A Specific Enzyme-Linked Immunosorbent Assay for Measuring β-Amyloid Protein Oligomers in Human Plasma and Brain Tissue of Patients With Alzheimer Disease

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Objective: To examine in vivo levels of β-amyloid (Aβ) oligomers (oAβ) vs monomeric Aβ in plasma and brain tissue of patients with sporadic and familial Alzheimer disease (AD) using a new enzyme-linked immunosorbent assay (ELISA) specific for oAβ.

Design: To establish the oAβ ELISA, the same N-terminal Aβ antibody was used for antigen capture and detection. Plasma and postmortem brain tissue from patients with AD and control subjects were systematically analyzed by conventional monomeric Aβ and new oAβ ELISAs.

Subjects: We measured oAβ species in plasma samples from 36 patients with clinically well-characterized AD and 10 control subjects. In addition, postmortem samples were obtained from brain autopsies of 9 patients with verified AD and 7 control subjects.

Main Outcome Measures: Oligomeric Aβ and 4 monomeric Aβ species in plasma samples from patients with AD and control subjects were measured by ELISA.

Results: The specificity of the oAβ ELISA was validated with a disulfide-crossed-linked, synthetic Aβ1-42Ser26Cys dimer that was specifically detected before but not after the dissociation of the dimers in β-mercaptoethanol. Plasma assays showed that relative oAβ levels were closely associated with relative Aβ42 monomer levels across all of the subjects. Analysis of sequential plasma samples from a subset of the patients with AD, including a patient with AD caused by a presenilin mutation, revealed decreases in both oAβ and Aβ42 monomer levels over a 1- to 2-year period. In brain tissue from 9 patients with AD and 7 control subjects, both oAβ and monomeric Aβ42 levels were consistently higher in the AD cases.

Conclusions: An oAβ-specific ELISA reveals a tight link between oAβ and Aβ42 monomer levels in plasma and brain. Both forms can decline over time in plasma, presumably reflecting their increasing insolubility in the brain.

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mient (MCI) and on to AD, albeit with unsatisfactory sensitivity and specificity. Another study found that patients with AD at baseline and those who developed AD later had significantly higher plasma Aβ40 levels; some of the patients with AD showed elevated levels of Aβ44 and Aβ38 before and during the early stages of AD, but plasma levels declined thereafter. Another study showed that nonde-
mended subjects with high levels of plasma Aβ42 were more than twice as likely to develop AD than those with low lev-
els and that patients with AD showed higher Aβ42 levels than control subjects without AD.

Patients with familial AD (FAD) having mutations in the presenilins, the catalytic subunits of the γ-secretase com-
plex that generates Aβ, have increased plasma levels of Aβ42. Plasma Aβ42 levels and the Aβ42/Aβ40 ratio were higher even in presymptomatic subjects carrying FAD mutations in pre-
senilin 1 (PS1) or APP, and Aβ42 levels may decrease with disease progression prior to symptom onset. Elevated plasma Aβ42 levels have been linked to a locus on chromo-
some 10 in some typical (late-onset) AD cases, and some first-degree relatives of patients with late-onset AD have elevated Aβ42 levels. These suggest that an increased plasma Aβ level is a heritable trait.

Plasma Aβ is a potentially promising but understud-
ied candidate marker for diagnosis and preclinical pre-
pidiction. However, plasma Aβ40 or Aβ42 was found not to be an optimal candidate in unbiased proteomic searches for AD fluid biomarkers. In one study, increased plasma Aβ42 levels were detected in patients with MCI, but a significant association was only observed in women. In a cohort of men at age 70 years, plasma Aβ40 and Aβ42 were not associated with incident AD at follow-up, whereas low plasma Aβ40 levels in another cohort of men at age 77 years were associated with a higher incidence of AD. Yet another study found that subjects with low plasma Aβ42/Aβ40 ratios had a higher risk of MCI or AD and greater cognitive decline. A correlation between increased plasma Aβ40 levels and an increased risk of de-
mencia has also been reported. A recent study of pa-
tients with MCI followed up for 7 years showed no signifi-
cant difference of plasma Aβ species between pa-
tients with MCI who later developed AD and patients with stable MCI or healthy control subjects.

