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Homology of the Amyloid Beta Protein Precursor in Monkey and Human Supports a Primate Model for Beta Amyloidosis in Alzheimer's Disease

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Progressive cerebral deposition of the amyloid β-protein (AβP) occurs in Alzheimer's disease and during aging of certain mammals (eg, human, monkey, dog) but not others (eg, mouse, rat). The authors cloned and sequenced a full-length cDNA encoding the β-protein precursor (βAPP) of cynomolgus monkey. The predicted amino acid sequence of the 695-residue protein is completely homologous to that of human. The alternatively transcribed exons encoding the Kunitz protease inhibitor region in monkey were cloned, showing only a single conservative amino acid substitution in the 751-residue form of βAPP and four substitutions in $βAPP_{770}$. Immunoblots of cerebral cortex with antibodies to various βAPP domains showed highly similar βAPP polypeptides in human and monkey, in contrast to those of mouse and rat. The latter differences reflect sequence substitutions, transcriptional regulation, and possibly post-translational modifications that may decrease the amyloidogenic potential of rodent βAPP. Immunocytochemistry of aged cynomolgus brain showed AβP deposited in blood vessels and diffuse and compacted plaques closely resembling those of humans, and the presence of β-amyloid–associated proteins ($α_1$-antichymotrypsin; complements C1q and C3c) characteristic of AβP deposits in Alzheimer's disease. The authors' findings demonstrate that cynomolgus monkey and perhaps other primates provide a close animal model for examining the early transcriptional and post-translational processing of βAPP that precedes AβP deposition during aging and in Alzheimer's disease. (Am J Pathol 1991, 138:1423–1435)

Growing evidence indicates that altered proteolytic processing of the amyloid β-protein precursor (βAPP) and progressive accumulation of a ~40-residue fragment, the amyloid β-protein (AβP), is an early pathogenetic event in Alzheimer's disease (AD). This process also occurs to a limited extent in virtually all aged humans, suggesting that the complete biosynthetic and catabolic pathways necessary to produce stable AβP fragments are normally present. Understanding the biology of βAPP, which is encoded by a gene on human chromosome 21,[1–4] and the molecular mechanism of AβP deposition in AD, should thus shed light on a prominent feature of cerebral aging in general. In this regard, we and others have previously shown that aged primates, dogs, and certain other mammals develop microvascular and plaquelike AβP deposits in cerebral cortex that closely resemble those found in aged humans and patients with AD.[5–9]

It is widely acknowledged that the rigorous molecular analysis of AD, a chronic, slowly evolving brain disorder, is hampered by the lack of an appropriate animal model of the disease. Virtually all of the advances in understanding the neuropharmacology, biochemistry, and molecular biology of AD in recent years have emerged from studies of human brain tissue taken at autopsy. The limitations of biochemical studies conducted on postmortem brain obtained at the end of the disease make the identification of an animal model of the early pathogenetic features of AD important. Amorphous deposits of AβP (referred to as 'diffuse' or 'preamyloid' deposits) are found in the brains of younger subjects with trisomy 21 (Down's syndrome) before the other cytopathologic features of AD that these patients invariably develop.[10,11]

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Such diffuse AβP deposits also occur in aged humans and patients with AD.[12,13] In view of this evidence that AβP deposition may precede the characteristic brain lesions of AD, we have pursued comparative studies of the molecular characteristics of βAPP in species that are either prone or resistant to cerebral β-amyloidosis during aging. Here we report the complete nucleotide and deduced amino acid sequences of the homologs in cynomolgus monkey of all three major transcripts of human βAPP: $βAPP_{695}$ (subscript indicates number of amino acids),[1] $βAPP_{751}$,[14,15] and $βAPP_{770}$.[16] Furthermore we characterize the detailed patterns of βAPP polypeptides in cynomolgus brain and compare them with those in the human and those in the rat and mouse, species that do not undergo AβP deposition with age. We also demonstrate many AβP deposits in aged cynomolgus brain that have the morphology and immunochemical reactivities of those found in aged humans and patients with AD, including diffuse plaques. Taken together, our data recommend the cynomolgus monkey and perhaps other shorter-lived primates as a close model for the dynamic study of the transcriptional and post-translational processing of βAPP that precedes AβP deposition and senile plaque formation in cerebral aging and AD.

Materials and Methods

Cloning of Cynomolgus Monkey $βAPP_{695}$ cDNA

pGEM-4 cDNA libraries from cortex or cerebellum of adult cynomolgus monkey *(Macaca fascicularis)*[17] were screened with cDNA fragments of human βAPP. Probes were β6 (0.7 kb),[18] corresponding to bases 1797 to 2451 of $βAPP_{695}$; pFB5 (provided by R. Tanzi), corresponding to bases 158 to 1795; and pFB5 digested with *Pst*1 (FB5/Pst; bases 243 to 1217). High-density plasmid screening[19] of 3×10^6 colonies was conducted using 5 ng of the plasmid library transformed into 0.5 ml of DH5 or HB101 competent cells (BRL). All probes were gel purified and labeled with $α^{32}P$-dCTP using random primers[20] to a specific activity of $\sim 4 \times 10^8$ cpm/μg; hybridization was carried out at $\sim 1 \times 10^7$ cpm/ml. Hybridization conditions were 5X Denhardt's/6X saline sodium citrate (SSC)/0.3% sodium dodecyl sulfate (SDS)/100 μg/ml ssDNA at 65° for 12 to 16 hours. Filters were washed at 42°C for 30 minutes each in 3X SSC/0.1% SDS, 2X SSC/0.1% SDS, 1X SSC/0.1% SDS, and 0.5X SSC/0.1% SDS, and were dried and exposed to Kodak XAR-5 film for 12 to 48 hours with an intensifying screen. Duplicate positive colonies were selected, replated at low density, and gridded in microtiter dishes[21] for secondary screening and purification. Duplicate filters were differentially screened with βAPP fragments specific for the 3′ region (β6 0.7 kb) and the 5′ region (pFB5 or FB5/Pst). cDNAs positive with both 3′ and 5′ probes were analyzed further by restriction mapping, and the longest clones subcloned into M13 for sequencing. ssDNA was sequenced[22] using the sequenase kit (USB) with either the universal primer or synthetic 16- to 20-base oligonucleotide primers specific for human βAPP. All subclones were overlapping, and 90% of the 3.1-kb $βAPP_{695}$ full-length cDNA was sequenced on both strands.

Cloning of Inserted Exons in $βAPP_{751}$ and $βAPP_{770}$ by PCR

Total RNA was purified from 50 mg of cynomolgus heart and kidney tissue using RNasol B (Cinna/Biotecx, Houston, TX), extracted with phenol and phenol/chloroform, ethanol precipitated in the presence of sodium acetate, and resuspended in 10 mmol/l (millimolar) TRIS/1 mmol/l ethylenediaminetetra-acetic acid (EDTA), pH 7.6. First-strand cDNA synthesis used a βAPP-specific primer (KPI-R), corresponding to the reverse of Kang bases 921-901, with a *Bam*HI site at the 5′ end (GGCGGATC-CAGGTG-TCTEGAGATACTTGT). The 20-μl reaction contained 1 μg RNA, 100 ng KPI-R primer, 2 μl RNasin (Promega), 1 μl (19.5 U) AMV Reverse transcriptase (Lifesciences), 1.25 mmol/l dNTPs in 1× Taq reaction buffer (Perkin-Elmer Cetus) and was incubated at 42°C for 1 hour. The reaction was stopped by addition of 180 μl H_2O and the products were used directly in the polymerase chain reaction (PCR). The forward primer used in PCR was KPI-F, corresponding to Kang bases 834 to 853 with an *Eco*RI site at the 5′ end (GGCGAATTCCACCA-CAGAGTCTGTGGAAG), and the reverse primer was KPI-R. Polymerase chain reactions (100 μl) contained 20 μl diluted cDNA products, 1 μmol/l (micromolar) each KPI-F and KPI-R primers, 200 μmol/l deoxynucleoside 5′-triphosphate (dNTP), 1 μl Taq polymerase (Cetus) in 1X Taq buffer overlayed with 3 drops mineral oil. Samples went through 30 cycles of 94°C for 1 minute; 55°C for 2 minutes; 72°C for 3 minutes, followed by a 10-minute extension at 72°C. The reaction was chloroform extracted to remove the mineral oil and phenol-extracted four times to remove the Taq polymerase. The final aqueous layer was ethanol precipitated in 2.5 mol/l (molar) ammonium acetate, and the resulting pellet resuspended in water and digested with *Eco*RI and *Bam*HI for 1 hour at 37°C. The reaction was run on a 4% GTG-agarose (FMC) gel in TAE buffer, stained with ethidium bromide, and the 320-bp and 280-bp bands corresponding to the $βAPP_{751}$ and $βAPP_{770}$ inserted exons, respectively, were cut out and purified by phenol extraction and ethanol precipitation. Each insert was ligated to gel-purified *Bam*HI and *Eco*RI

double-digested M13 vector for 3 hours at 14°C and transformed into XLI Blue (Stratagene) competent cells. Fragments were sequenced and analyzed as described above.

