Massive CA1/2 Neuronal Loss with Intraneuronal and N-Terminal Truncated Aβ42 Accumulation in a Novel Alzheimer Transgenic Model

Caty Casas,* Nicolas Sergeant,† Jean-Michel Itier,‡ Véronique Blanchard,*, Oliver Wirths,§ Nicolien van der Kolk,qli Valérie Vingtdeux,† Evita van de Steeg,q Günther Ret,‡ Thierry Canton,*, Hervé Drobecq,‰ Allan Clark,‡ Bruno Bonici,* André Delacourte,‡ Jesús Benavides,* Christoph Schmitz,‡ Günter Tremp,‡ Thierry Canton,* Hervé Drobecq,† Patrick Benoit,* and Laurent Pradier*

From the Departments of Central Nervous System/Alzheimer Disease* and Functional Genomics,‡ Aventis-Pharma Paris Research Center, Vitry sur Seine, France; INSERM U422,† Groupe Vieillissement Cérébral et Dégénérescence Neuromale, Équipe Proteomique, Lille, France; UMR8525,¶ Centre National de la Recherche Scientifique, Institut de Biologie de Lille, Université de Lille II, Lille, France; the Department of Psychiatry,§ Division of Neurobiology, Saarland University, Homburg/Saar, Germany; and the Department of Psychiatry and Neuropsychology,¶ Division of Cellular Neuroscience, Maastricht University, Maastricht, The Netherlands

Alzheimer’s disease (AD) is characterized by a substantial degeneration of pyramidal neurons and the appearance of neuritic plaques and neurofibrillary tangles. Here we present a novel transgenic mouse model, APP50-PS1KI that closely mimics the development of AD-related neuropathological features including a significant hippocampal neuronal loss. This transgenic mouse model carries M233T/L235P knocked-in mutations in presenilin-1 and overexpresses mutated human amyloid-β (Aβ) precursor protein. Aβ42 is the major form of Aβ species present in this model with progressive development of a complex pattern of N-truncated variants and dimers, similar to those observed in AD brain. At 10 months of age, an extensive neuronal loss (>50%) is present in the CA1/2 hippocampal pyramidal cell layer that correlates with strong accumulation of intraneuronal Aβ and thioflavine-S-positive intracellular material but not with extracellular Aβ deposits. A strong reactive astrogliosis develops together with the neuronal loss. This loss is already detectable at 6 months of age and is PS1KI gene dosage-dependent. Thus, APP50-PS1KI mice further confirm the critical role of intraneuronal Aβ42 in neuronal loss and provide an excellent tool to investigate therapeutic strategies designed to prevent AD neurodegeneration. (Am J Pathol 2004, 165:1289–1300)

Neurodegenerative diseases are morphologically characterized by loss of vulnerable neuronal subpopulations of the central nervous system. Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by extensive neuronal degeneration and the development of neuritic amyloid plaques and neurofibrillary tangles. Neuronal and synaptic losses in AD are correlated with dementia and occur in specific brain areas involved in memory processing.1–3 The core of senile plaques is mainly composed of a heterogeneous amalgam of amyloid-β (Aβ) peptides comprising the full-length, N-terminal truncated, and posttranslationally modified isovariants. Aβ peptides are normally generated by successive proteolysis of the amyloid precursor protein (APP), a large transmembrane glycoprotein that is initially cleaved by the β-site APP-cleaving enzyme 1 (BACE1) and subsequently in the transmembrane domain by γ-secretase.4 The γ-secretase is a multimeric protein complex that includes presenilin (PS), nicastrin, Aph-1, and Pen-2.5,6 Genetic evidences also support a central role for APP processing in AD neurodegeneration.7

