Reduced β-Amyloid Production and Increased Inflammatory Responses in Presenilin Conditional Knock-out Mice*

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Mutations in presenilins (PS) 1 and 2 are the major cause of familial Alzheimer’s disease. Conditional double knock-out mice lacking both presenilins in the postnatal forebrain (PS cDKO mice) exhibit memory and synaptic plasticity impairments followed by progressive neurodegeneration in the cerebral cortex. Here we further investigate the molecular events that may underlie the observed phenotypes and identify additional neuropathological markers in the PS cDKO brain. Enzyme-linked immunosorbent assay analysis showed reduced levels of the toxic β-amyloid (Aβ) peptides in the cerebral cortex of PS cDKO mice. Interestingly, the reduction in Aβ40 and Aβ42 peptides is similar in PS1 conditional knock-out and PS cDKO mice. We further examined the gene expression profile by oligonucleotide microarrays in the PS cDKO cerebral cortex and found that a high number of genes are differentially expressed, most notably a group of up-regulated inflammatory genes. Quantitative real-time reverse transcription PCR and Western analyses confirmed the elevated levels of glial fibrillary acidic protein, complement component C1q, and cathepsin S, up-regulation of which has been associated with inflammatory responses in various neurodegenerative processes. Immunohistochemical analysis revealed that the increase in complement component C1q is confined to the hippocampal formation, whereas glial fibrillary acidic protein and cathepsin S are up-regulated throughout the entire neocortex and hippocampus. In addition, strong microglial activation occurs in the hippocampus and the deeper cortical layers of PS cDKO mice. These results indicate that the memory impairment and neurodegeneration in PS cDKO mice are not caused by Aβ accumulation and that loss of PS function leads to differential up-regulation of inflammatory markers in the cerebral cortex.

Alzheimer’s disease (AD) is the most common form of dementia in the elderly population. Mutations in presenilins 1 and 2 (PS1 and PS2) are the major cause of familial Alzheimer’s disease (1). Studies on PS1-null mice have revealed essential roles of PS1 in cortical development and γ-secretase cleavage of the amyloid precursor protein (APP) and Notch1 (Ref. 2–6). In contrast to PS1-null mice, which are perinatally lethal (2), PS2-null mice are viable (7, 8), but mice lacking both presenilins display early embryonic lethality (8, 9). To study PS function in the adult cerebral cortex, which is the most relevant experimental system with respect to AD pathogenesis, we have previously generated presenilin conditional double knock-out (PS cDKO) mice lacking both presenilins in excitatory neurons of the forebrain after about postnatal day 18 (10, 11). These mice exhibit memory and synaptic plasticity deficits at the age of 2 months that become more severe as the mouse ages and are accompanied by neuronal degeneration in the cerebral cortex in an age-dependent manner (11). These impairments are associated with reductions in N-methyl-D-aspartate receptor-mediated responses and in the expression of CBP and of CREB/CBP downstream genes (11). These findings demonstrate that presenilins have important functions in the adult cerebral cortex.

The purpose of the current study is to investigate further what alterations at the molecular level underlie the impairments caused by presenilin inactivation in the adult brain and to search for additional neuropathological markers in the cerebral cortex of PS cDKO mice. Because β-amyloid (Aβ) peptides, which have neurotoxic properties at increased levels, are products of the presenilin-dependent γ-secretase cleavage of APP, we examined Aβ levels in the cerebral cortex of PS cDKO mice and found a significant decrease of both Aβ40 and Aβ42. As an approach to search for gene expression changes in the cerebral cortex of PS cDKO mice, we performed oligonucleotide microarray analysis and found that a high number of genes are differentially regulated. We further confirmed by quantitative real-time RT-PCR (qRT-PCR) and Western analyses the increase in the levels of a group of genes that are involved in inflammatory responses, and we characterized histologically their up-regulation pattern in the brain of PS cDKO mice.

**EXPERIMENTAL PROCEDURES**

**Mice**—The generation of PS1 conditional knock-out (PS1 cKO), PS2−/−, and PS cDKO mice has been described previously (7, 10, 11). Neural progenitor cell (NPC)-specific PS cDKO mice were generated by crossing floxed PS1 mice (10), nestin-Cre transgenic mice (12), and PS2−/− mice (7). The genetic background of all experimental groups used in this study was C57BL6/129 hybrid.

**Western Blot**—Mouse cerebral cortex was dissected and homogenized in cold buffer (for Fig. 1A: 50 mM Tris (pH 7.4), 150 mM NaCl, 2 mM

ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; NPC, neuronal progenitor cell; PBS, phosphate-buffered saline; PS, presenilin; qRT-PCR, quantitative real time reverse transcription PCR; RT-PCR, reverse transcription PCR.