Antibodies

Three carboxyl terminal antisera, αC_1,[23] αC_7, and αC_8 were raised against synthetic peptide C: $\beta APP_{732-751}$ (numbering of Ponte et al, 1988[14]). Three amino terminal antisera, αN_1,[24] αN_2,[24] and αN_3 were raised against synthetic peptide N (βAPP_{45-62}). Antisera αKPI_1 to $\beta APP_{300-315}$, and αKPI_2[25] to $\beta APP_{324-336}$, both specifically recognize the KPI domain in βAPP_{751} and βAPP_{770}. Rabbits αC_7, αC_8, and αN_3 were each initially injected with 2 mg peptide conjugated to keyhole limpet hemocyanin. At 3-week intervals, each rabbit was boosted with 1 mg or 0.25 mg peptide conjugated to rabbit serum albumin to minimize the production of antibodies to the carrier protein. Rabbits developed titers to unconjugated peptide of more than 1:100,000 (αC_7 and αC_8) or more than 1:25,000 (αN_3) as measured by dot blots. αKPI_2, αN_1 and αN_2 were generously provided by M. Palmert and S. Younkin (Case Western Reserve School of Medicine). αB5[26] is an affinity-purified polyclonal antiserum made to a recombinantly expressed construct comprising $\beta APP_{500-648}$. αB5 and αKPI_1 were provided by D. Schenk and T. Oltersdorf (Athena Neurosciences). Standard dilutions used were as follows: αB5, 1:2500; αC_7, 1:1500; αC_8, 1:750; αN_3 and αKPI_1, 1:500; αC_1, αN_1, αN_2, and αKPI_2, 1:300.

Antiserum A[27] was raised against native AβP purified by high-pressure liquid chromatography (HPLC) from amyloid-rich fractions of AD cerebral cortex. Antiserum Y[28] was raised against a synthetic peptide of $A\beta P_{1-38}$. Both antisera specifically recognize AβP deposits without formic acid pretreatment. Antiserum P,[8] raised to PHF-rich fractions of AD cortex, and antiserum DJ,[29] raised to heat-stable microtubule-associated proteins (including tau) from fetal human brain, both strongly label neurofibrillary tangles (NFT) and the paired helical filament (PHF)-containing neurites of human senile plaques. RT97[30] and SMI 34 (Sternberger-Meyers) are monoclonal antibodies to the neurofilament high molecular weight protein. Anti-α_1-antichymotrypsin (ICN, Dako, and Atlantic), αC1q (Accurate), αC3c (Accurate), and anti-glial fibrillary acidic protein[31] also were used. A standard dilution of 1:250 was applied for all these antisera except Y (1:500) and SMI34 (1:10,000).

The specificities of all synthetic peptide antisera were checked by peptide absorption. Specificity of antiserum A was checked by absorption with SDS-purified amyloid core fractions[27] or with AβP synthetic peptides. Typically 15 to 45 μg of the relevant unconjugated peptide per μl of undiluted antiserum was incubated (overnight at 4°C, or 3 hours at 25°C) at 1:250 to 1:1500 dilution in phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 0.02% NaN_3. A control, nonabsorbed aliquot of the antiserum was treated identically, with the exception that water was added instead of synthetic peptide. After absorption, samples were centrifuged at 16,000*g* for 20 minutes, and the supernatants were used as primary antisera.

Immunoblotting

Homogenates of frozen brain, kidney, adrenal, or heart from adult cynomolgus monkey, human, mouse, or rat were essentially prepared as described previously.[23] Briefly, tissue aliquots (~0.2 to 1 g wet weight) were pulverized in liquid nitrogen and homogenized at 4°C in 2 volumes of isotonic buffer containing protease inhibitors. Homogenates were analyzed either directly by Western blotting or, in some cases, further fractionated at 200,000*g* and the pellets solubilized in 2% Triton X-100.[23] Freshly drawn EDTA-treated plasma samples from aged rhesus monkeys and humans (AD and normal patients) were selectively precipitated by extensive dialysis against 50 mmol/l TRIS, pH 7.6, in the presence of protease inhibitors and resolubilized in PBS as described previously.[32] Human kidney 293 cells transfected with βAPP cDNAs have previously been described[23,25,33] and were provided by T. Oltersdorf. Equal protein loading was determined in duplicate by the dye-binding assay (Biorad).[34] Samples were electrophoresed[35] on 5% to 20%, 10% to 25%, or 7.5% polyacrylamide 8-cm minigels and blotted[36] onto Immobilon-P (Millipore). Immunodetection used goat anti-rabbit gamma G immunoglobulin (IgG) coupled to alkaline phosphatase (Promega) at 1:7500 dilution.

Immunocytochemistry

Formalin-fixed coronal slices from the frontal cortex of three 19-year-old female cynomolgus monkeys were embedded in paraffin, cut into sequentially numbered 6-μ sections, and immunostained using the avidin-biotin-peroxidase system (Vector) with hematoxylin counterstaining. All staining runs included sections of AD occipital cortex run in parallel as positive controls.

Results

cDNA Cloning and Sequencing of Cynomolgus βAPP_{695} Shows Complete Amino Acid Homology to Human

Two pGEM-4 cDNA libraries from cynomolgus monkey cortex or cerebellum[17] were screened with various frag-

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HUMAN
MONKEY                            -143   GTTTCCTCGGCAGCGGTAGGCGAGAG  -121

HUMAN      G    G    T      -  G               CGG
MONKEY  CACTCGGAGCAGCGCGCGGGGGCCCCCGGAGACGGCGGCGGTGG---CGCGGGCAGAG  -61

HUMAN
MONKEY  CAAGGACGCGGCGGATCCCACTCGCACAGCAGCGCACTCGGTGCCCCGCGCAGGGTCGCG  -1

HUMAN
MONKEY  ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGTA  60
MONKEY  M  L  P  G  L  A  L  L  L  L  A  A  W  T  A  R  A  L  E  V   20

HUMAN     C      T                         T
MONKEY  CCTACTGATGGCAATGCTGGCCTGCTGGCTGAACCCCAGATCGCCATGTTCTGTGGCAGA  120
MONKEY  P  T  D  G  N  A  G  L  L  A  E  P  Q  I  A  M  F  C  G  R   40

HUMAN
MONKEY  CTGAACATGCACATGAATGTCCAGAATGGGAAGTGGGATTCAGATCCATCAGGGACCAAA  180
MONKEY  L  N  M  H  M  N  V  Q  N  G  K  W  D  S  D  P  S  G  T  K   60

HUMAN
MONKEY  ACCTGCATTGATACCAAGGAAGGCATCCTGCAGTATTGCCAAGAAGTCTACCCTGAACTG  240
MONKEY  T  C  I  D  T  K  E  G  I  L  Q  Y  C  Q  E  V  Y  P  E  L   80

HUMAN                                                              C
MONKEY  CAGATCACCAATGTGGTAGAAGCCAACCAACCAGTGACCATCCAGAACTGGTGCAAGAGG  300
MONKEY  Q  I  T  N  V  V  E  A  N  Q  P  V  T  I  Q  N  W  C  K  R   100