Gene-targeted and transgenic mice have proven valuable for modeling various aspects of AD amyloid pathology and associated cognitive changes.8 However, no mouse model recapitulates the complete human neuro-pathological spectrum. In particular, there has been little...
PS1KI were bred with APPSL mice, which overexpress and resulted in viable and fertile animals. The mixed 129SV and mixed 129SV-C57BL/6 genetic backgrounds gous allele. The PS1KI line was established in both pure were established and referred to as PS1KI. For gene sion of the mutant 230-bp Ps1 allele was determined by Southern hy-プロブインク M146L transgenic mice that occurred in 17-month-old animals. 10 The paucity of neuronal loss in AD transgenic models has been recently reviewed. 11 Here we describe the develop-ment of a novel double-transgenic mouse model, APP<sup>PSL</sup>-PS1KI carrying four FAD-linked mutations, which develop a massive hippocampal neuronal loss as early as 6 months of age. We first generated a new PS1 knock-in (PS1KI) mouse model carrying the M233T and L235P mutations into the endogenous presenilin locus that was then crossed with transgenic APP<sup>PSL</sup> mice. 12 APP<sup>PSL</sup>-PS1KI mice develop an accelerated amyloid deposition, as reported for other similar bигenic mice 9 and present a complex pattern of Aβ<sub>1-42</sub> isovariants highly similar to that described in AD brain. 13 Quite uniquely, APP<sup>PSL</sup>-PS1KI mice display an early and massive neuronal loss in the CA1/2 pyramidal cell layer preceded by the presence of abundant intraneuronal Aβ peptide and in-tracellular thioflavin-S-positive material. Strong astro-gliosis also develops in the affected pyramidal layer.

**Materials and Methods**

**Generation of the PS1 Mutant Knock-In Mouse Line**

A PS1 knock-in mouse line was derived using a two-step mutagenesis strategy based on the creation of a target-ing vector that bears base changes in the coding region at codons M233T and L235P and surrounding introns of the Ps1 gene, 14, 15 as described in Figure 1. Presence of the mutated Ps1 allele was determined by Southern hy-bridization of EcoRl-restricted genomic DNA with a 230-bp Ps1 probe indicated in Figure 1A, bottom dia gram. Five chimeric mice exhibited germline transmission of the mutant Ps1 allele. Homozygous (Ho) mice were established and referred to as PS1KI. For gene dosage analysis, PS1KI (He) designates the heterozygous allele. The PS1KI line was established in both pure 128SV and mixed 129SV-C57BL/6 genetic backgrounds and resulted in viable and fertile animals. The mixed PS1KI were bred with APP<sup>PSL</sup> mice, which overexpress human APP<sub>751</sub> carrying the London (V717I) and Swedish (K670N/M671L) mutations under the control of the Thy1 promoter 12 on a mixed C57BL/6-CBA genetic back-ground. All animals used for this study, including non-transgenic littermate controls, were generated from the same founders (APP<sup>PSL</sup> and PS1KI mice) in two generations and have statistically the same genetic background: C57BL/6 50%-CBA 25%–129SV 25%. When present, the APP transgene was heterozygote.

All experiments on animals were performed in compli ance with and following the approval of the Aventis Ani-imal Care and Use Committee, in accordance with stand-ards for the care and use of laboratory animals (Centre National de la Recherche Scientifique-Institute of Labo-ratory Animal Resources) in accordance with French and European Community rules.

**Antibodies**

For Western blotting or immunohistochemistry analysis the following primary antibodies were used: anti-PS1, mAb 1563 (Chemicon, Souffliewyersheim, France); anti-tubulin (Sigma, Saint Quentin Fallavier, France); polyclonal rabbit anti-mouse GFAP (DAKO, Glostrup, Denmark), polyclonal antiserum 23850 against APP, 16 anti-serum APP-CTF C17, 17 and rabbit polyclonal antibody against Hspa5 (alias BiP) (SPA-826; Stressgen/TEBU, Perray en Yvelines, France); 6E10 monoclonal antibody directed against hu-man Aβ<sub>42</sub>–15 (Senetek/Biovalley, Marne la Vallée, France) biotinylated 4G8 monoclonal antibody against human Aβ<sub>17-24</sub> (Senetek); 692 rabbit polyclonal antiserum against human Aβ (generous gift from Gerd Multhaup, Freie Uni-versität Berlin, Berlin, Germany); G2-10 monoclonal anti-body to the C-terminus of Aβ<sub>40</sub> (Genetics Company, Schlieren, Switzerland); 22F9, 18 G2-13 (Genetics Com-pany), and 21F2 (Athena Neurosciences, San Francisco, CA) 15 monoclonal antibodies to the C-terminus of Aβ<sub>42</sub>.

