 8 and 9). Either PS1+/+ versus PS1−/− or slow-internalizing APP mutant versus APP wild-type transfected controls is shown (mean ± S.D., four independent experiments).

According to Fig. 8C, total Aβ showed a PS1-dependent average reduction of 87 ± 4.2% for the wild-type and 66 ± 5.6% for APP-Δct, whereas Aβ2–42 was reduced by only 43 ± 15.8% (Aβ2–42 versus total Aβ species, p = 0.029) and 22 ± 18% (Aβ2–42 versus total Aβ species, p = 0.029), respectively. The PS1-dependent reductions for the other single Aβ peptide species aside from Aβ2–42 were similar to total Aβ. They ranged between 92 ± 4.2% (Aβ1–38) and 84 ± 4.6% (Aβ1–42) for the wild-type and 70 ± 5.4% (Aβ1–40) and 57 ± 5.6% (Aβ1–42) for the carboxyl-terminal-deleted APP construct (Fig. 8C).

Fig. 8, A and B, compares the effect of trafficking mutants on Aβ peptide secretion for PS-deficient neurons and controls. According to Fig. 8A, which depicts the slow-internalizing mutant (APP-Δct) versus wild-type APP in PS1+/+ neurons, we observed an APP-Δct-dependent reduction in the range of 69 ± 12.6% (Aβ1–40) to 79 ± 10.1% (Aβ1–38) for all Aβ peptide species except Aβ2–42, which was reduced in the order of only 25 ± 29.1%. The APP-Δct-dependent reduction of Aβ2–42 was...
and APP-C, APP processing. According to Fig. 9 the absence of 42 amino acids of the cytoplasmic domain. Radioactive material was performed with a PhosphorImager. Note that the APP-Δct holo-forms always run lower than the other constructs due to the absence of 42 amino acids of the cytoplasmic domain. Notably, as depicted in Fig. 9B, the differential effect of APP-Δct on the secretion of Aβ2–42 as compared with Aβ1-x was not significant any more in PS1-deficient neurons.

**FIG. 6.** Similar expression levels in PS1+/+ and PS1−/− mouse neurons virus-transfected with human APP-trafficking mutants. Representative analyses of full-length APP are shown. PS1+/+ or PS1−/− primary neuronal cultures were infected with pSFV bearing APP-WT, APP-Δct, or APP-KK as described and metabolically labeled with [35S]methionine for 4 h at 37 °C. Cells extracts were immunoprecipitated using antibodies against the APP ectodomain. The precipitates were separated by SDS-PAGE on 10–20% Tris-tricine gradient gels, and detection of radioactive material was performed with a PhosphorImager. Note that the APP-Δct holo-forms always run lower than the other constructs due to the absence of 42 amino acids of the cytoplasmic domain.

**FIG. 7.** Aβ peptides in PS1+/+ and PS1−/− neurons infected with APP-trafficking mutants. The image represents a typical secretion pattern of four independent experiments. Lanes 1 and 4, mouse neurons expressing human APP-WT; lanes 2 and 5, mouse neurons expressing human APP-Δct; lanes 3 and 6, mouse neurons expressing human APP-KK. PS1+/+ primary neuronal cultures were infected with pSFV-APP-WT as described. After a 6-h postinfection at 37 °C, incubation media were recovered, and Aβ peptides were enriched by amino-terminal-selective immunoprecipitation (mAb 1E8) and analyzed by Aβ SDS-PAGE/immunoblot.

significantly less pronounced as compared with total Aβ peptide species (p = 0.029). Thus, a reduced secretion of Aβ peptides was observed for impaired endocytosis of APP in PS1+/+ neurons (Fig. 8A) as well as PS1 deficiency (Fig. 8C), and both effects also differentially affected the secretion of amino-terminal-truncated Aβ2–42 and the Aβ1-x species. For the APP-KK construct, we observed low levels of Aβ peptides for all Aβ peptide species, which were too close to the limit of detection to allow quantification.

Fig. 8B depicts the Aβ peptide ratios for APP-Δct versus wild-type APP in PS1−/− neurons. In contrast to PS1+/+ neurons, the reduction of Aβ peptide secretion due to the slow-internamizing mutant was much less pronounced and ranged only from 14 ± 25.1% (Aβ1–37) to 26 ± 14.3% (Aβ1–42). In this case, the secretion of Aβ2–42 was not reduced at all by the APP-Δct mutant, but the differential effect of the slow-internamalizing mutant on the secretion of Aβ2–42, as compared with that of the Aβ1-x peptide species, was not significant any more.