HUMAN                                                C
MONKEY  GGCCGCAAGCAGTGCAAGACCCATCCCCACTTTGTGATTCCCTACCGCTGTTTAGTTGGT  360
MONKEY  G  R  K  Q  C  K  T  H  P  H  F  V  I  P  Y  R  C  L  V  G   120

HUMAN           T
MONKEY  GAGTTTGTAAGCGATGCCCTTCTCGTTCCTGACAAGTGCAAATTCTTACACCAGGAGAGG  420
MONKEY  E  F  V  S  D  A  L  L  V  P  D  K  C  K  F  L  H  Q  E  R   140

HUMAN                                                 A
MONKEY  ATGGATGTTTGCGAAACTCATCTTCACTGGCACACCGTCGCCAAAGAGACGTGCAGTGAG  480
MONKEY  M  D  V  C  E  T  H  L  H  W  H  T  V  A  K  E  T  C  S  E   160

HUMAN       T                                   C    T  C
MONKEY  AAGAGCACCAACTTGCATGACTACGGCATGTTGCTGCCCTGTGGAATCGATAAGTTCCGA  540
MONKEY  K  S  T  N  L  H  D  Y  G  M  L  L  P  C  G  I  D  K  F  R   180

HUMAN                             A
MONKEY  GGGGTAGAGTTTGTGTGTTGCCCACTGGCTGAGGAAAGTGACAATGTGGATTCTGCTGAT  600
MONKEY  G  V  E  F  V  C  C  P  L  A  E  E  S  D  N  V  D  S  A  D   200

HUMAN                                                          T
MONKEY  GCGGAGGAGGATGACTCGGATGTCTGGTGGGGCGGAGCAGACACAGACTATGCAGACGGG  660
MONKEY  A  E  E  D  D  S  D  V  W  W  G  G  A  D  T  D  Y  A  D  G   220

HUMAN                                              T
MONKEY  AGTGAAGACAAAGTAGTAGAAGTAGCAGAGGAGGAAGAAGTGGCCGAGGTGGAAGAAGAA  720
MONKEY  S  E  D  K  V  V  E  V  A  E  E  E  E  V  A  E  V  E  E  E   240

HUMAN
MONKEY  GAAGCCGATGATGACGAGGACGATGAGGATGGTGATGAGGTAGAGGAAGAGGCTGAGGAA  780
MONKEY  E  A  D  D  D  E  D  D  E  D  G  D  E  V  E  E  E  A  E  E   260

HUMAN                                      T                   A
MONKEY  CCCTACGAAGAAGCCACAGAGAGAACCACCAGCATCGCCACCACCACCACCACCACCACG  840
MONKEY  P  Y  E  E  A  T  E  R  T  T  S  I  A  T  T  T  T  T  T  T   280

HUMAN                              ↓
MONKEY  GAGTCTGTGGAAGAGGTGGTTCGAGTTCCTACAACAGCAGCCAGTACCCCTGATGCCGTT  900
MONKEY  E  S  V  E  E  V  V  R  V  P  T  T  A  A  S  T  P  D  A  V   300

HUMAN         T  C                    T
MONKEY  GACAAGTACCTTGAGACACCTGGGGATGAGAATGAACACGCCCATTTCCAGAAAGCCAAA  960
MONKEY  D  K  Y  L  E  T  P  G  D  E  N  E  H  A  H  F  Q  K  A  K   320

HUMAN        G
MONKEY  GAGAGACTTGAGGCCAAGCACCGAGAGAGAATGTCCCAGGTCATGAGAGAATGGGAAGAG  1020
MONKEY  E  R  L  E  A  K  H  R  E  R  M  S  Q  V  M  R  E  W  E  E   340

HUMAN                A
MONKEY  GCAGAACGTCAGGCAAAGAACTTGCCTAAAGCTGATAAGAAGGCAGTTATCCAGCATTTC  1080
MONKEY  A  E  R  Q  A  K  N  L  P  K  A  D  K  K  A  V  I  Q  H  F   360

HUMAN
MONKEY  CAGGAGAAAGTGGAATCTTTGGAACAGGAAGCAGCCAACGAGAGACAGCAGCTGGTGGAG  1140
MONKEY  Q  E  K  V  E  S  L  E  Q  E  A  A  N  E  R  Q  Q  L  V  E   380

HUMAN
MONKEY  ACACACATGGCCAGAGTGGAAGCCATGCTCAATGACCGCCGCCGCCTGGCCCTGGAGAAC  1200
MONKEY  T  H  M  A  R  V  E  A  M  L  N  D  R  R  R  L  A  L  E  N   400

HUMAN            C           T                                A
MONKEY  TACATCACTGCTCTGCAGGCCGTTCCTCCTCGGCCTCGTCACGTGTTCAATATGCTGAAG  1260
MONKEY  Y  I  T  A  L  Q  A  V  P  P  R  P  R  H  V  F  N  M  L  K   420
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   T                                                 A             G
AAGTACGTCCGCGCAGAACAGAAGGACAGACAGCACACCCTGAAGCATTTCGAGCATGTA  1320
K  Y  V  R  A  E  Q  K  D  R  Q  H  T  L  K  H  F  E  H  V    440

                          C
CGCATGGTGGATCCCAAGAAAGCTGCTCAGATCCGGTCCCAGGTTATGACACACCTCCGT  1380
R  M  V  D  P  K  K  A  A  Q  I  R  S  Q  V  M  T  H  L  R    460

           T                T
GTGATTTACGAGCGCATGAATCAGTCCCTCTCCCTGCTCTACAACGTGCCTGCAGTGGCC  1440
V  I  Y  E  R  M  N  Q  S  L  S  L  L  Y  N  V  P  A  V  A    480
                     *

GAGGAGATTCAGGATGAAGTTGATGAGCTGCTTCAGAAAGAGCAAAACTATTCAGATGAC  1500
E  E  I  Q  D  E  V  D  E  L  L  Q  K  E  Q  N  Y  S  D  D    500
                                               *

                                                            A
GTCTTGGCCAACATGATTAGTGAACCAAGGATCAGTTACGGAAACGATGCTCTCATGCCG  1560
V  L  A  N  M  I  S  E  P  R  I  S  Y  G  N  D  A  L  M  P    520

                            C        C
TCTTTGACCGAAACGAAAACTACCGTGGAGCTTCTTCCCGTGAATGGAGAGTTCAGCCTG  1620
S  L  T  E  T  K  T  T  V  E  L  L  P  V  N  G  E  F  S  L    540

                                T
GACGATCTCCAGCCGTGGCATTCTTTCGGGGCTGACTCTGTGCCAGCCAACACAGAAAAC  1680
D  D  L  Q  P  W  H  S  F  G  A  D  S  V  P  A  N  T  E  N    560

GAAGTTGAGCCTGTTGATGCCCGCCCTGCTGCCGACCGAGGACTGACCACTCGACCAGGT  1740
E  V  E  P  V  D  A  R  P  A  A  D  R  G  L  T  T  R  P  G    580

                           G
TCTGGGTTGACAAATATCAAGACAGAGGAGATCTCTGAAGTGAAGATGGATGCAGAATTC  1800
S  G  L  T  N  I  K  T  E  E  I  S  E  V  K  M [D  A  E  F]   600

              A                                     A
CGACATGACTCAGGTTATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCCGAAGATGTG  1860
[R  H  D  S  G  Y  E  V  H  H  Q  K  L  V  F  F  A  E  D  V]  620

                             A                          G
GGTTCAAACAAAGGTGCAATCATTGGTCTCATGGTGGGCGGTGTTGTCATAGCAACAGTG  1920
[G  S  N  K  G  A  I  I  G  L  M  V  G  G  V  V] I  A  T  V   640

                                                A
ATCGTCATCACCTTGGTGATGCTGAAGAAGAAACAGTACACGTCCATTCATCATGGTGTG  1980
I  V  I  T  L  V  M  L  K  K  K  Q  Y  T  S  I  H  H  G  V    660

GTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCACCTGTCCAAGATGCAGCAGAAC  2040
V  E  V  D  A  A  V  T  P  E  E  R  H  L  S  K  M  Q  Q  N    680

   C
GGCTATGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAACTAGACCCCCGCCACA  2100
G  Y  E  N  P  T  Y  K  F  F  E  Q  M  Q  N  *                695

GCAGCCTCTGAAGTTGGACAGCAAAACCATTGCTTCACTACCCATCGGTGTCCATTTATA  2160

 A  G           ----
GAATACTGTCGGAAGAAACAAACCCCTCTGTTTTATGATTTACTCATTATCGCCTTTTGA  2220

                                                 C
CAGCTGTGCTGTAACACAAGTAGATGCCTGAACTTGAATTAATCCACAAATCAGTAATGT  2280

 A                   T
ATTCTCTCTCTCTTTACATTTTGGTCTCTACACTACATTATTAATGGGTTTTGTGTACTG  2340

          T
TAAAGAATTTAGCCGTATCAAACTAGTGCATGAATAGATTCTCTCCTGATTATTTATCA  2400

CATAGCCCCTTAGCCAGTTGTATATTATTCTTGTGGTTTGTGACCCAATTAAGTCCTACT  2460

                                                       C
TTACATATGCTTTAAGAATCGATGGGGGATGCTTCATGTGAACGTGGGAGTTTAGCTGCT  2520

TCTCTTGCCTAAGTATTCCTTTCCTGATCACTATGCATTTTAAAGTTAAACATTTTTAAG  2580

T                                T  C
TACTTCAGATGCTTTAGAGAGATTTTTTTTCCACGATTGCATTTTACTGTACAGATTGCT  2640

                                   AC                C
GCTTCTGCTATATTTGTGATATAGGAATTAAGAGGAT--ACACGTTTGTTTCTTTGTGCC  2700

                              A    TC
TGTTTTATGTGCACACATTAGGCATTGAGACTTCGAGCTTT--TTTTTTTGTCCACGTAT  2760

                                                         C
CTTTGGGTCTTTGATAAAGAAAAGAATCCCTGTTCATTGTAAGCACTTTTACGGGGTGGG  2820

                            C                  C  T
TGGGGAGGGGTGCTCTGCTGGTCTTAAATTACCAAGAATTCTCCAAAAAAA-TTTCTGCA  2880

GGATGATTGTACAGAATCATTGCTTATGACATGATCGCTTTCTACACTGTATTACATAAA  2940

TAAATTAAATAAAAT-poly(A) tail                                  2955
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ments of human βAPP cDNA. Initially, the cortex library was screened with a mixture of a 3′ fragment (β6[18]) and a 5′ fragment (pFB5; provided by R. Tanzi). A 1.7-kb cDNA corresponding to bases 1279 through 2955 and amino acids 427 through 695 of human $βAPP_{695}$[1] was isolated. All 268 deduced amino acids of this 3′ clone were homologous to the human protein. The cerebellar library then was probed with a 5′ fragment of pFB5 and yielded a 3.1 kb cDNA (Mcm2), which was shown by sequencing to correspond to nucleotides −143 to 2955 of human $βAPP_{695}$ (Figure 1). Sequence comparison of this full-length clone with human βAPP showed 97% nucleotide homology overall, and 98% nucleotide homology within the coding region. The deduced amino acid sequence was 100% homologous to human $βAPP_{695}$ (Figure 1).[1] This identity contrasts to the 18- and 22-amino acid substitutions, respectively, reported in $βAPP_{695}$ of rat[37] and mouse[38] (Table 1). The AβP region itself has three substitutions in rat and mouse but is completely conserved in monkey (Table 1).

Cloning of the Alternatively Spliced Exons in $βAPP_{751}$ and $βAPP_{770}$ of Cynomolgus Shows Few Amino Acid Substitutions

$βAPP_{751}$ and $βAPP_{770}$ are alternatively spliced transcripts, containing either 168- or 225-bp fragments inserted at nucleotide 865 of human $βAPP_{695}$. The resulting 56- and 75-amino acid inserts contain a region homologous to the Kunitz family of serine protease inhibitors.[14–16] Homologous fragments were amplified from cynomolgus cDNA using PCR.[39,40] Oligonucleotide primers were synthesized to 19 base sequences flanking the insert site. Polymerase chain reaction with these primers should produce 106-, 274-, and 331-bp fragments derived from $βAPP_{695}$, $βAPP_{751}$, and $βAPP_{770}$, respectively. Reactions performed on the cDNA libraries from cynomolgus brain showed the 106-bp band but did not contain the 274- or 331-bp fragments (data not shown).

Because kidney and heart are known to contain $βAPP_{751}$ and $βAPP_{770}$ mRNA and proteins,[3,23] total RNA was isolated from frozen cynomolgus heart and kidney. The cDNA synthesized from this RNA was used in PCR, which yielded the 106-, 274-, and 331-bp fragments. Sequence analysis of two independent clones containing the 274- and 331-bp fragments showed 99.3% and 98.4% nucleotide homology, corresponding to 1 and 4 amino acid substitutions, respectively, within the 56- and 75-residue inserts of $βAPP_{751}$ and $βAPP_{770}$ (Figure 2). The single amino acid substitition in $βAPP_{751}$ was outside of the active site of the Kunitz protease inhibitor (KPI) domain. Cynomolgus $βAPP_{751}$ and $βAPP_{770}$ proteins are 99.9% and 99.5% homologous, respectively, to the corresponding human βAPPs (Table 1).

βAPP Polypeptides Show Highly Similar Electrophoretic Patterns in Human and Monkey But Not Rodent Brain

Using antibodies to various regions of βAPP (Figure 3), homogenates of cynomolgus monkey brain were compared on Western blots with those of humans, which develop AβP deposits during brain aging, and those of rat and mouse, which do not. All βAPP antibodies detected a polypeptide pattern in monkey brain that was highly similar to that previously reported in human brain.[23,24] Three antisera to a carboxy-terminal synthetic peptide, $βAPP_{732–751}$ (numbering of $βAPP_{751}$), specifically labeled proteins in monkey cortical homogenates migrating at 106 to 139 kd and at ~11 kd (Figure 4A). Homogenates of monkey kidney, adrenal gland, and heart contained a qualitatively similar set of βAPP isoforms. Subcellular fractionation demonstrated the presence of these proteins in the Triton-solubilized membrane fraction (data not shown). Further analyses on 7.5% and 10% to 25% gradient polyacrylamide gels showed discrete bands migrating at 139, 127, 116, 111, 106, 12, and 10 kd. These polypeptides were compared with the well-characterized βAPP proteins overexpressed in kidney 293 cells transfected with $βAPP_{751}$ or $βAPP_{695}$ human cDNAs.[23,25,33] The overexpressed N- plus O-glycosylated and N-glycosylated βAPP isoforms migrating at 127 and 106 kd, respectively, in $βAPP_{695}$-transfected cells and at 139 and 116 kd, respectively, in $βAPP_{751}$-transfected cells comigrated with the corresponding bands in monkey and human brain homogenates (Figure 4B; lanes 1 through 4). The 111-kd band in the brain homogenates did not comigrate with any overexpressed protein in the cell lines. The 10- to 12-kd C-terminal–reactive peptides in monkey and human brain comigrated with βAPP fragments overexpressed in both

Figure 1. *Nucleotide and deduced amino acid sequence of cynomolgus monkey $βAPP_{695}$ cDNA compared with human $βAPP_{695}$ cDNA. The nucleic acid sequence of a full-length cDNA encoding monkey $βAPP_{695}$ is shown in the second line. Nucleotide substitutions found in human $βAPP_{695}$ are indicated in the first line. The deduced amino acid sequence in monkey is shown in the third line and is completely homologous to that of human.[1] The region comprising the 40-amino acid AβP fragment in humans[61] is boxed. The arrow (nucleotide 865) indicates the site at which additional exons are inserted in $βAPP_{751}$ and $βAPP_{770}$. Large asterisks indicate potential N-glycoslylation sites and the bracket indicates a potential phosphorylation site.[62] The first of the two polyadenylation sites (underlined) found in human cDNAs was used in both monkey cDNAs sequenced. Sequence translation and alignments were done on the University of Wisconsin Sequence Analysis software package.[63]*