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The abbreviations used are: AD, Alzheimer’s disease; Aβ, β-amyloid; APP, amyloid precursor protein; CREB, cAMP-response element-binding protein; CBP, CREB-binding protein; cDKO, conditional double knock-out; cKO, conditional knock-out; CTF, APP C-terminal fragment;
EDTA, 1% Nonidet P-40, 0.5% Triton X-100, and protease and phosphatase inhibitors; for Fig. 2B, 50 mM Tris (pH 7.4), 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 1% SDS, and protease and phosphatase inhibitors). Same amounts of total protein were resolved by SDS-PAGE electrophoresis and transferred to polyvinylidene difluoride membranes as described (10). For Fig. 1A, blots were incubated with the Saeko antisera (13) followed by 125I-labeled anti-rabbit antibody (ICN Biomedicals). For Fig. 2B, blots were incubated with antibodies against glial fibrillary acidic protein (GFAP) (1:100,000; Sigma), cathepsin S (1:1000; kind gift of Guo-Ping Shi, Boston, MA), or complement component C1q (1:1000; Quidel) and developed with enhanced chemiluminescence (PerkinElmer Life Sciences). For Fig. 2B, blots were probed with an antibody against α-tubulin (1:100,000; Sigma) as a loading control. Films exposed by blots probed for cathepsin S were subjected to densitometric analysis using NIH Image software, values were normalized to α-tubulin, and significance was tested post hoc using one-factor analysis of variance with Scheffe’s S test.

Sandwich Aβ Enzyme-linked Immunosorbent Assay (ELISA)—The sandwich Aβ ELISA was developed as described (14). In brief, NUNC Maxisorb immunoassay plates (96-well) were coated with the antibodies 2G3 (anti-Aβ40) or 21F12 (anti-Aβ42) at 0.3 μg/well in PBS overnight at 4 °C. Plates were subsequently blocked with Block ACE (1:4 dilution of original solution; Snow Brand Milk Products) for 2 h at room temperature. Plates were resuspended with PBS-Tween 20 briefly. The samples were loaded in the wells and incubated overnight at 4 °C. After washing with PBS-Tween 20, the plates were incubated in a solution of biotinylated alkaline phosphatase, 1:5000 dilution; Amersham Biosciences) for 1.5 h at 4 °C. The signal was amplified by adding 100 μl of AttoPhos and measured with a Fluoroskan (PerkinElmer Life Sciences). The detection limits are 3.125 pg/ml for both Aβ40 and Aβ42. The final values were normalized to the loading amount of wet tissue and analyzed for significance using the Student’s t test.

Aβ Extraction from Brain Tissue—Tissue was dissected, weighed, and homogenized in Tris-buffered saline (50 mM Tris and 150 mM NaCl, pH 7.4). The homogenates were centrifuged at 100,000 g for 20 min. The resulting pellets were resuspended in 5M guanidine, 50 mM Tris, pH 8, and incubated at room temperature for 10–30 min. The suspension was then diluted 1:10 with loading buffer (10% Block ACE) and centrifuged at 11,000 × g for 10 min. The supernatant was subjected to Aβ detection.

Microarray Analysis—Total RNA was purified from the cerebral cortex of a PS cDKO and a control mouse using the TRI reagent (Sigma). RNA was reverse-transcribed, amplified, labeled, and hybridized onto murine MG-U74A GeneChip probe arrays (Affymetrix) following the protocol detailed by Affymetrix. Analysis of differential expression was performed using DNA-Chip Analyzer (dChip) (15). Genes with mean value >60 were analyzed further by cross-comparison between PS cDKO and control.

Quantitative Real-time RT-PCR—Quantitative real-time RT-PCR was performed as described (11). Briefly, total cortical RNA was treated with DNase I and reverse transcribed in the presence of random hexamers. PCR reactions were performed using SYBR Green PCR Master Mix in an ABI PRISM 7700 Sequence Detector (Applied Biosystems) with 10 μl of diluted (1:25) cDNA and gene-specific primers. Reactions were performed in duplicate, and threshold cycle values were normalized to 18 S RNA. All procedures were carried out together for PS cDKO and control in gender-matched pairs. The PCR products were analyzed by electrophoresis to confirm their correct sizes. Significance was tested post hoc using one-factor analysis of variance with a Scheffe’s S test.