In APP-KK-transfected and PS1-deficient neurons, decreased secretion of all Aβ peptide species was observed (Fig. 7). However, the validity of this effect is again limited by the low amount of Aβ peptides secreted.

**FIG. 9.** Comparison of the amino-terminal-truncated Aβ2–42 with the other Aβ peptide species to highlight differential effects on APP processing. According to Fig. 9C, in both APP wild-type- and APP-Δct-transfected neurons, Aβ2–42 is significantly less reduced due to PS1 deficiency as compared with the total of all other Aβ peptide forms (p = 0.029). In PS1+/+ control neurons (Fig. 9A), Aβ2–42 is decreased by only 25 ± 29.1%, whereas the total of all other Aβ forms is reduced by 71 ± 10.9% (p = 0.029).

**DISCUSSION**

**High Resolution Analysis of Aβ Peptides**—One- and two-dimensional Aβ SDS-PAGE/immunoblot combined with immunoprecipitation offers a detection sensitivity comparable to enzyme-linked immunosorbent assay methods and a high resolution separation of a complex mixture of APP metabolites. We further refined the separation gel matrix and detection sensitivity of the Aβ SDS-PAGE/immunoblot, which allows improved separation of the amino- and carboxyl-terminal-truncated Aβ peptide and detection of only 0.3–0.6 pg of Aβ peptides. The monoclonal antibody 1E8 used in this study is highly specific for the Aβ peptide amino terminus, and Aβ peptides amino-terminally truncated by more than 2 amino acids are barely detected (cross reactivity <5%).

**Aβ Peptide Quintet and Carboxyl-terminal-truncated Aβ Peptides**—Our finding of a highly conserved Aβ peptide quintet in various biological fluids and PS1-dependent γ-secretase processing of Aβ1–37, Aβ1–38, and Aβ1–39 provides further evidence for alternative γ-secretase activities (60–62). Interestingly, the relative Aβ peptide quantities of the latter five Aβ peptides were nearly identical in human CSF and supernatants from human APP-transfected primary mouse neurons. In CSF samples, we identified disease-specific patterns of this Aβ peptide quintet in subjects with AD; specifically, the relative amounts of Aβ1–38 and Aβ1–42 were closely correlated. In the RIPA fraction of brain homogenates, Aβ1–38 was elevated in AD patients as compared with controls, whereas this was not observed for Aβ1–37 and Aβ1–39. This may be of pathophysiological relevance because Aβ1–38 was reported to induce autoggregation of α-synuclein (63, 64).

**Specific Elevation of an Additional Amino-terminal-truncated Aβ Peptide Species in AD and Its Identification as Aβ2–42**—Investigating the RIPA-soluble fraction of brain homogenates and CSF samples from AD patients, we observed an additional Aβ peptide species migrating below (anodically) Aβ1–42. Using IR-MALDI mass analysis, gas-phase sequencing, and Aβ IPG-2D-PAGE/immunoblot conformation experiments with synthetic Aβ peptides, we identified the latter Aβ peptide as Aβ2–42, i.e. an Aβ peptide species lacking the amino-terminal aspartate.

Like Aβ1–42, this additional Aβ peptide species was strikingly and consistently up-regulated in the frontal lobe and up-regulated to a much lesser degree in the cerebellum of patients with AD. Interestingly, a patient with familial AD due to a PS1 mutation (T115C) also showed the striking elevation of Aβ2–42 and an Aβ peptide pattern otherwise identical to the sporadic AD cases. An Aβ peptide pattern similar to the one observed for patients with sporadic AD was observed for LBD
patients, but only for those cases in which Lewy body neuropathology was paralleled by a heavy β-amyloid plaque burden (LBD CERAD C). Aβ2–42 was also detectable on a very low level in patients with other dementias and in nondemented controls.