These often inconsistent reports on the association of plasma Aβ levels with AD may reflect the fact that mea-
surements to date only represent the pools of mono-
nomic Aβ and were measured by different Aβ enzyme-
linked immunosorbent assays (ELISAs). To understand the relationship among different Aβ species in vivo, we developed an ELISA that can detect oAβ and simulta-
neously measured both Aβ monomers and oAβ in hu-
man plasma or postmortem brain tissue. Levels of hu-
man oAβ detected by our new oAβ-specific ELISA were closely associated with the levels of monomeric Aβ42. Levels of both soluble and insoluble Aβ42 and oAβ species were significantly higher in brain tissue of patients with AD compared with those in brain tissue of control sub-
jects. We also observed decreases in plasma Aβ levels in follow-up samples from the same patients 1 to 2 years later. The development of an oligomer-specific ELISA ap-
licable to plasma extends Aβ measurement to a highly relevant neurototoxic form of this pathogenic peptide.

**METHODS**

**PLASMA AND BRAIN LYSATE PREPARATION**

Blood samples were collected in potassium-EDTA–containing collection tubes and centrifuged at 1600g for 15 minutes. The plasma supernatant was aliquoted and stored at –80°C until mea-
sured. Average ages for patients with AD and control subjects at the time of blood drawing were 72 years (n=36) and 62 years (n=10), respectively. Brain lysates from postmortem human brains (n=16) were prepared as recently described.

**ELISAS AND ANTIBODIES**

Sandwich ELISAs for monomeric Aβ were performed as described. The use of C-terminal capturing antibodies and N-terminal or midregion detecting antibodies has been a standard format for measuring monomeric Aβ species in many studies. The capture antibodies 2G3 (to Aβ residues 33-
40) and 21F12 (to Aβ residues 33-42) were used for Aβ40 and Aβ42 species, respectively. The detecting antibodies were bio-
tinylated 3D6 (to Aβ1-40) or biotinylated 266 (to Aβ residues 13-28) for Aβ40/4042 species. These antibo-
dies were kindly provided by Peter Seubert, PhD, and Dale Schenk, PhD (Elan Corp, plc, South San Francisco, California).

To detect oAβ species, the same N-terminal antibody, either 82E1 (to Aβ residues 1-16; Immuno-Biological Laboratories, Inc, Minneapolis, Minnesota) or 3D6, was used for both cap-
ture and detection. A sandwich ELISA procedure identical to that for the traditional monomer assays was followed to mea-
sure relative oAβ levels, which were calculated using standard curves of synthetic Aβ40 peptide captured by 2G3 antibody and measured by the same detecting antibodies (82E1 or 3D6).

**STABLE CELL LINES AND CELL CULTURE**

Chinese hamster ovary cells stably expressing wild-type (wt) human APP751 plus either wt or mutant PS1 (resulting in lines PS1VT1, PS1VT3, PS1VT73, PS1M146L, PS1M146L-2, PS1M146L-3 [M146L missense mutation], PS1L46V, PS1C410Y-1, PS1C410Y-2, PS1C410Y-3 [C410Y missense mutation]) were maintained in 200-µg/mL G418 (In-
vitrogen Corp, Carlsbad, California) plus 25-µg/mL puromy-
cin (for PS1). We also examined Chinese hamster ovary cell lines singly transfected with wt APP751 or APP751 bearing the V717F (“Indiana”) missense mutation. Cells were incubated in methionine-free, fetal bovine serum–free media for 45 minutes before labeling with 200-µCi/mL sulfur 35 ([15S]–
labeled methionine for 38 hours (to convert microcuries per milliliter to becquerels per milliliter, multiply by 37,000). Conditioned media were collected and immunoprecipitation was performed as described.