Immunohistochemistry—For cathepsin S, complement component C1q, and CD45 immunostainings, mice were sacrificed with CO2 and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were dissected, post-fixed for 2 h at room temperature, and cryoprotected in 30% sucrose for 48 h at 4 °C. Cryostat sagittal sections were cut (10 μm for cathepsin S and 30 μm for complement component C1q and CD45 immunostaining) and incubated with antibodies against cathepsin S (1:30, Santa Cruz Biotechnology), complement component C1q (1:100, HyCult Biotechnology), or CD45 (1:1000; BD Pharmingen) followed by 1:1000 goat anti-rabbit (Dako) or 1:1000 donkey anti-goat (Jackson ImmunoResearch Laboratories) IgG and 1:500 donkey anti-mouse (Jackson ImmunoResearch Laboratories) IgG secondary antibodies. After washing, sections were incubated with 1:2000 rhodamine-conjugated anti-goat (Molecular Probes) or 1:2000 fluorescein-conjugated anti-mouse (Molecular Probes) IgGs for 2 h at room temperature. After washing with PBS, the sections were mounted on gelatin-coated slides and covered with mounting medium (Vector Laboratories) without Ni2+ (brown color) for cathepsin S and CD45 detection. Images were captured using an Olympus BX40 light microscope.

RESULTS

To evaluate the potential involvement of altered APP processing in the phenotypes observed in PS cDKO mice, we examined APP processing in the cerebral cortex of PS cDKO mice. The substrates for γ-secretase-mediated proteolysis of APP are the APP C-terminal fragments (CTFs), which are products of α- and β-secretase activities and accumulate in the absence of γ-secretase cleavage. To determine the levels of CTFs in the cerebral cortex of PS cDKO mice, we performed Western analysis using an antisera (Saeko) that detects the C-terminal region of APP (13). At 2 and 6 months of age we observed accumulation of CTFs in PS cDKO mice in comparison with control and PS2−/− mice (Fig. 1A). The degree of accumulation was slightly higher than that seen in PS1 cKO mice, with similar patterns at 2 and at 6 months of age (Fig. 1A).

Next, we used an ELISA to determine the levels of Aβ peptides in the cerebral cortex of PS cDKO mice. We first measured generation of Aβ40 and Aβ42 peptides in the cerebral cortex of 6-month-old mice and found a significant reduction of both species in PS cDKO as compared with control and PS2−/− mice (Fig. 1B). Interestingly, similar reductions were found in PS cDKO and PS1 cKO mice (Fig. 1B). Given the difference in the severity of the phenotypes between 2-month-old and 6-month-old PS cDKO mice (11), we next asked whether the degree of reduction in Aβ levels is also different between these two time points. ELISA measurements at the age of 2 months also showed a significant decrease of Aβ40 and Aβ42 in the PS cDKO cerebral cortex, as compared with control, at similar levels as the decrease observed at 6 months (Fig. 1C). These results demonstrate that the reduction in the presenilin-mediated γ-secretase cleavage of APP in PS cDKO mice is similar to that in PS1 cKO mice and is age-independent.

Because a small amount of Aβ was still detected in the cortex of PS cDKO mice, which could be due to expression of PS1 in glial cells and/or interneurons, we measured Aβ levels in another line of nervous system-specific PS cDKO mice (NPC-PS cDKO mice) in which Cre recombinase is expressed under the control of the nestin promoter (12); thus, PS1 expression is inactivated in neural progenitor cells and NPC-derived neurons and glia. We found that Aβ40 peptides could no longer be detected in whole brain extracts from NPC-PS cDKO embryos at day 15.5 (Fig. 1D). However, a small amount of Aβ42 was still detected (Fig. 1D), suggesting that its production may not be entirely regulated by presenilins.

We next aimed to determine what changes in gene expression are potentially associated with the progressive development of neuropathology in PS cDKO mice. We performed oligonucleotide microarray analysis using RNA from the cerebral cortex of 6-month-old mice as an approach to search for differentially expressed genes in large scale. At 6 months of age, PS cDKO mice have lost 18% of their cortical neurons and display very severe impairments of learning and memory and synaptic plasticity (11). The gene expression profiles of PS cDKO and a control mouse were compared using oligonucleotide microarrays representing ~12,500 murine characterized genes and expressed sequence tags. Analysis of differential expression was performed using DNA-Chip Analyzer (dChip), a model-based approach computing expression levels for oligonucleotide arrays (15). This algorithm assigns proper weight to each probe within the set of 16 probes that interrogate each gene and influences the calculation of the fold change. 37 characterized genes and expressed sequence tags were found to be >2-fold up-regulated in PS cDKO mice (Table 1). Classification showed that ~25% of these genes are involved in inflammatory or
immune responses, such as GFAP, chains of complement component C1q, and members of the cathepsin family. Ion and gap junction channels, transport/cytoskeleton-related genes, and genes involved in oxidation, signal transduction, or transcription were also among the genes that showed increased levels of expression. There were 26 characterized genes and expressed sequenced tags that were 2-fold down-regulated in PS cDKO mice (Table II). As in the case of the up-regulated genes, functional groups included ion channels, transport/cytoskeleton-related genes, and genes involved in transcription. In addition, genes involved in cell growth or metabolism were also decreased.2