In AD brain samples, Aβ2–42 was consistently increased, but detectable CSF levels were observed only in a subset of AD patients. Nonetheless, the presence of Aβ2–42 in CSF was specific for AD because the peptide was observed significantly less frequently in non-AD patients. Interestingly, one of those patients was an LBD case, and four of the remaining five patients presented with cognitive impairment of unknown etiology as part of their psychiatric symptomatology. At present, we cannot explain why only a subset of AD patients had detectable CSF levels of Aβ2–42. Further experiments will clarify whether Aβ2–42 CSF levels are sensitive to the preanalytical handling of the samples. Alternatively, CSF Aβ2–42 may correlate with the clinical course of AD or indicate a specific phenotype of the disease. It is noteworthy that the subgroup of AD patients with Aβ2–42-positive CSF samples was also characterized by decreased and elevated relative abundances of Aβ1–37 and Aβ1–39, respectively.

An increased ratio of amino-terminally modified Aβx–42: Aβ1–42 in CSF samples of AD patients has been suggested previously (65), whereas the ratio of Aβx–40:Aβ1–40 did not discriminate between AD and non-AD patients. However, the latter enzyme-linked immunosorbent assay-based study did not further specify the nature of these amino-terminal modifications. Taken together, the data suggest that the ratio Aβ2–42:Aβ1–42 is a promising surrogate marker for the neurochemical diagnosis of AD.

**Amino-terminal- and Carboxyl-terminal-truncated Aβ Species in PS1-deficient Neurons and APP-trafficking Mutants**—There is increasing evidence that APP processing in the transmembrane region by γ-secretase(s) is closely related to presenilin activity (20). Therefore, we investigated to what extent the generation of the amino-terminal- and carboxyl-terminal-truncated Aβ peptides was also dependent on presenilin activity. In addition, we were interested in studying the generation of the amino-terminal- and carboxyl-terminal-truncated Aβ peptide species in addition to Aβ1–40 and Aβ1–42 in APP-trafficking mutants.

We observed a striking PS1-dependent reduction of the carboxyl-terminal-truncated Aβ peptides 1–37, 1–38, and 1–39 that was at least as pronounced as that initially described for Aβ1–40/42 (16). Surprisingly, this was contrasted by a significantly less pronounced reduction of Aβ2–42. Furthermore, the Aβ peptide secretion pattern of neurons carrying the slow-internalizing mutant (PS1+/+ and APP-Δct) closely resembled the Aβ peptide secretion profile of PS1−/− neurons. By contrast, a striking reduction of all secreted Aβ peptides, including Aβ2–42, was observed for the construct carrying the ER retention motif (APP-KK). However, this finding is less valid due to the low level of secreted Aβ peptides. Taken together, our data show that the generation of Aβ2–42 is less dependent on PS1 activity and endocytosis of APP, and they suggest that both Aβ peptide species (Aβ2–42 and Aβ1–x) were not generated in the ER.

**Amino-terminal-truncated Aβ Peptides**—The generation of

![Fig. 8. Normalization: secretion of different Aβ peptide species by PS1+/+ or PS1−/− neurons infected with APP-trafficking mutants. Results as shown in Fig. 7 and from three other independent experiments were quantified. Data obtained for PS1+/+ (A) or PS1−/− (B) transfected with APP-Δct were compared with data obtained from cells expressing APP-WT, which constitutes the 100% reference. In C, original data obtained in A and B were expressed as the ratio of PS1−/−:PS1+/+ for all APP constructs independently. * Aβ2−42 versus total Aβ peptide species, p < 0.025; n.s., Aβ2−42 versus total Aβ peptide species, p > 0.05.](image-url)
Aβ2–42 Is Elevated in AD

Accordingly, Aβ2–42 is the direct precursor of Aβ peptides 3–40/42 and their pyroglutamate derivatives 3pyro-40/42, which are not amenable to further degradation by aminopeptidase(s) (see review in Ref. 74). Moreover, pyroglutamyl aminopeptidase activity is low in human cortical extracts, which explains the relative prominence of Aβ3pyro-40/42 (75). The latter two amino-terminal-truncated Aβ peptide species are deposited before Aβ peptides 1–x and represent a dominant species in early stages of β-amyloid plaque formation (76, 77).

Under physiological conditions, Aβ2–42 should be metabolized rapidly to Aβ3–42 due to a high activity of cortical aminopeptidase N, which exceeds the activity of the glutaryl-aminopeptidase by severalfold (75). The high activity of aminopeptidase N might explain why Aβ2–40, among several aminoterminal-elongated or -truncated Aβ peptide species (Aβ2–40) that were generated as recombinant Aβ peptides in a cell culture system, was selectively prone to amino-terminal degradation (9).