**PRODUCTION AND CHARACTERIZATION OF CROSS-LINKED Aβ DIMERS**

β-Amyloid dimers (dAβ) were generated by atmospheric oxida-
tion of a 20µM solution of synthetic Aβ1-40Ser26Cys in 20mM ammonium bicarbonate, pH 8.0, for 4 days at room tempera-
ture. To facilitate disassembly of aggregates formed during the oxidation reaction, the peptide solution was lyophilized and the lyophilate was incubated in 5M guanidine hydrochloride, 50mM TRIS hydrochloride, pH 8.0, for 4 hours. Disulfide-
cross-linked dAβ were isolated from untreated monomer and higher aggregates by size exclusion chromatography using a Su-
perdex 75 10/30 high-resolution column (GE Healthcare, Mil-
waukee, Wisconsin) eluted with 50mM ammonium acetate, pH

**DENATIVE DATA**

An oligomer–specific ELISA applicable to plasma extends Aβ measurement to a highly relevant neurototoxic form of this pathogenic peptide.
8.5, at a flow rate of 0.8 mL/min. Fractions (0.5 mL) were collected, an aliquot of each was electrophoresed on 16% TRIS-tricine polyacrylamide gels, and protein was detected by silver staining. Size exclusion chromatography fractions found to exclusively contain dAβ were pooled and used as the dimer stock. The concentration of peptides in stock solutions was determined by comparison with wt Aβ1-40 of known concentration. Once collected, all of the samples were stored at −80°C until used. To disrupt the disulfide bonds linking the monomers, dAβ were treated with 3% β-mercaptoethanol (βME), followed by serial dilutions for quantification by ELISA. The diluted, residual amount of βME did not interfere with the capture and detecting antibodies. The Aβ1-40Ser26Cys was synthesized by the Biopolymer Laboratory, Department of Neurology, UCLA Medical Center, Los Angeles, California, and the correct sequence and purity were confirmed by amino acid analysis, reverse-phase high-performance liquid chromatography, and mass spectrometry.

ESTABLISHING AN oAβ-SPECIFIC ELISA

To analyze oAβ species in human samples, we established a sensitive and specific oAβ ELISA. The assay relies on the use of a single monoclonal antibody for both capture and detection. Thus, for oAβ to be detected by this sandwich ELISA, an Aβ assembly must contain at least 2 exposed copies of the same epitope that is accessible by the identical capturing and detecting antibody.27,28 This means that this assay will recognize only Aβ assemblies that contain at least 2 Aβ molecules. Two monoclonal antibodies, 82E1 and 3D6, that each recognize this sandwich ELISA, an Aβ assembly must contain at least 2 Aβ molecules. Two monoclonal antibodies, 82E1 and 3D6, that each recognize the same epitope of human Aβ were tested.

To confirm the specificity of the ELISA, we used a synthetic Aβ peptide, Aβ1-40Ser26Cys, that is capable of reversibly forming covalently cross-linked dAβ under oxidizing conditions.25,29 Disulfide-crossed-linked dAβ were

Figure 1. Detection of low-molecular-weight oligomeric β-amyloid (Aβ) (di-Cys synthetic dimer) by enzyme-linked immunosorbent assay (ELISA). A, Size exclusion chromatography fractions of Aβ dimers (dAβ) and synthetic monomeric Aβ (mAβ) were visualized on the same sodium dodecyl sulfate electrophoresis gel by silver staining. Based on the absolute amount of synthetic Aβ peptide loaded on the same gel, the concentration of dAβ was calculated. βME indicates β-mercaptoethanol; wt, wild-type. B, In the presence of βME, dAβ were dissociated into mAβ. Serial dilutions of dAβ with and without βME treatment were analyzed by ELISA using antibody 82E1 (C) or 3D6 (D). Error bars indicate SEM.

Figure 2. Measurements of the relative concentrations of oligomeric β-amyloid (oAβ) from patients with Alzheimer disease (AD) and control subjects by enzyme-linked immunosorbent assay. Most of the control subjects (7 of 10 subjects) have plasma levels of oAβ below the detection limit, but many of the patients with AD (19 of 36 patients) have detectable oAβ levels.
The overall difference between the patients with AD and control subjects is minimal for Δβ. Most of the control subjects (9 of 10 subjects) have detectable Δβ. A majority of subjects who carried high plasma levels of Δβ did not reveal a clear separation of patients with AD from control subjects. When the levels of Δβ were assayed using either antibody 82E1 or antibody 3D6, we estimated that the concentration of our synthetic Δβ stock was 81µM. In the presence of 3% βME, the disulfide bonds linking the monomers that form Δβ were disrupted, producing a reduced monomer (Figure 1B). In the absence of βME, no monomeric Δβ was detected and the vast majority of Δβ species were Δβ (Figure 1B).