**Western Blot Analysis**

Frozen half brains (minus cerebellum) were homoge-nized in 10 vol of buffer containing 4 mmol/L Tris, pH 7.4, 0.32 mol/L sucrose, and a proteinase inhibitor cocktail (Complete; Roche Diagnostics, Myelan, France). Equal amounts of proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and trans-ferred to nitrocellulose. Membranes were revealed with primary (anti-PS1 C-term or anti-APP C-term) and corre-sponding horseradish peroxidase-conjugated secondary antibody (New England Biolabs/Ozyme, Montigny, France) followed by enhanced chemiluminescence (Pierce, Asnières, France). Membranes were either ex-posed to Hyperfilm (Amersham, Saclay, France) or digi-talized and analyzed with a GeneGnome 16-bit charge-coupled device video camera and Genetools software (Syngene). The analysis of the full-length and the APP carboxy-terminal fragments (APP-CTFs) was performed, as previously described. 17

**Aβ Electrochemiluminescence Immunoassay**

Aβ peptides were detected in brain homogenates by electrochemiluminescence assay using different anti-Aβ antibodies and Origen M8 Analyzer (IGEN Europe Inc.), as previously described. 12 Briefly, the ruthenylated 4G8 antibody (Aβ epitope 17-24) was used in combination with biotinylated 6E10 antibody (Aβ epitope 5-15) to de-tect total Aβ. To specifically measure Aβ<sub>42</sub> species, the 6E10 antibody was replaced by 22F9. Therefore, the Aβ<sub>42</sub> assay can detect N-terminal truncated forms of Aβ whereas the total Aβ assay does not.
Two-Dimensional Gel Electrophoresis

Brain homogenates were centrifuged at 100,000 × g for 1 hour at 4°C. The pellet was treated with 1 vol of pure formic acid and sonicated. Formic acid was evaporated under nitrogen and the protein pellet homogenized in two-dimensional lysis buffer (10 mmol/L Tris, 2 mol/L thiourea, 7 mol/L urea, 4% Triton X-100, 20 mmol/L di-thiothreitol, 0.4% Pharmalytes, pH 4 to 6.5). Protein concentration was quantified using the 2D Quant protein quantification kit (Amersham). One hundred μg of protein were equilibrated in a ReadyStrip IPG strip, pH 4 to 7 (Bio-Rad, Marnes-la-Coquette, France) and two-dimensional gel electrophoresis and Western blotting were performed, as previously described. Peptide identity was confirmed by mass spectrometry as described, see supplemental Figure S1 available at www.anipathol.org.

Immunohistochemistry and Histology

Histopathological analysis was in part performed on hemi-brain from single APPSL or PS1KI and bigenic mice as well as littermate nontransgenic controls at 2, 6, and 10 months of age (four to eight mice per genotype). Mice were sacrificed by cervical dislocation. After postfixation for 1 week in solution containing 4% paraformaldehyde and 0.1 mol/L phosphate-buffered saline (PBS), pH 7.5, the hemi-brains were stored in 18 hours in PBS containing 20% sucrose and finally frozen at −30°C. Sagittal cryostat floating sections (25 μm-thick) were preincubated in blocking buffer (10% normal goat serum in PBS) and then incubated in 0.03% hydrogen peroxide at 19°C for 30 minutes and then in primary antibody solution (biotinylated 4G8 1/200 or anti-Hsap5 1/100). For Hsap5 immunostaining, an incubation (1 hour) with the biotin-coupled anti-rabbit IgG antibody (1/400; Vector Laboratories, Oxford, UK) was performed before incubation with avidin-horseradish peroxidase (Vector Laboratories). Diaminobenzidine tetrahydrochloride was used as a substrate for the peroxidase. Immunostained sections were exhaustively cut into series of 30-μm-thick frontal sections on a cryostat. One series of every tenth section per animal was stained with cresyl violet as described. Briefly, the left brain halves of the transcardially perfused mice were postfixed in 4% buffered formalin at 4°C and were then cryoprotected by immersion in 30% sucrose in Tris-buffered saline at 4°C overnight. Afterward, brain halves were quickly frozen and stored at −80°C until further processing. Hemi-brains were exhaustively cut into series of 30-μm-thick frontal sections on a cryostat. One series of every tenth section per animal was stained with cresyl violet as described. On all sections showing the hippocampus, the pyramidal cell layer CA1/2 was delineated. Total numbers of neurons were investigated with the Optical Fractionator (Micro Bright Field; Williston, VT). The details of the counting procedure were as follows. Objective used for delineating the pyramidal cell layer CA1–2, ×10; objective used to count the pyramidal cells, ×100; base and height of the unbiased virtual counting spaces used to count neurons, 400 μm² and 4 μm, respectively; distance between the unbiased virtual counting spaces in orthogonal directions, x and y, 75 μm; measured actual average section thickness after histological processing, 8.0 μm; average sum of unbiased virtual counting spaces used per animal, 299; average sum of neurons counted per animal, 871; average predicted coefficient of error of the estimated total numbers of neurons, 0.034 (for details see Schmitz and Hof). Differences between groups were tested with analysis of variance followed by post hoc Bonferroni’s multiple comparison tests for pairwise comparisons. Statistical significance was established at P < 0.05. All calculations were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA).