Table 1. *Amino Acid Substitutions in Monkey, Rat, and Mouse βAPP Compared with Human*

	APP_{695}		APP_{751}		APP_{770}	
	No. subs.	% homol.	No. subs.	% homol.	No. subs.	% homol.
Monkey	0	100	1	99.9	4	99.5
Rat	18	97.4	20	97.3	23	97.0
Mouse	22	96.8	25	96.7	30	96.1

```
Human    DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
Monkey
Rat           G   F R
Mouse         G   F R
```

The protein sequence homologies of monkey, rat[37,50] and mouse[38,42] βAPPs are compared with that in human.[1,14–16] The number of amino acid substitutions and the resulting percent homology are shown for each of the three major βAPP transcripts: βAPP_{695}, βAPP_{751}, and βAPP_{770}. The location of the substitutions in mouse βAPP_{751} are diagramed in Figure 3. Three amino acid substitutions in mouse and rat occur within the AβP region itself, as is shown in the lower half of the table. The histidine at residue 13, which in rat and mouse is changed to arginine, has been postulated to be important in forming the characteristic β-pleated sheet structure of AβP.[51]

cell lines and represent membrane-retained C-terminal fragments of βAPP after proteolytic cleavage.[23,33,41]

In contrast to the highly similar βAPP pattern in monkey and human, rat and mouse brain showed a distinctive pattern of immunoreactive proteins. Robust bands at 106 to 121 kd and a faint band at 127 kd were specifically labeled by the C-terminal antisera in rodent brain homogenates (Figure 4B; lanes 5 and 6). The presence of only very low levels of the 127-kd band (N- plus O-glycosylated βAPP_{695}) in rodent brain, and the detection of an additional 121-kd βAPP isoform in rodent but not primate brain (Figure 4B, lanes 3 through 6) suggest that different post-translational modifications of βAPP_{695} occur in rodent than in primate brain. Antibodies to the KPI domain did not specifically label any proteins in rat and mouse brain; the virtual absence of the βAPP_{751} polypeptides (139 and 116 kd) in the rodent homogenates (Figure 4B, lanes 5 and 6) is consistent with the low level of βAPP_{751} mRNA reported in mouse and rat brain.[42,43] These differences between primate and rodent βAPP isoforms observed with the C-terminal antisera were confirmed with three antisera to the N-terminal synthetic peptide, βAPP_{45-62} (data not shown). The 10- to 12-kd C-terminal fragments were detected similarly in rodent and primate brains.

Comparative immunoblotting of primate and rodent tissue homogenates with αB5 (an affinity-purified antiserum to a midregion construct, $\beta APP_{500-648}$) demonstrated striking species differences. In monkey and human brain, αB5 labeled the full-length, membrane-associated βAPP polypeptides (Figure 4C; lanes 1 and 2). αB5 also specifically identified cytosolic forms of βAPP that migrated from ~95 to 125 kd and were not detected by C-terminal antisera (Figure 4C; lanes 1 and 2). In contrast, αB5 detected no forms of βAPP in rat and mouse brain (Figure 4C; lanes 3 and 4). Similarly, αB5 failed to label βAPP isoforms detected by our C-terminal antisera in rat non-neural tissues and in the rat adrenal-derived cell line, PC12 (data not shown). These results suggest that the $\beta APP_{500-648}$ region containing the αB5 epitope has a distinct conformation in rodent *versus* monkey and human, due to the rodent amino acid substitu-

```
HUMAN            ↓                              G
MONKEY    GTTCGAGAGGTGTGCTCTGAACAAGCCGAGACGGGGCCATGCCGAGCAATGATCTCCCGC    918
MONKEY    V  R  E  V  C  S  E  Q  A  E  T  G  P  C  R  A  M  I  S  R      306
HUMAN

HUMAN
MONKEY    TGGTACTTTGATGTGACTGAAGGGAAGTGTGCCCCATTCTTTTACGGCGGATGTGGCGGC    978
MONKEY    W  Y  F  D  V  T  E  G  K  C  A  P  F  F  Y  G  G  C  G  G      326
HUMAN

HUMAN                                                       C ↓
MONKEY    AACCGGAACAACTTTGACACAGAAGAGTACTGCATGGCCGTGTGTGGCAGCGTCATGTCC    1038
MONKEY    N  R  N  N  F  D  T  E  E  Y  C  M  A  V  C  G  S  V  M  S      346
HUMAN                                                      A

HUMAN               T           A          G                  ↓
MONKEY    CAAAGTTTACGCAAGACTACCCGGGAACCTCTTACCCGAGATCCTGTTAAACTTCCTACA    1098
MONKEY    Q  S  L  R  K  T  T  R  E  P  L  T  R  D  P  V  K  L  P  T      366
HUMAN              L           Q           A
```

Figure 2. *Nucleotide and deduced amino acid sequences of alternatively spliced exons in cynomolgus βAPP_{751} and βAPP_{770} compared with human βAPP_{770}. The alternative transcripts, βAPP_{751} and βAPP_{770}, contain 168-bp and 225-bp fragments encoded on one or two exons, respectively, which are inserted into βAPP_{695} at nucleotide 865 (Figure 1). The nucleic acid sequence for monkey βAPP_{770} (beginning at nucleotide 859) is shown in the second line, with nucleotide substitutions found in human βAPP_{770} shown in the first line (numbering according to Kitaguchi[16]). The deduced amino acid sequence in monkey is shown in the third line, and the amino acid substitutions found in the human are shown in the fourth line. Arrows depict the borders of the two alternatively spliced exons. The second exon (nucleotides 1034-1090) is not present in βAPP_{751}. Residues 291-342 encode a Kunitz protease inhibitor domain[14–16] and are completely homologous in monkey and human.*

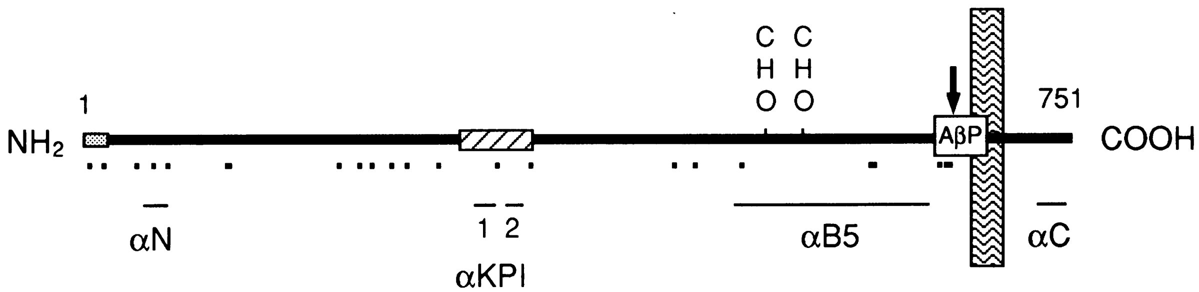

Figure 3. *Schematic diagram of primate βAPP_{751} depicting the epitopes of antibodies used in this study and the location of amino acid substitutions in the mouse. The bold black line represents βAPP_{751}; the extramembraneous amino-terminus (NH_2) containing a 17-residue signal sequence and the cytoplasmic carboxyl-terminus (COOH) are indicated. The putative transmembrane domain is depicted by the vertical hatched bar.[1] The proposed 40-amino acid AβP region is shown by the white box. The arrow depicts the βAPP constitutive proteolytic cleavage site (defined in transfected 293 cells[41]) within the AβP region, resulting in a large, glycosylated N-terminal fragment, which is secreted, and a ~10 kd C-terminal fragment, which is retained in the membrane. The 56-amino acid insert encoding the Kunitz protease inhibitor (KPI) is depicted by the lined box. CHO indicates putative N-linked glycoslylation sites. The location of each amino acid substitution in mouse βAPP_{751}[38,42] is represented by dots directly below the line. The lower lines represent the epitopes of the antibodies used in this study; antibodies were raised to synthetic peptides (αN, αKPI_1, αKPI_2, and αC) or to a bacterial construct (αB5) (see Materials and Methods).*

tions or different post-translational modifications in this portion of the molecule, which contains the two consensus sequences for N-glycosylation (Figure 3).