Serial dilutions of Δβ were made after the Δβ stock was treated with or without βME, and the diluted samples were assayed using either antibody 82E1 (Figure 1C) or antibody 3D6 (Figure 1D) in a sandwich format. When the relative levels of the diluted Δβ (800pM) of the Δβ stock were normalized to 1, either monoclonal antibody detected a clear linear reduction in the levels of Δβ when further diluted to 400pM and 200pM (Figure 1C). In the presence of βME, dissociation of the disulfide bond markedly reduced the amount of Δβ signal; the levels of remaining Δβ were less than 10% of the same fraction without βME, with antibody 82E1 showing even greater specificity than antibody 3D6 in this regard (Figure 1C and D). Therefore, our new ELISA accurately measured the levels of Δβ in a linear fashion and is highly sensitive to the dissociation of Δβ to monomers.

**Figure 3.** Measurements of the absolute concentrations of β-amyloid (Δβ) species Δβ1-40 (A), Δβ1-42 (B), Δβx-40 (C), and Δβx-42 (D) from patients with Alzheimer disease (AD) and control subjects by enzyme-linked immunosorbent assay. Levels of Δβ1-40, Δβ1-42, Δβx-40, and Δβx-42 were measured by separate sandwich enzyme-linked immunosorbent assays using C-terminal–specific antibodies. The overall difference between the patients with AD and the control subjects is minimal for Δβ. Most of the control subjects (9 of 10 subjects) have plasma levels of Δβ1-42 below the detection limit, but many of the patients with AD (17 of 36 patients) have detectable Δβ1-42 levels.

**QUANTIFYING PLASMA Δβ FROM PATIENTS WITH AD**

Using the Δβ-specific ELISA, we screened plasma samples obtained from 36 patients with well-characterized AD and 10 control subjects. The ages of the patients with AD ranged from 50 to 90 years at the time of blood sampling, with an average age of 72 years. The age of control subjects ranged from 52 to 68 years, with an average age of 62 years. Conventional β ELISAs were applied to measure monomeric Δβ species.

We analyzed plasma levels of Δβ as a function of subject age but did not observe an age-dependent alteration of Δβ levels (Figure 2). There were a number of subjects who carried high plasma levels of Δβ-reactive species, with a wide age distribution between 60 and 90 years. Overall, we found that most control subjects (7 of 10 subjects) had plasma levels of Δβ below our detection limit, whereas more than half of the patients with AD (19 of 36 patients) had detectable Δβ levels (Figure 2).

From the same aliquot of plasma, we measured 4 monomeric Δβ species using 4 distinct ELISAs: Δβ1-40, Δβ1-42, and N-terminally heterogeneous Δβx-40 and Δβx-42 species (ie, Δβx-40 and Δβx-42). The levels of Δβ1-40 and Δβx-40 did not reveal a clear separation of patients with AD from control subjects (Figure 3A and B). On average, the levels of Δβx-40 were higher than those of Δβ1-40 (note scales on the ordinates of Figure 3A and B), indicating that a variable portion of plasma Δβx-40 is N-terminally truncated. When the levels of Δβx-42 (Figure 3C) and Δβ1-42 (Figure 3D) were analyzed from the same aliquots of plasma, we found that the levels of Δβ1-42 species were...
We calculated the average plasma Aβ levels for each of the 4 ELISAs in all of the patients with AD and control subjects. Plasma levels of Aβ42 and Aβ40 did not differ significantly between patients with AD and control subjects (Figure 4A). However, the average plasma levels of both Aβ42 and Aβ were found to be significantly higher in patients with AD than in control subjects (Figure 4A). Furthermore, the relative levels of Aβ42 and Aβ were closely associated. For this, we chose a threshold of 10pM for Aβ42 (ie, readily detectable above baseline) and identified 7 subjects who carried levels of Aβ42 greater than 10pM (Figure 4B). Next, we identified 7 subjects who carried higher levels of Aβ than the remaining subjects (Figure 4C). We found that the subjects who carried levels of Aβ42 greater than 10pM (Figure 4B) were the same subjects who carried high levels of Aβ (Figure 4C), and the relative levels of Aβ42 and Aβ for each subject were tightly linked. Except in 1 patient who had the highest Aβ level (Figure 4C) but had a relatively low Aβ42 level (Figure 4B), our Aβ ELISA clearly detected Aβ-reactive species whose levels were tightly associated with those of monomeric Aβ42.