Results

Generation of APPSL/PS1KI Transgenic Mice and Analysis of APP Metabolism

A PS1 knock-in mouse model carrying two FAD-linked mutations (PS1M233T and PS1L235P) in the mouse endogenous presenilin-1 gene has been generated (Figure 1).
Large Heterogeneity of N-Terminally Truncated Aβ<sub>x-42</sub> Variants in APP<sup>Sl</sup>-PS1KI Mice

Because the total Aβ quantification assay does not detect most N-truncated forms of Aβ, unlike the Aβ<sub>x-42</sub> assay, an Aβ<sub>x-42</sub>/total Aβ ratio value greater than unity suggested the presence of N-truncated species. We therefore analyzed the Aβ<sub>x-42</sub> biochemical characteristics in the APP<sup>Sl</sup>-PS1KI mouse brain using two-dimensional gel electrophoresis. In young bigenic mice (2.5 months of age), the proteomic pattern of Aβ<sub>x-42</sub> accumulation and the Aβ<sub>x-42</sub>/total Aβ ratio. By 10 months of age, total Aβ levels were similar in APP<sup>Sl</sup> and APP<sup>Sl</sup>-PS1KI mice.
plexity of the pattern of Aβ isovariants increased with stronger spot intensities and new N-terminal truncated forms progressively appearing. At 4 months of age, an additional spot (pl 5.8) appeared corresponding to human Aβx-42-truncated forms at position 2 and 3. A more acidic Aβx-42 isovariant (pl 4.3) also appeared, which could correspond to mouse Aβ1-42. At 6 months of age, we detected additional spots at pl 5.9 and 6.9 corresponding to the pyroglutamate modified N-terminal truncated form of Aβ at position 3 (Aβ(N3(pE)) and to the Aβx-42 species truncated at positions 12, 13, or 14, respectively (Figure 3). It should be noted that Aβ(N3(pE)) only appeared 2 months after the corresponding nonmodified Aβ3–42 variant. In 10-month-old mice, we observed an identical pattern with stronger intensities. By contrast, in APPSL mouse brain (10 months of age) with the same total Aβ levels as APPSLPS1KI mice, only very limited levels of Aβx-42 N-terminal truncated isovariants at positions 2, 3, 4, and 5 were detected (Figure 3). According to the characterization of Aβx-42 species performed in the human brain of patients with AD13 and confirmed by mass spectrometry analysis (see supplemental Figure S1), the identity of human Aβx-42 species (hAβ) is summarized at the bottom. Isoelectric points were determined using internal standards. Spot at pl 4.3 putatively corresponds to the endogenous mouse Aβ1-42 (8 kd). Arrowheads point (down leftwards) to the weak staining of spots at pl 5.8 and 6.3. Monomeric species of Aβx-42 are visualized at 4 kd, whereas dimeric species are resolved at 8 kd.

truncated isovariants and abundant oligomers in APPSLPS1KI mouse brain closely mimics the situation observed in AD pathology.

Neuronal Loss and Abundant Intracellular Aβ Accumulation in the Brain of APPSLPS1KI Mice

As expected from our biochemical studies, immunohistochemical detection of Aβ peptide using 4G8 antibody confirmed an accelerated rate of Aβ peptide deposition in APPSLPS1KI brain parenchyma. No Aβ deposits were detected in nontransgenic mice or in PS1KI mice. We detected the first signs of Aβ deposition in APPSLPS1KI mouse brain at 2.5 months of age compared to 6 months in APPSL mice as previously described (data not shown). At 6 months, we found widespread and numerous round compact Aβ deposits within the cortical, hippocampal, and thalamic areas of APPSLPS1KI mice whereas in age-matched...
APP<sub>SL</sub> mice only very few deposits were present and restricted to the subiculum and deeper cortical neuronal layers (Figure 4). In older mice (10 months of age) extracellular Aβ deposit distribution, density, and size were increased in the brains of both models that were reaching similar plaque load. However, we noted differences in the size of Aβ deposits that were more compact, smaller, and more numerous in APP<sub>SLPS1KI</sub> mice than in APP<sub>SL</sub> mice (Figure 4B). Similar to other APP transgenic models, astrocytic and microglial activation were present around amyloid plaques (data not shown) together with abundant dystrophic neurites (evidenced by characteristic periplaque APP immunostaining, Figure 5C).