Human and Monkey Plasma Contain Soluble KPI-positive Forms of βAPP Migrating at ~125 and ~105 kd

Plasma obtained from two aged rhesus monkeys and from humans was extensively dialyzed and the resulting precipitate examined on immunoblots. A strongly immunoreactive ~125-kd band was specifically detected in monkey plasma by anti-βAPP_{45-62} (Figure 5, lane 2), the KPI antisera (Figure 5, lane 6), and αB5 (not shown), but not by any C-terminal antiserum. Human plasma is known to contain an immunochemically identical protein[32] that comigrates with the monkey isoform (Figure 5, lanes 1 and 5). In addition, monkey plasma and some human plasma samples contained two to three tightly

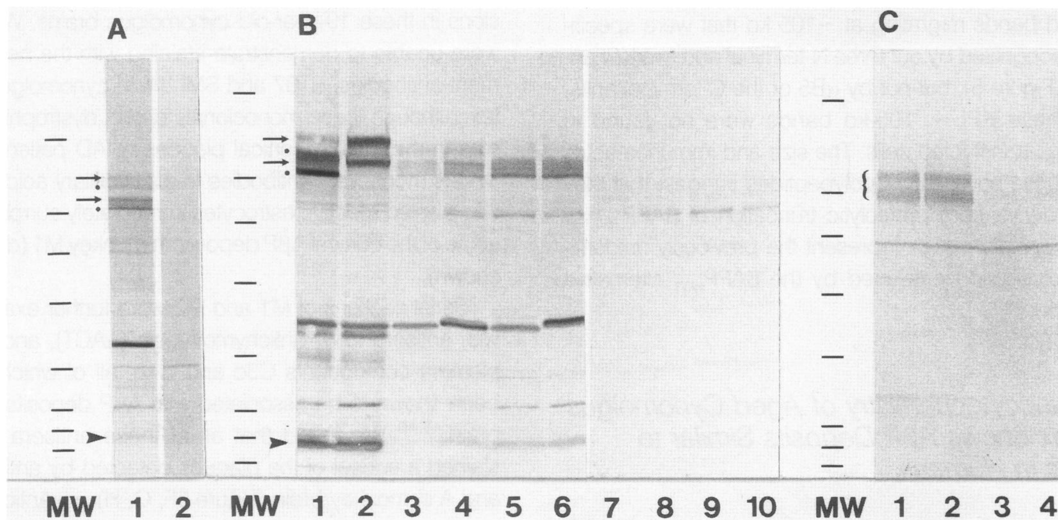

Figure 4. *Immunoblots of cerebral cortex in various species using region-specific βAPP antisera.* **A**: *Homogenates of adult cynomolgus monkey cortex (50 μg/lane) labeled by a C-terminal antiserum (αC_7 to $\beta APP_{732-751}$). Lane 1, αC_7 (1:1500); lane 2, αC_7 after absorption with peptide C (30 μg peptide/μl undiluted antiserum). Molecular weight (MW) markers (bars): 195, 106, 71, 44, 27, 18, 14, and 6 kd. The 106-139 kd βAPP polypeptides include 106 and 127 kd bands (dots) representing the N- and the N- plus O-glycolsylated forms of βAPP_{695}, respectively, and 116 and 139 kd bands (arrows) representing the N- and the N- plus O-glycolsylated forms of βAPP_{751}, respectively (see 4B). All of these full-length forms and the ~11 kd fragment (arrowhead) were specifically labeled by this and two other C antisera. Bands migrating at ~30 and ~18 kd were specifically labeled only by αC_7 and were not overexpressed in βAPP-transfected cells; they presumably represent unrelated proteins that cross-react with this synthetic peptide antiserum. Five to twenty percent polyacrylamide gradient minigels.* **B**: *Homogenates of primate and rodent cortex (50 μg protein/lane) and kidney 293 cells transfected with human βAPP cDNAs (12 μg/lane) labeled with αC_7. Lanes: 1, 293 cells transfected with βAPP_{695}; 2, 293 cells transfected with βAPP_{751}; 3 and 7, human cortex; 4 and 8, cynomolgus monkey cortex; 5 and 9, rat cortex; 6 and 10, mouse cortex. Staining in lanes 1–6 αC_7 (1:1500); lanes 7–10, αC_7 after absorption with peptide C. The 106–139 kd full-length βAPP polypeptides (dots: βAPP_{695}; arrows: βAPP_{751}) and the ~11 kd fragment (arrowhead) are indicated as in* **A**; *these bands were specifically labeled by this and two other C-terminal antisera. MW markers as in* **A**. *Five to twenty percent polyacrylamide gradient gel.* **C**: *Homogenates of primate and rodent cortex (50 μg protein/lane) labeled with affinity-purified αB5 ($\beta APP_{500-648}$; 1:2500). Lanes: 1, human cortex; 2, cynomolgus monkey cortex; 3, rat cortex; 4, mouse cortex. Bracket indicates βAPP forms detected in human and monkey, but not rodent brain; in some blots of rodent brain, very faint bands were visible. A monoclonal antibody (1G5) made to the same bacterial construct as αB5 produced an identical immunostaining pattern.*

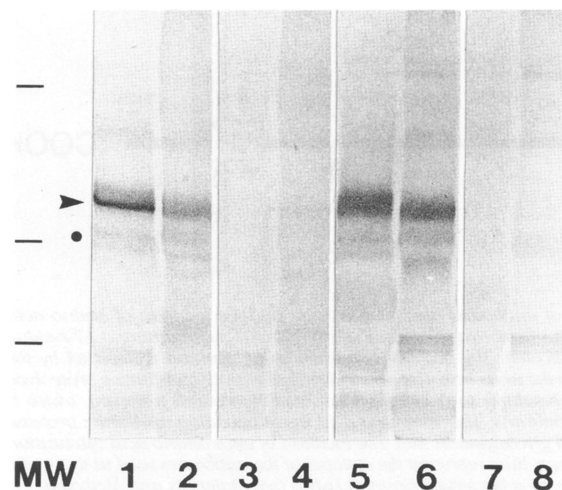

Figure 5. *Immunoblots of monkey and human plasma labeled with N-terminal and KPI-specific βAPP antisera. Plasma proteins were concentrated by dialysis-induced precipitation[32] and electrophoresed (40 μg/lane concentrated from ~0.25 ml plasma) on 7.5% acrylamide minigels. Lanes: 1, 3, 5, and 7, normal human plasma; 2, 4, 6, 8, rhesus monkey plasma. Staining in lanes: 1 and 2, αN_3 (βAPP_{45-62}; 1:500); 3 and 4, αN_3 after absorption with peptide N (45 μg/μl); 5 and 6, αKPI_1 (β$APP_{300-315}$; 1:500); 7 and 8, αKPI_1 after absorption with peptide KPI_1 (15 μg/μl). The constitutively secreted βAPP form migrating at ~125 kd (arrowhead) and the additional KPI-containing ~105 kd forms (dot) were also specifically labeled by αN_1, αN_2 and αKPI_2 but not by C-terminal antibodies. αB5 only recognized the ~125 kd form (data not shown). MW markers: 199, 105, and 71 kd.*

spaced bands migrating at ~105 kd that were specifically recognized by our three N-terminal and two KPI antisera (Figure 5), but not by αB5 or the C-terminal antisera. These KPI + 105-kd bands were not found in βAPP_{751}-transfected cells. The size and immunoreactivity of these novel KPI+ polypeptides suggest that they either derive from proteolytic truncation of βAPP_{751} or βAPP_{770} isoforms, or represent the previously unidentified polypeptide produced by the βAPP_{563} alternative transcript.[44]

Immunocytochemistry of Aged Cynomolgus Cortex Shows AβP Deposits Similar to Those in Humans