CONCOMITANT INCREASES OF MONOMERIC Aβ42 AND Aβ IN CULTURED CELLS

To further validate the close association of monomeric Aβ42 with Aβ detected by our Aβ ELISA, we analyzed these Aβ species generated from cell lines expressing FAD-causing mutations in either APP or PS1. These cell lines have elevated Aβ42 monomer levels and secrete sodium dodecyl sulfate–stable, low-n Aβ oligomers into the media.

Similar to our AD and control cases that carry different levels of plasma Aβ, we have found variable levels of Aβ produced from multiple stable cell lines expressing wt or mutant (M146L or C410Y) PS1 and wt or mutant (V717F) APP. These lines were metabolically labeled with [35S]methionine, and the conditioned media were immunoprecipitated with antibody 21F12 (to Aβ ending at residue 42) or 1282 (a pan-Aβ polyclonal antibody) and analyzed by gel fluorography. Antibody 21F12 immunoprecipitated both monomeric Aβ42 and the p342 species (generated by sequential α- and γ-secretase cleavages of APP) from the conditioned media of all of the cell lines. In media from APPV717F and PS1146L-2 cells, additional Aβ-immunoreactive bands migrating at approximately 5 kDa, 8 to 10 kDa, and 12 to 14 kDa (collectively designated sodium dodecyl sulfate–stable, low-n oligomers) were also detected (Figure 5A). Light exposure of blots separating Aβ42 monomers from Aβ indicated that more Aβ42 was produced from cells expressing APPV717F or mutant PS1 (PS1146L-2) (Figure 5B). The levels of Aβ were correlated with the levels of monomeric Aβ42, ie, the cell lines secreting elevated Aβ42 also had higher levels of oligomers in their media. We further examined the levels of total Aβ precipitated by antisemirou 1282 but did not observe an elevation in total Aβ signal corresponding to those of Aβ42 and Aβ. Thus, the amounts of total Aβ generated by cells expressing APPV717F or mutant PS1 (PS1146L-2) were comparable to those from the other cell lines (Figure 5C). Moreover, no Aβ species were detected in these immunoprecipitates of total Aβ, suggesting that the Aβ species made by the cells are principally composed of Aβ42. These results in well-defined cultured cell lines support our finding in human plasma that levels of Aβ are closely associated with those of monomeric Aβ42.

DECLINING PLASMA Aβ42 LEVELS OVER 1 TO 2 YEARS

The close association of monomeric Aβ42 and Aβ levels suggests a dynamic conversion between these 2 Aβ
The monomeric Aβ in cultured cells expressing familial Alzheimer disease mutations in amyloid precursor protein (APP) or presenilin 1 (PS1). Conditioned media from radiolabeled wild-type (wt) or familial Alzheimer disease–linked mutant APP- or PS1-expressing Chinese hamster ovary cells were immunoprecipitated with antibody 21F12 (for Aβ expressing V717F APP, the clonal line PS1M146L-2 expressing M146L PS1 produces high levels of monomeric Aβ). Aβ appearance of oligomeric Aβ (A). The increased oligomeric Aβ levels were not linearly associated with total levels of Aβ as measured by immunoprecipitation using polyclonal Aβ antibody 1282 (B).

Figure 5. Increased oligomeric β-amyloid (Aβ) levels are closely associated with increasing levels of monomeric Aβp342 in cultured cells expressing familial Alzheimer disease mutations in amyloid precursor protein (APP) or presenilin 1 (PS1). Conditioned media from radiolabeled wild-type (wt) or familial Alzheimer disease–linked mutant APP- or PS1-expressing Chinese hamster ovary cells were immunoprecipitated with antibody 21F12 (for Aβ42 [A and B] or 1282 (for total Aβ [C]), followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis to visualize different Aβ species by autoradiography. Similar to the cell line expressing V717F APP, the clonal line PS1M146L-2 expressing M146L PS1 produces high levels of monomeric Aβ42 (B is a light exposure of A) correlated with the appearance of oligomeric Aβ (A). The increased oligomeric Aβ levels were not linearly associated with total levels of Aβ as measured by immunoprecipitation using polyclonal Aβ antibody 1282 (C).