The accelerated Aβ peptide deposition and the presence of abundant Aβ<sub>42</sub> prompted us to evaluate neuronal survival in APP<sub>SLPS1KI</sub> mouse brain. Microscopic analysis of cresyl violet-stained brain sections from APP<sub>SL</sub> or PS1KI mice showed no gross alteration compared to age-matched nontransgenic mice up to 10 months of age (Figure 5A). Strikingly, detailed analysis of the hippocampal CA1-3 subfields and of the dentate gyrus showed that APP<sub>SLPS1KI</sub> mice developed a marked reduction of the hippocampal pyramidal cell layer thickness that was particularly prominent in the CA1/2 region at 10 months of age in both males and females (seven of seven bigenic mice, four males and three females; Figure 5, A and B). A marked neuronal loss in 10-month-old APP<sub>SLPS1KI</sub> mice was also observed with a second histological marker (methyl green dye) and with heat shock protein A5 (Hspa5, alias Bip) immunolabeling (data not shown). APP immunostaining in the brain of 2- and 10-month-old APP<sub>SLPS1KI</sub> mice further confirmed the age-dependent cell loss in CA1/2 hippocampal subfields (Figure 5C) and indicated that the neuronal loss correlated with a particularly high expression in CA1/2 neurons of the human APP transgene driven by the Thy-promoter (Figure 5C) as previously reported. No overt neuronal loss was observed either in the dentate gyrus or CA3 subfield at this age.

Neuronal loss was quantitatively assessed by high-precision design-based stereology. We found a substantial loss of pyramidal cells within hippocampal layer CA1/2 in 10-month-old APP<sub>SLPS1KI</sub> mice compared to 2-month-old APP/PS1KI mice (−49%; P < 0.001, in both sexes) as well as compared to 2-month-old and 10-month-old APP<sub>SL</sub> mice (−54% (P < 0.01) and −53% (P <
0.01), respectively] and compared to 2-month-old and 10-month-old PS1KI mice [56% (P < 0.001) and 59% (P < 0.001), respectively; Figure 6]. There was a striking difference between the substantial neuron loss in the hippocampal CA1/2 field (50%) and the almost lack of amyloid plaques within the CA1/2 pyramidal cell layer.

Interestingly, macroscopic analysis indicated that some CA1/2 neuronal cell loss was present as early as 6 months of age in the brains of APPSLPS1KI female mice (n = 5 of 5; see also Figure 8B) but not in three males suggesting that females were affected earlier than male mice. Furthermore, the neuronal loss was also observed in 15-month-old APPSLPS1KI heterozygous (He) mice, confirming the existence of a PS1KI gene-dosage effect (data not shown). The disruption of deep cortical layers suggests that the neuronal loss might extend well beyond the CA1/2 region, but further analysis will be necessary to document it throughout the brain.

Analysis of adjacent brain sections stained with Aβ antibodies did not demonstrate an obvious relationship between CA1/2 cell death and extracellular Aβ peptide deposition in APPSLPS1KI mice (Figure 7A and Figure 8). Whereas neuronal loss was observed throughout the entire length of the CA1/2 pyramidal layer, most extracellular Aβ deposits were essentially sparsely distributed on either side of the CA1/2 subfield (ie, in the stratum radiatum, lacunosum-molecular, and oriens) but not within the pyramidal neuronal layer. Moreover, the finding that 10-month-old APPSL mice with, for some, a hippocampal Aβ deposit load quite similar to APPSLPS1KI (Figure 7A, left) did not display neuronal loss in CA1/2 subfield further suggests that cell death in APPSLPS1KI mice is not apparently linked to the deposition of extracellular Aβ peptides. By contrast, the neuronal loss in the CA1/2 region was closely correlated with marked intraneuronal Aβ immunostaining that was present as early as 2 months of age (Figure 7A and Figure 8A). Within the pyramidal neuronal layer, both the density and the intensity of Aβ peptide-immunostained granular bodies were higher in APPSLPS1KI mice compared to APPSL mice (Figure 7A) and much stronger in the CA1/2 subfield in agreement with APP transgene expression. A very significant astroglisis developed in the area of strong intraneuronal Aβ immunoreactivity and neuronal loss (Figure 7B). High magnification in 4-μm-thin tissue sections using a large array of pan-Aβ (Figure 8; A to C and F) and Aβ42 (Figure...
The APPSL-PS1KI transgenic mouse model therefore develops an early neuronal loss within the hippocampal CA1/2 pyramidal cell layer, which correlates with the presence of abundant intraneuronal Aβ peptide and intracellular thioflavine-S-positive material.