Because AβP deposits had not previously been described in cynomolgus monkey, we characterized the neuropathologic changes found in three aged (19 years old) cynomolgus brains (M1, M2, M3). Immunostaining of formalin-fixed cortical sections with AβP antisera showed AβP deposits in two of the three brains. M1 had abundant microvascular and plaquelike AβP deposits scattered throughout the cortical neuropil (Figure 6A). The deposits were intensely and specifically labeled by both antiserum A, raised to native AβP purified from AD cerebral cortex, and antiserum Y, raised to a synthetic $A\beta P_{1-38}$ peptide. The morphology of the plaques included diffuse, finely granular deposits that were not compacted, as well as deposits that had a compacted center and a peripheral halo of immunoreactivity and thus resembled mature or "classical" senile plaques in AD cortex. In addition, AβP was deposited in the walls of some capillaries (Figure 6B) and in some larger microvessels that appeared to be arterioles. Within a single cortical gyrus in monkey M1, this entire morphologic spectrum of AβP deposits could be observed. Similar cortical deposits were immunolabeled in aged nondemented humans (Figure 6D), and to a much greater extent, in patients with AD (Figure 6E). M2 had far fewer AβP deposits; these were usually located around microvessels, although a few small, partially compacted plaques were found in certain cortical regions (Figure 6C). Although Thioflavin S staining labeled the majority of monkey AβP deposits, reaction with Congo red detected very few of them. Silver staining with a modified Bielschowsky method showed some but not all of the AβP-immunoreactive plaques. Only a few plaques in monkey M1 contained small numbers of linear, silver-positive profiles resembling the dilated neurites found around some AβP plaques in rhesus monkey[7,8] and in the neuritic plaques of AD cortex.

Antibodies to PHF and human tau, which detect neurofibrillary tangles and some of the dystrophic neurites of senile plaques in AD brains, demonstrated no similar lesions in these 19-year-old cynomolgus brains. We also were unable to demonstrate staining with the neurofilament antibodies, RT97 and SMI 34, in cynomolgus cortex, although these monoclonals detect dystrophic neurites in the mature cortical plaques of AD patients and rhesus monkeys.[8] Antibodies to glial fibrillary acidic protein labeled reactive astrocytes immediately surrounding some of the cortical AβP deposits in monkey M1 (data not shown).

Serial sections of M1 and M2 were further examined with antisera to α_1-antichymotrypsin (αACT), and complement components C3c and C1q, all of which have been shown to be associated with AβP deposits in AD brain.[45,46] We found that all of these antisera faintly stained a subset of the plaques detected by antisera Y and A in monkey brain (Figure 6F, G, H). α_1-Antichymotrypsin recognized the greatest number of AβP-positive plaques. There were no obvious morphologic differences between AβP deposits that did or did not react with these antisera to β-amyloid–associated proteins.

Discussion

The identification of a close animal model of the early neuropathologic changes of AD would enable rigorous examination over time of the molecular and cellular alterations that precede the classical amyloid-bearing senile

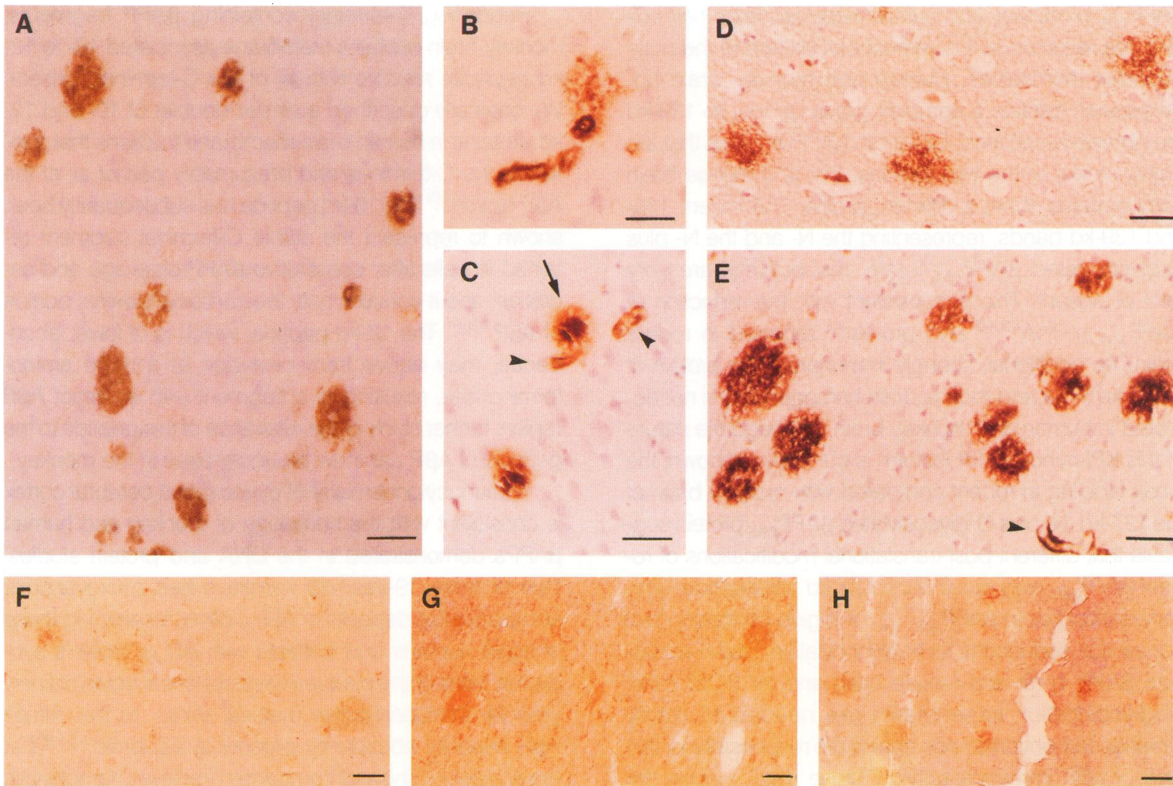

Figure 6. *Immunocytochemistry of aged cynomolgus cortex compared with aged human and Alzheimer's disease cortex.* **A**: *Representative field of senile plaques in the frontal cortex of a 19-year-old cynomolgus monkey (M1) specifically labeled by antiserum Y (1:500) to synthetic* $A\beta P_{1-38}$. **B**: *Antiserum Y also detected AβP deposits in the walls of cortical capillaries and in pericapillary plaques in M1.* **C**: *Another 19-year-old cynomolgus monkey (M2) had a small number of tightly clustered cortical AβP deposits, usually in microvessels (arrowheads). Some of the microvessel deposits were associated with plaques (arrow). Antiserum A (1:250) to native AβP purified from AD cerebral cortex.* **D**: *Diffuse plaques in occipital cortex of normal aged human labeled by antiserum A (1:250) were morphologically similar to diffuse plaques in the monkey.* **E**: *Alzheimer's disease occipital cortex containing multiple AβP deposits in diffuse and compacted senile plaques and in blood vessel walls (arrowhead) were specifically labeled by antiserum A (1:250).* **F–H**: *Cortical plaques in cynomolgus monkey M1 stained by antibodies to:* **F**, *α1-antichymotrypsin (αACT, 1:250);* **G**: *complement factor, C3c (1:250); and* **H**: *complement factor, Clq (1:250). More than 50% of the cynomolgus plaques detected by AβP antibodies were labeled by αACT. A smaller percentage of plaques was detected by αC3c or αClq (Bars = 40 μ).*

plaques and neurofibrillary lesions of AD. Recent observations from several laboratories indicate that deposition of AβP in the form of 'diffuse' plaques can occur before or in the absence of detectable structural changes in surrounding neurons or glia.[10–13,47–49] Thus diffuse AβP deposits appear to represent the earliest histologic change yet detected in the brains of patient's with Down's syndrome or AD. Changes in the transcription, translation, or post-translational processing of βAPP are likely to play a role in the excessive AβP formation found in AD and Down's syndrome. Such changes are difficult to detect and study in postmortem human brain. Rats and mice have not been found to develop AβP-containing brain lesions with age. The data presented here, however, demonstrate that aged monkeys provide a highly relevant model for the study of β-amyloidosis in AD.