Table. Declines in Plasma β-Amyloid Levels in a Patient With Alzheimer Disease Carrying a Presenilin 1 Mutation

<table>
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<tr>
<th>Age (yr)</th>
<th>Aββ3-40, pM</th>
<th>Aββ4-40, pM</th>
<th>Aββ1-42, pM</th>
<th>Aββ3-40/Aββ4-40, %</th>
<th>Aββ4-40/Aββ1-42, %</th>
<th>Aββ1-42/Aββ4-40, %</th>
<th>Aββ3-40/Aββ4-40, %</th>
<th>Aββ4-40/Aββ1-42, %</th>
<th>Aββ1-42/Aββ4-40, %</th>
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<tr>
<td>58 y</td>
<td>26</td>
<td>48</td>
<td>84</td>
<td>7</td>
<td>36</td>
<td>13</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60 y</td>
<td>ND</td>
<td>36</td>
<td>48</td>
<td>ND</td>
<td>15</td>
<td>NA</td>
<td>24</td>
<td></td>
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</tr>
<tr>
<td>Loss, %</td>
<td>Approximately 100</td>
<td>25</td>
<td>43</td>
<td>Approximately 100</td>
<td>58</td>
<td>NA</td>
<td>NA</td>
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</tbody>
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Abbreviations: Aββ, β-amyloid; NA, not applicable; ND, nondetectable; oAβ, oligomeric β-amyloid.

species. To further examine the relationship of Aβ1-42 and oAβ in vivo, we obtained sequential blood samples from a patient with symptomatic FAD carrying a mutant PS1. Blood samples were drawn at ages 58 and 60 years. Levels of Aβ were quantified by separate ELISAs for oAβ and the 4 monomeric Aβ species (Table). For oAβ species, we observed a significant decrease such that oAβ was no longer detectable at age 60 years. For monomeric Aβ, the plasma concentrations of Aβ1-42 and Aβx-42 were 48pM and 84pM, respectively, at age 58 years. The concentration of Aβ1-42 was 7pM, and that of Aβx-42 was 36pM. The plasma Aβx-42/Aβx-total ratio was in the same range as those reported in patients carrying FAD-causing PS1 mutations.8 Plasma samples collected 22 months later had significantly lower Aβ concentrations: the level of Aβx-42 measured 36pM (25% reduction), and Aβx-42 was now undetectable (Table). Concentrations of Aβ1-42 and Aβx-42 declined to 48pM (43% reduction) and 15pM (58% reduction), respectively. Thus, while the levels of both Aβ species decreased over 2 years, Aβx-ending species were reduced more dramatically than Aβ1-ending species. The complete loss of the Aβx-42 and oAβ signals is consistent with our findings that the levels of these 2 species are closely associated.

We also obtained sequential blood samples from a subset of our patients with AD (n = 12, including the patient with FAD described earlier) and compared their Aβ levels over a 1- to 2-year span. All of the 5 Aβ species were again measured by ELISA and the changes in Aβ levels were calculated. A similar number of subjects showed increased or decreased levels of Aβ1-42 over the course of 1 to 2 years (Figure 6A), failing to show a clear temporal trend in Aβ1-42. Comparison of Aβx-40 levels revealed that 3 of 11 subjects had an increased Aβx-40 level and the remaining 8 subjects had a reduced Aβx-40 level (Figure 6B). In the 4 patients with AD who had detectable plasma Aβ1-40 levels (Figure 6C), 3 subjects showed a dramatic reduction in the Aβ1-40 level after 1 year but 1 subject had almost identical levels of Aβ1-40 at both times. The latter subject also maintained similar levels of oAβ after 1 year, whereas 4 patients with AD with detectable oAβ levels had a reduction in the relative plasma oAβ level (Figure 6E). Thus, the decrease of Aβ1-42 levels occurred in subjects who also showed a similar decrease in oAβ levels. Strikingly, all but 1 subject showed decreases in Aβx-42 in the second plasma samples (Figure 6D).

ELEVATED LEVELS OF MONOMERIC Aβ1-42 AND oAβ IN BRAIN TISSUE OF PATIENTS WITH AD

To investigate the association of monomeric Aβ1-42 and oAβ directly in human brains, we obtained postmortem brain...
tissue from 9 patients with AD and 7 age-matched control subjects distinct from the subjects examined in the plasma Aβ studies described earlier. We performed sequential extractions of brain tissue to obtain “soluble” (TRIS-buffered saline [TBS]) and “insoluble” (guanidine hydrochloride) extracts and measured their Aβ contents by ELISA.