**Discussion**

The generation of transgenic mouse models based on mutant APP and Ps1 genes have enabled major advances in our understanding of the amyloid cascade hypothesis.23 These models recapitulate several features of AD, including amyloid plaques with dystrophic neurites, synaptic dysfunction and behavioral deficits but fail to develop extensive neuronal death. In view of the much higher sensitivity to Aβ neurotoxicity in older versus younger primates,24 the lack of neuronal loss in transgenic mice with a high amyloid burden was attributed to their short life span. Here we describe a novel transgenic mouse model, APPSL-PS1KI, which carries two PS1 knocked-in and two APP FAD-linked mutations. In addition to the expected acceleration of extracellular Aβ peptide deposition, the APPSL-PS1KI model develops an age-dependent massive neuronal loss in the hippocampus, a structure involved in learning and memory processes. Specific neurodegeneration in the hippocampal CA1 subfield and entorhinal cortex is an early event in the AD pathology that correlates directly with the severity of the disease.1 Interestingly, APPSL-PS1KI mice show extensive neuronal loss in the CA1/2 subfield at 10 months of age in both male and female mice with detection as early as 6 months in female mice. In this model, the neuronal loss is definitely biased by the APP transgene expression pattern (very high expression in CA1/2 but not in CA3) but is likely more widely distributed with further aging, especially in subiculum and cortical regions (see below). Additional stereological analysis will be necessary to further document neuronal loss in such areas as entorhinal cortex and other cortical regions. The CA1/2 neuronal loss in APPSL-PS1KI mice extends homogeneously throughout the pyramidal layer and is not related to the local proximity of extracellular Aβ peptide deposits. It is therefore distinct from the neuronal loss observed in most other transgenic models, which has been limited to the close vicinity of Aβ deposits.9 Previously, APP23 transgenic mice were shown to develop a moderate loss of CA1 neurons in older animals (14 to 18 months of age) in close correlation with amyloid plaque load.25 We have also recently described a significant neuronal loss extending beyond amyloid plaques but less pronounced and again in significantly older (>17 months of age) bigenic APPSL-PS1KI mice using stereological methods.10,11 By contrast, the early neuronal loss in the present APPSL-PS1KI mice is very prominent as early as 6 to 10 months of age. Remarkably, the neuronal loss distribution closely parallels the strong intraneuronal Aβ immunostaining and the accumulation of intracellular thioflavine-S-positive material present throughout the pyramidal cell layer but does not correlate with extracellular deposits. Strong astrogliosis is also occurring in proximity of Aβ-

Figure 7. Relationship between neuronal loss and intraneuronal Aβ peptide accumulation in the CA1/2 subfield. Representative photomicrographs of Aβ immunostaining (4G8 antibody) on sagittal brain sections of 10-month-old APPSL (left) and APPSL-PS1KI (right) mice. Two different mice per group are illustrated. A: Within the CA1/2 hippocampal subarea Aβ deposits are mainly present on either side of rather than within the pyramidal cell layer in both APPSL and APPSL-PS1KI. Both the intensity and frequency of granular Aβ immunostaining within the remaining CA1/2 pyramidal cells were increased in APPSL-PS1KI mice compared to APPSL mice. Arrows indicate the localization of the CA1/2 neuronal cell layer. B: Astrocytic staining against GFAP (blue) and Aβ staining (antiserum 692, brown) in CA1 of a 6-month-old APPSL (left) and APPSL-PS1KI (right) 4-μm brain sections. Note the astrogliosis in the pyramidal cell layer in APPSL-PS1KI mice. Scale bars: 100 μm (A), 50 μm (B).
positive neurons. Both intraneuronal Aβ and thioflavine-S-positive material stainings preceded neuronal loss. It will be important in future experiments to confirm that the thioflavine-S-positive material is indeed Aβ as suggested by the present results. There is growing evidence that intraneuronal Aβ accumulation is important for the pathogenesis of both AD, and Down syndrome, with one report of thioflavine-S-positive material in AD.