We have sequenced a full-length cDNA encoding the non–KPI-containing form of βAPP from cynomolgus monkey and found 100% amino acid homology to the human. Furthermore, sequencing of the KPI-containing insert of βAPP_{751} shows only a single conservative substitution (val for ala) at the last of the 56 amino acids in this exon, ie, not affecting the active site of the inhibitor. Within the additional 19-amino acid insert found in βAPP_{770}, we detected three substitutions in cynomolgus, suggesting less evolutionary pressure for conservation of this exon. The essentially complete homology of βAPP_{695} and βAPP_{751} in monkey and human contrasts with the 22 and 18 amino acid substitutions, respectively, found in mouse and rat βAPP_{695} and the 25 and 20 substitutions, respectively, found in mouse and rat βAPP_{751}.[38,42,37,50] These changes in the primary structure of rodent βAPP (see Figure 3) may alter post-translational modifications, particularly proteolytic cleavage sites, thereby making rodent βAPP less prone to release of the amyloidogenic AβP fragment. Furthermore there are three amino acid changes within rodent AβP itself (Table 1) that could affect its polymerization into cross-β–pleated sheet filaments[51] or its catabolism in brain.

The homologous primary structures of βAPP_{695} and

βAPP$_{751}$ in monkey and human deduced from their cDNAs are reflected in the immunoblot pattern of the polypeptides themselves. Human and monkey brain homogenates contain a complex group of 106- to 139-kd membrane-associated, full-length βAPP isoforms that are highly similar in the two species. Rat and mouse brain homogenates show a different profile. The rodent 116- and 139-kd bands, representing the N- and the N- plus O-glycosylated βAPP$_{751}$ forms, respectively, are very faint or absent. This is consistent with the reduction of βAPP$_{751}$ mRNA[42,43] and protein[43] reported in rodent brain. An additional, strongly immunoreactive protein at ~121 kd in rat and mouse brain homogenates is not detected in human and monkey brain. Although the nature of this KPI-minus βAPP isoform is currently unknown, the weak labeling in rodent compared with monkey brain of the 127-kd, N- plus O-glycoslylated βAPP$_{695}$ protein suggests that different post-translational modifications of rodent βAPP$_{695}$ may result in the 121-kd polypeptide. Similarly antibodies to βAPP$_{500-648}$, a region containing two consensus sequences for N-glycosylation,[1] fail to recognize βAPP in rat and mouse brain and rat PC12 cells. Deglycosylation experiments are needed to clarify whether carbohydrate side chains are responsible for this lack of αB5 immunoreactivity. These data support the hypothesis that differences in the primary structure of rodent βAPP result in changes in its proteolysis and other enzymatic modifications that could account in part for the absence of detectable AβP deposition in rodents. Differences in the expression of βAPP alternative transcripts[43] or in βAPP-modifying enzymes, including proteases, also may contribute to the lack of β-amyloidosis in rodents. These considerations have important implications for current attempts to produce a transgenic mouse showing AβP deposition.

In addition to full-length βAPP, we have detected cytosolic, C-terminal truncated isoforms ranging from ~95 to 125 kd in monkey brain (Figure 4C); similar bands are found in human tissue and fluids.[24,25,52] Monkey plasma contains a large βAPP$_{751}$ N-terminal fragment of ~125 kd (Figure 5) with identical immunoreactivities to that found in human plasma.[32] This soluble form of βAPP (ie, PN-II[26]) presumably contains the amino-terminal portion of AβP, as has been demonstrated immunochemically in human CSF[53] and by direct sequencing in the media of βAPP-transfected cells.[41] In contrast, the novel ~105-kd KPI-containing polypeptides we have detected in monkey plasma and some human plasma samples (Figure 5) may arise from an alternate cleavage amino-terminal to AβP that could leave the AβP intact as part of an ~20-kd C-terminal fragment. Some C-terminal βAPP antisera have detected an ~20-kd band in tissue homogenates.[23] Alternatively, the 105-kd fragment could derive from the alternate transcript, βAPP$_{563}$, which lacks the carboxy-terminal 208 residues of the βAPP$_{751}$ form.[44]

Regarding C-terminal–containing βAPP fragments, monkey brain contains membrane-associated 10- to 12-kd peptides reactive with all of our C-terminal antisera. We originally described a similar doublet of 10- and 12-kd proteins in human brain membrane fractions that contained the C-terminus and presumably part or all of the AβP region.[23] The 10-kd peptide has subsequently been shown to represent the stable C-terminal fragment retained in cells after constitutive βAPP cleavage and secretion, and it contains only the carboxyl-terminal portion of AβP.[33,41] The 12-kd peptide, which is of lower abundance, may derive from cleavage at a more amino-terminal site, resulting in a fragment with an intact AβP region; further study of the relevance of this peptide to the genesis of AβP can now be undertaken in the monkey.

Immunocytochemistry of cynomolgus cerebral cortex is consistent with the homology of monkey and human βAPPs demonstrated in the DNA and protein studies. Two of three 19-year-old monkeys had microvascular and plaquelike deposits of AβP closely resembling those of aged humans and patients with AD or Down's syndrome. Most of the plaques were spherical areas of finely granular immunoreactive material similar to the diffuse AβP deposits abundantly present in AD brain.[12,13] Far fewer plaques showed compacted centers characteristic of the so-called 'mature' or 'classical' senile plaques of humans, perhaps because the cynomolgus brains we obtained were only from late middle-aged animals. Similarly the 19-year-old animals we studied had very little evidence of silver-positive dystrophic neurites surrounding the AβP deposits. Thioflavin S detected a majority of plaques, whereas the less sensitive Congo red stain labeled only a few. This staining profile is consistent with that used to define preamyloid or diffuse plaques in humans. In accord with previous studies of aged monkey brain,[6,8] no neurofibrillary tangles or neurites reactive with antibodies to PHF were detected.

Another shared property of human and cynomolgus plaques is the presence of certain β-amyloid-associated proteins, ie, αACT and the complement factors, C1q and C3c. α_1-Antichymotrypsin has previously been shown in humans to be associated with β-amyloid and not other types of amyloid,[45,54] and has been localized to the AβP deposits of aged rhesus monkeys.[9] Complement C1q and C3c have been immunochemically detected in human plaques.[46,55] In our study, only a subset of cynomolgus AβP deposits showed reaction with antibodies to αACT and the two complement components. This finding is again consistent with analyses of human plaques, which suggest that only ~50% of neuritic plaques and ~30% or 10% of diffuse plaques in AD patients are labeled by αACT or complement antibodies, respectively.[56]

The very close similarity of monkey and human βAPP and AβP that we document here indicates that aged non-

human primates undergo a proteolytic processing of βAPP into stable AβP fragments that is qualitatively highly similar to that occurring in early stages of Down's syndrome, AD, and cerebral aging in humans. Cynomolgus monkeys, and perhaps other shorter-lived primates, thus provide a compelling animal model of a principal and early pathogenetic feature of AD. Several kinds of questions can be approached in non-human primates that would be difficult to study rigorously in postmortem AD brain. For example, the sequence of cellular responses (eg, microglial activation, astrocytosis, neuritic dystrophy) that apparently follow the formation of diffuse AβP deposits can be assessed over time in monkeys maintained for other experimental reasons and perfused at intervals during middle and late life. Also, sensitive analyses of βAPP mRNA levels in fresh monkey brain using S1 nuclease protection assays[57] or *in situ* hybridization will help clarify the currently conflicting data about which, if any, βAPP transcripts are expressed differently during cerebral β-amyloidosis. Fresh monkey tissue also would be an excellent source for purification of candidate proteases involved in the alternative processing of βAPP that generates an intact AβP-containing fragment. Finally the focal microinjection of AβP (or βAPP fragments containing AβP) into the association cortex of the monkey, a species that undergoes spontaneous age-related β-amyloidosis, could lead to a manipulable model of the cellular and molecular changes underlying senile plaque formation in AD. The latter experiment is of particular interest in light of recent studies demonstrating that synthetic AβP may induce trophic[58–60] or toxic[60] responses in cultured rat neurons.

Acknowledgments

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