The soluble pool of brain Aβ extracted by TBS had relatively low levels of Aβ1-40 (Figure 7A); an average of 1.1 pmol/g of Aβ1-40 was detected in the patients with AD vs 0.7 pmol/g in the control subjects (P = .14). The average level of TBS-soluble Aβ1-42 was significantly higher in patients with AD than in control subjects (Figure 7B). Likewise, the relative levels of TBS-soluble Aβ1-42 were significantly higher in patients with AD as most control subjects had almost undetectable levels of soluble Aβ1-42 (Figure 7C).

After the extraction of soluble Aβ by TBS, the resultant pellets were further extracted in guanidine hydrochloride to obtain the insoluble pools of Aβ. Overall, insoluble Aβ1-42 levels (Figure 7E) were several hundred-fold higher than soluble Aβ1-42 levels (Figure 7B), and they were almost 10-fold higher than insoluble Aβ1-42 levels (Figure 7D). This is consistent with numerous previous reports that amyloid deposits in brain tissue of patients with AD are mainly composed of Aβ1-42. The levels of both insoluble Aβ1-40 and insoluble Aβ1-42 were significantly higher in brain tissue from patients with AD than those in brain tissue from control subjects. Furthermore, a substantial and significant increase in the relative levels of insoluble oAβ was observed in brain tissue of patients with AD (Figure 7F). Importantly, brain tissue levels of insoluble Aβ1-42 were closely associated with the levels of oAβ in both patients with AD and control subjects (for AD: correlation coefficient = 0.88; for control subjects, correlation coefficient = 0.98). For example, 1 patient with AD (patient 4) showed relatively low levels of Aβ1-42, and accordingly the oAβ level was low. A control subject (subject 3) showed a relatively high level of Aβ1-42, corresponding to a high level of oAβ (Figure 7F). On average, significantly higher levels of all of the 3 insoluble Aβ species (Aβ1-40, Aβ1-42, and oAβ) were found in brain tissue of patients with AD compared with those in brain tissue of control subjects (Figure 7D-F).

Given the burgeoning evidence that small oligomeric assemblies of Aβ may be principally responsible for neu-
Figure 7. Increased levels of monomeric and oligomeric β-amyloid (Aβ) in the brain tissue of patients with Alzheimer disease (AD). Soluble pools (TRIS-buffered saline [A-C]) and insoluble pools (guanidine hydrochloride [D-F]) of Aβ were prepared by sequential TRIS-buffered saline extraction and then guanidine hydrochloride extraction. A, The concentrations of soluble Aβ_{1-42} in TRIS-buffered saline–extracted brain tissue of patients with AD are slightly higher than those in control subjects. The mean absolute levels of soluble Aβ_{1-42} (B) and the mean relative levels of soluble oligomeric Aβ (oAβ) (C) are significantly higher in brain tissue from patients with AD. Insoluble Aβ_{1-40} (D), Aβ_{1-42} (E), and oAβ (F) levels are much higher in brain tissue from patients with AD compared with those in brain tissue from control subjects, and the differences are statistically significant. Error bars indicate SEM. The differences between patients with AD and control subjects for all of the Aβ species (except soluble Aβ_{1-40}) are statistically significant (P < .05).

In this study, we oxidized synthetic Aβ_{1-40}Ser26Cys peptide to generate disulfide–crossed-linked dimers to test our new oAβ ELISA. The Aβ_{1-40}Ser26Cys dimer preparation was treated with guanidine hydrochloride and therefore was free of any higher-molecular-weight aggregates (Figure 1B). Subsequent separation of the stable dimers from un–cross-linked monomers was achieved by size exclusion chromatography. Detection of the synthetic dimers only in the absence of βME clearly demonstrated the specificity of our oAβ ELISA, with far less ability to detect monomers.