Intraneuronal Aβ accumulation (but not thioflavine-S-positive) has also been documented in several amyloid transgenic mouse models, including our previous APPSLPS1KI model, in association with neuronal stress and synaptic alterations. In addition, it has been shown that microinjection of Aβ42, but not Aβ40, into cultured human primary neurons is drastically more toxic than its extracellular application. As demonstrated by direct immunochemistry and because Aβ42 is the predominant Aβ isoform produced in APPSLPS1KI mice, the intraneuronal pool of Aβ42 in the pyramidal cell layer most likely contributes to the neuronal loss observed, especially if present in an aggregated conformation as suggested by the thioflavine-S staining. Alternatively, Aβ42 oligomers are highly abundant in the APPSLPS1KI brain and might also participate to the CA1/2 neuronal loss in APPSLPS1KI mice. Indeed, Kim and colleagues reported a selective neurotoxicity in the CA1 area and entorhinal cortex promoted by Aβ oligomers or amyloid diffusible ligands. Both the intraneuronal Aβ and the intracellular thioflavine-S stainings extended to cortical regions, strongly suggesting that neuronal loss in other brain areas such as entorhinal cortex could be detected in APPSLPS1KI mice of older age.

Two distinctive biochemical features of the Aβ42 peptide accumulating in APPSLPS1KI brain could contribute to the observed phenotype. First, it is remarkable that Aβ42 is the major form accumulated with a ratio of Aβ42/total Aβ close to 1 compared to a ratio of 0.2 to 0.3 in the APPSL mice. Certainly, this ratio might be slightly overestimated in aged mice because of the presence of N-terminal truncated Aβ species (not detected in the total Aβ assay) observed from 4 months of age onwards, but not in 2.5-month-old mice (ratio value of 0.85). In comparison, the same APPSL mouse line bred with standard overexpressing PS1M146L transgenic mice leads to an Aβ42/total Aβ ratio of only 0.3 to 0.4, similar to the range of values reported for a large number of other APP-based transgenics, even with PS1 knock-in muta-
tions.21,35 This might result from the specific combination of double PS1 and APP mutations. In addition, the mutant form of PS1 is expressed at physiological levels by all cells (PS1 knock-in) in the present model unlike some of the previously characterized PS1 knock-in mice that demonstrated a lower expression of the mutant allele.23,24 Thus, the vast unbalanced mix in favor of \( A\beta_{42} \) is a key factor for acceleration of \( A\beta \) aggregation and would likely contribute to the presence of intracellular thioflavine-S-positive material and the subsequent neuronal toxicity in APP\(^{SL}\)-PS1KI mice. An additional difference with our previously described bigenic APP\(^{SL}\)-PS1\(^{M146L}\) mice is that the levels of APP C-terminal fragments are increased in APP\(^{SL}\)-PS1KI whereas they are decreased in APP\(^{SL}\)-PS1\(^{M146L}\) mice (unpublished data). The latter is consistent with the observed large overexpression of mutant PS1\(^{M146L}\) fragments (and therefore of \( \gamma \)-secretase activity) whereas in PS1 knock-in mutants, the increase in APP C-terminal fragments could be indicative of a partial loss of \( \gamma \)-secretase activity. Intriguingly, PS1 FAD mutations have also been presented as partial loss-of-function mutations36 and recently the conditional PS1 knockout transgenic mice has been shown to develop major neuronal loss as in the present report.35 It is tempting to speculate that in PS1 mutant overexpressing transgenic, the partial loss of function is offset by the large PS1 overexpression leading only to the relative increase in \( A\beta_{42} \) but not to neuronal loss. In all instances, the PS1-mutant KI model is by construction a better simulation of the human PS1 mutant FAD.