In view of the comparatively high percentage of Aβ2–39/40 in cerebrovascular β-amyloid and the vasoactive properties of Aβ peptides (78, 79), it is of interest to note that pericytes and periendothelial cells of brain parenchyma vessels coexpress aminopeptidase N, aminopeptidase L, and nestin as part of the autonomous angiotensin system of the brain (80). Soluble aminopeptidase N (CSF and plasma) and aminopeptidase A (glutamyl-aminopeptidase activity; plasma) have been reported to be decreased in AD (75, 81). Aminopeptidase N also has alanine-aminopeptidase activity, which may be relevant for the elevated CSF levels of Aβ2–42 in a subgroup of our AD patients.

The pathogenetic role of amino-terminal-truncated Aβ peptide has been reviewed recently (82, 83), and Saido (84) proposed an aminopeptidase hypothesis for Aβ peptide catabolism; however, the pathophysiological role of Aβ2–42 was not addressed.

In conditioned media of PS1+/+ neurons, Aβ2–42 amounts only to ~10% of Aβ1–42. Thus, we would expect a severalfold increase of Aβ2–42 if the prominent decrease of Aβ1–42 in PS1- and endocytosis-deficient neurons is due to its increased catabolism by aminopeptidase A generating Aβ2–42. However, we observe an attenuated decrease of A2–42. Therefore, Aβ1–42 is not likely to be a precursor of Aβ2–42. This does not exclude the possibility that Aβ2–42 might be a rapidly metabolized (e.g. by aminopeptidases) precursor of other amino-terminal-truncated Aβ peptide species, e.g. the highly amyloidogenic Aβ3–42 and Aβ3pyro-42.

Taken together, our data suggest that Aβ2–42 is generated due to an alternative βAspAla-secretase activity rather than a standard BACE-MetUasp and aminopeptidase A (aspartyl) activity, as in the latter case, a reduction of the precursor Aβ1–42 in PS1-deficient neurons should have been paralleled by a much more pronounced decrease of Aβ2–42.

Investigating BACE-1 knockout mice by the methods described here will help to clarify whether the generation of Aβ2–42 is due to BACE activity.

Secreted Aβ peptides were also analyzed by urea-based Aβ SDS-PAGE/immunoblot in human embryonic kidney cell lines stably expressing human APP mutants defective in endocytosis (9). The slow-endocytizing mutants comprised APP lacking the entire cytoplasmic domain or APP with both tyrosine residues of the motif GYENPTY mutated to alanine. In response to the impaired endocytosis of APP, a reduction of Aβ1–40 secretion was observed, which was paralleled by elevated levels of two amino-terminal-truncated Aβ peptides (Aβx/y-40). These were identified as Aβ peptides 3–40 (y-40) and 5–40 (x-40) by comi-
Aβ2–42 Is Elevated in AD

igration with a large panel of recombinant standard Aβ peptides that included Aβ2–40. Interestingly, only Aβ2–40 was partially amino-terminally-degraded in vivo when it was generated as recombinant standard. Our synthetic Aβ2–40 was stable and, according to its electrophoretic migration pattern, might also comigrate with Aβ peptide y-40. Thus, it is not clear to what extent human embryonic kidney cell lines may also produce Aβ2–40 in response to slow-internalizing APP mutants. In accordance with our data from the APP-KK construct, the amino-terminal-truncated Aβ peptides were not generated when the APP mutants were retained in the ER by treatment with brefeldin. Cescco et al. (9) conclude from their data that cleavage at position 1 of Aβ peptides occurs predominantly in endosomes, whereas β-secretase cleavage at alternative sites takes place at the plasma membrane.