With this new oAβ ELISA and previously established monomeric Aβ ELISAs, we obtained several lines of evidence for a close association between the levels of
monomeric Aβ, and oAβ species in human plasma and brain tissue. First, the close association of Aβ1-42 and oAβ was observed in a subset of patients with AD who had readily detectable levels of plasma Aβ1-42 (Figure 4). The average levels of these 2 Aβ species were significantly higher in the patients with AD than in the control subjects. Indeed, only 1 of 10 control subjects showed such readily detectable levels of Aβ1-42. Second, our studies of brain tissue from patients with AD and control subjects showed the same pattern, ie, levels of insoluble Aβ, associated closely with the relative levels of insoluble oAβ across individual cases (Figure 7). Third, among all of the patients with AD and control subjects, higher levels of oAβ were not linked to lower levels of monomeric Aβ, suggesting that the conversion of monomeric Aβ into oAβ per se is not the major contributor to the observed reduction of monomeric Aβ42. Overall, our oAβ-specific ELISA allowed us to establish a close quantitative relationship between the levels of Aβ1-42 and oAβ in human plasma and brain tissue.

This association was also observed in cultured cells expressing human APP. We found that the appearance of soluble oAβ in the medium occurred exclusively in those clonal cell lines with significantly increased Aβ42 monomer production (Figure 5). The FAD mutations in PS1 or APP lead to enhanced Aβ42 generation, and the occurrence of oAβ is attributable to enhanced γ-secretase cleavage of APP at the 42nd residue of Aβ, which is facilitated by AD-causing mutations in either the substrate (APP) or the protease (PS1). Thus, levels of Aβ1-42 are closely associated with the formation of oAβ.

Our longitudinal comparisons of individual patients with AD provide new insight into the dynamic changes in plasma Aβ levels without the problem of intersubject variation. Using monomeric Aβ and oAβ ELISAs, we have provided 2 snapshots of Aβ levels within a relatively short period. Whereas we saw no clear directional change of Aβ1-42 levels, the levels of the remaining 4 Aβ species all showed a reduction over the course of 1 to 2 years. About three-quarters of cases showed a reduction in Aβ42 levels. Among the cases with detectable plasma levels of Aβ1-42 and oAβ, all but 1 showed a decrease in Aβ levels. In the case of Aβ1-42, 9 patients showed a reduction over the 1- to 2-year period, whereas 1 patient showed an increase. Importantly, the individuals with decreasing Aβ1-42 monomer levels were the same subjects who showed decreases in oAβ levels.

While our oAβ ELISA provides an accurate method to measure such species in human blood, our results do not yet validate any one Aβ species as a biomarker for AD. Currently, the CSF tau/Aβ42 ratio has been the best predictor of the development of AD-type cognitive decline in still-nondemented subjects. Changes in the levels of tau and Aβ42 in CSF may reflect dysfunction in the cerebrum that can be measured by the electroencephalographic rhythm, and low Aβ42 levels in CSF correlate well with positive Pittsburgh Compound B uptake by positron emission tomographic scanning. Precise measurement of Aβ in plasma and CSF and subsequent correlation with levels in postmortem brain tissue should yield a clearer picture of Aβ metabolism in vivo. Mathematically modeling the equilibrium between monomeric Aβ and oAβ would also help elucidate the catabolic turnover of Aβ in the peripheral and central nervous systems. Considering a wide range of factors that could contribute to variations in plasma Aβ levels, it is currently difficult to obtain a clear separation of patients with AD from control subjects simply by measuring the levels of plasma Aβ species. However, our findings suggest that measuring plasma Aβ and oAβ over 1 to 2 years or more can reveal a significant reduction in plasma Aβ levels, especially Aβ42 levels, and this finding raises the possibility of a direct relationship of plasma Aβ to brain amyloid formation.

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Author Contributions: All of the authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Xia, Yang, Shankar, Walsh, and Selkoe. Acquisition of data: Xia, Yang, Smith, Shen, and Selkoe. Analysis and interpretation of data: Xia, Yang, Shankar, Smith, and Selkoe. Drafting of the manuscript: Xia. Critical revision of the manuscript for important intellectual content: Xia, Yang, Shankar, Smith, Shen, Walsh, and Selkoe. Statistical analysis: Xia and Yang. Obtained funding: Xia and Selkoe. Administrative, technical, and material support: Xia, Yang, Shankar, Smith, and Selkoe. Study supervision: Xia, Walsh, and Selkoe.

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