Another possible contributing factor to neuronal loss in APP\(^{SL}\)-PS1KI is the presence of a highly heterogeneous population of N-terminal truncated \( A\beta_{42} \) forms in brain. Truncated \( A\beta \) peptides at the N-terminus are known to aggregate more readily and to accumulate in the brain of sporadic AD patients, in early onset FAD patients, especially in PS1 mutation carriers,38,39 and in Down syndrome brain.40–42 The major forms of N-truncated \( A\beta \) species in AD senile plaques are those modified by cyclization at residues 3 and 11 with pyroglutamate.38,43 In APP\(^{SL}\)-PS1KI mice, N-truncated \( A\beta_{42} \) species appearance follows \( A\beta_{1-42} \) accumulation with subsequent detection of modified forms, such as \( A\beta_{3-42} \) at an age with the first signs of hippocampal neuronal loss. The time sequence suggests that \( A\beta \) N-terminal truncations and modifications are temporally related to plaque maturaion. However, in APP\(^{SL}\) mice, with a similar amyloid burden as APP\(^{SL}\)-PS1KI mice (10 months of age), N-truncated forms of \( A\beta \) are far less abundant, indicating that they do not simply result from postdeposition N-terminal exoproteolysis. This data indicates that PS1 mutations not only affect the specificity of the \( A\beta \) peptide cleavage at its C-terminus, as part of the \( \gamma \)-secretase complex, but could also alter N-terminus cleavage. We cannot exclude the possibility that N-terminal truncated \( A\beta \) forms could result from de novo alternative \( \beta \)-cleavages. BACE1 is the main \( \beta \)-secretase cleaving APP at positions 1 or 11 of the \( A\beta \) peptide.44–46 Because interactions between BACE1, PS1, and the \( \gamma \)-secretase complex have been recently reported,47,48 PS1 mutations could induce, by altering BACE1 selectivity or recruiting other \( \beta \)-secretases, an array of different truncated \( A\beta_{x-42} \) forms, as previously suggested by Russo and colleagues.39 Altogether, the complex pattern of \( A\beta \) N-terminal truncated forms in APP\(^{SL}\)-PS1KI mice closely resembles that found in AD brain13 and represents the first report of such species in APP-based transgenic models. Because these \( A\beta \) species aggregate more readily and are more toxic, they might play a key role in the neurotoxicity observed in this model. The APP\(^{SL}\)-PS1KI mice could enable a further characterization of the process whereby \( A\beta \) truncated forms are generated and a further elucidation of their pathological role.

In summary, APP\(^{SL}\)-PS1KI is the first transgenic AD model to our knowledge showing early onset and severe neuronal loss. The neuronal loss is correlated with the presence of abundant intraneuronal \( A\beta \) and intracellular thioflavine-S-positive material rather than extracellular \( A\beta \) deposits. \( A\beta_{1-42} \) is the major \( A\beta \) form produced in this model with progressive appearance of N-truncated and dimeric species. The present data add further evidence for a pathological role of \( A\beta_{42} \) species, especially the \( A\beta_{x-42} \) intraneuronal pool, which could therefore represent a prime target for therapeutic intervention. Additionally, the massive neuronal loss observed in an APP-based transgenic model provides a yet-missing link between \( A\beta \) peptide and neuronal toxicity in vivo in strong support for the \( A\beta \) peptide hypothesis of AD, but further highlighting that amyloid plaques might not be a critical factor. The relevance of this multi-FAD mutant transgenic model to sporadic AD remains to be confirmed. However, the major involvement of intraneuronal \( A\beta \) has been previously highlighted in AD26,27 and \( A\beta_{42} \) levels are strongly increased after head trauma, a major AD environmental risk factor. Similarly, the N-terminal truncated forms of \( A\beta_{42} \) have first been detected in sporadic short-term AD cases.13 The multiple mutations in the present model are likely critical to recapitulate in a few months a pathological process taking decades in man. In conclusion, APP\(^{SL}\)-PS1KI mice represent a significant novel AD model and a unique tool to investigate therapeutic strategies designed to prevent AD-related neuronal death.

Acknowledgments
We thank Zakia Bouaiche and Antoine Ghestem for technical assistance, Dominique Santier-Baron for discussion, Dr. Thomas Rooney for detailed editing of the manuscript, Dr. Charles Babinet (Pasteur Institute, France) for kindly providing CK35 ES cells, and Dr. Campion for early communication of novel PS1 FAD mutations.

References