Interestingly, human embryonic kidney cells did not generate significant amounts of Aβ2/3–42, and, vice versa, we did not identify elevated levels of Aβ2/3–40 in the RIPA-soluble fraction of Aβ peptides in brain homogenates. The latter observations suggest that non-neuronal cells such as human embryonic kidney cells or cerebral endothelial cells favor the generation of amino-terminal- and carboxyl-terminal-truncated Aβ39/40, whereas neuronal cells favor the processing of amino-terminal-truncated but carboxyl-terminal-elongated Aβ peptides, e.g. Aβ2–42. This would be of pathophysiological relevance for fibrillogenesis because amino-terminal truncation and carboxyl-terminal elongation act synergistically in increasing the aggregation potential of Aβ peptides. Moreover, our finding of a prominent elevation of Aβ2–42 in the RIPA-soluble fraction of brain homogenates indicates that Aβ2–42 does not originate from the β-amyloid plaque core because the latter aggregates will not be disaggregated by mild detergents. Because β-sheet structure can be induced in amyloidogenic peptides by neutralization of aspartate (85, 86), Aβ2–42 may serve as a first nidus for β-amyloid nucleation preceding the formation of plaque core β-amyloid. Moreover, in neuronal cells, amino-terminal-truncated Aβ peptides may also be generated intracellularly (87). If Aβ2/3–42 are generated by alternative β-secretase activity in a mildly acidic subcellular compartment, as known for BACE activity, then their aggregation potential should be specifically high at this site due to a less acidic isoelectric point of Aβ2/3–42 (Ip = 6.37) as compared with that of Aβ1–42 (Ip = 5.37).

We cannot exclude that Aβ3–42 is also generated in our neuronal cell culture system because both peptides migrate at the same position and are not separated by isoelectric focusing. However, our quantification of Aβ2–40/42 is not confounded by Aβ2–40/42 because during SDS-PAGE/immunoblot, mAb 1E8 does not detect significant amounts of Aβ peptides that are truncated aminoterminaly by more than 1 amino acid. A comparative analysis of the detergent-soluble fraction of immunoprecipitates from the frontal lobe of AD patients revealed that Aβ2–42 is the most prominent Aβ peptide species aside from Aβ1–42, exceeding the abundance of additional amino-terminal-truncated Aβ peptides such as 3p-40/42 and 3–42.

We observed a striking elevation of Aβ2–42 in the RIPA-soluble fraction of Aβ peptides from the frontal lobe of a patient with a PS1 mutation (T115C), and according to more recent studies, the generation of amino-terminal-truncated Aβ peptides is a general feature of familial AD.

Russo et al. (88) also investigated the detergent-soluble fraction of Aβ peptides from brain homogenates of subjects with sporadic AD and familial AD, which were linked to mutations in either the PS1 or APP gene. In AD, amino-terminal-truncated Aβ peptides (3/4–42 and 11–42) were more abundant than the full-length Aβ peptide 1–42, and the most prominent relative amounts of amino-terminal-truncated Aβ peptides were observed for the pathogenic PS1 mutations. The authors conclude that both γ-secretase cleavage and β-secretase cleavage are affected by PS1 mutations, but they also consider that PS1 mutations might affect these secretases indirectly by interfering with the trafficking of APP in the cell.

Furthermore, Kumar-Singh et al. (46) recently described an aggressive form of familial AD caused by a novel missense mutation in APP (T114I). Thus, the γ-secretase cleavage generating Aβ1–42 is directly involved. This mutation resulted in the most drastic increase (11-fold) of the Aβ1–42:Aβ1–40 ratio reported thus far and coincided in brain with the deposition of abundant and predominant nonfibrillar preamyloid plaques composed primarily of Aβx2-k in the absence of Aβx-40. Interestingly, carboxyl-terminal-truncated Aβ peptides 1–37, 1–38, and 1–39 and x-37, x-38, and x-39 were also elevated and were most pronounced for Aβ38. This pattern closely resembles the one we observed in our brain homogenates of AD patients, i.e. striking elevation of Aβ2–42 and Aβ1–42, surprisingly low amounts of Aβ1–40, and elevated levels of Aβ1–38. The authors conclude that Aβ2–42 as diffuse nonfibrillar plaques has an essential but underdetermined role in AD pathology.

Conclusions—Taken together, our data indicate that the amino-terminal truncated and carboxyl-terminal-elongated Aβ2–42 was generated within a post-ER secretory pathway, where most of the Aβ2–42 is generated by βγ-secretase activity, possibly as alternative BACE activity, and not due to a standard BACEactivity with secondary catabolic aminopeptidase activity. Moreover, the generation of Aβ2–42 by γ-secretase activity seems to be less PS1-dependent.

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