PS cDKO mice exhibit several morphological hallmarks of synaptic and neuronal loss (11). Based on the PS cDKO gene expression profile as revealed by microarray analysis, we investigated further what additional molecular alterations that have been associated with neurodegenerative processes are present in the cerebral cortex of PS cDKO mice. GFAP, an intermediate filament protein, is abundantly expressed during astrocytic activation and is present at increased levels in the brain of AD patients (16, 17). As shown in Table I, GFAP was found to be one of the most highly up-regulated genes in the cerebral cortex of PS cDKO mice by oligonucleotide microarrays. We performed qRT-PCR analysis using RNA from the cerebral cortex of seven PS cDKO and seven control mice to further test the levels of GFAP expression with an independent technique. GFAP was found to be 10-fold elevated in the PS cDKO cerebral cortex (p < 0.0005; Fig. 2A), confirming the microarray result and demonstrating strong astrocytic activation. Western analysis using total cerebral cortical lysates showed a strong GFAP signal in the PS cDKO samples, whereas the band in the control samples was out of detection with the antibody dilution used, further verifying the microarray and qRT-PCR results at the protein level (Fig. 2B).

Of the inflammation-related genes that were shown higher than 2-fold up-regulated in PS cDKO mice (Table I), three genes (complement component C1q α and β polypeptides and complement component 3α receptor 1) are involved in complement system activation. The γ polypeptide of complement component C1q was also found up-regulated by 1.6-fold (data not shown). The complement system is an important part of the immune system, and its activation has been associated with AD pathology and cognitive dysfunction (18). qRT-PCR analysis for the α polypeptide of complement component C1q further showed increased mRNA levels in the cerebral cortex of PS cDKO mice by ~4-fold (p < 0.00001; Fig. 2A). By Western blot

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2 The PS cDKO and control lists of hybridization values for all genes included in the microarrays can be accessed through the Gene Expression Omnibus (GEO) web site at www.ncbi.nlm.nih.gov/geo under accession number GSE1375.
using an antibody against C1q, we observed a strong increase in the PS cDKO lysates in the intensity of a band with a molecular mass of 24 kDa, was under detection in both cDKO and seven control mice, after normalizing for cathepsin S and GFAP we observed no colocalization pattern, reflecting its lysosomal distribution. By double labeling for cathepsin S and GFAP we observed no colocalization (data not shown). Up-regulated cathepsin S was found in a punctate morphology of their activated state (Fig. 3). Immunohistochemistry using an antibody against cathepsin S showed a dramatic elevation of cathepsin S-positive cells in PS cDKO mice compared with control mice, where immunoreactivity was virtually absent (Fig. 3). Staining was observed throughout the neocortex (Fig. 3A) as well as in the stratum oriens and stratum radiatum of the hippocampus proper (Fig. 3B), the lacunosum molecular layer, and the dentate gyrus molecular layer (data not shown). Up-regulated cathepsin S was found in a punctate pattern, reflecting its lysosomal distribution. By double labeling for cathepsin S and GFAP we observed no colocalization (data not shown), suggesting that the cells bearing increased levels of cathepsin S in PS cDKO mice may not be astrocytes.

Interestingly, immunostaining for complement component C1q revealed no immunoreactivity in the neocortex (Fig. 3A) but a marked increase in immunoreactivity in the PS cDKO hippocampus compared with the control (Fig. 3B). Staining in PS cDKO mice was observed in a diffuse pattern and was present in the stratum oriens, stratum radiatum, and molecular layers of the hippocampus (Fig. 3B and data not shown). In control mice, staining was clearly observed only in the molecular layers of the hippocampus at levels similar to or lower than those in PS cDKO mice (data not shown) and was very weak or absent in the stratum oriens and stratum radiatum (Fig. 3B).

Given the high inflammatory responses observed in PS cDKO mice, we next addressed whether microglial activation,
another important inflammatory mechanism, is also present in the PS cDKO brain. By immunostaining using an antibody against CD45, a transmembrane glycoprotein widely used as a microglial marker, we observed a strong increase in immuno-reactivity in the hippocampus of PS cDKO compared with control mice, with a cellular staining pattern typical of the morphology of activated microglia (Fig. 3B). Staining was also observed at increased levels in the neocortex, mainly at the deeper layers (Fig. 3A). Labeled activated microglia in the hippocampus of PS cDKO mice were present in the stratum oriens and stratum radiatum (Fig. 3B), the lacunosum molecular layer, and the dentate gyrus molecular and polymorph layers (data not shown).

**DISCUSSION**

We have shown previously that inactivation of presenilins in the adult cerebral cortex results in impairments of learning and memory, synaptic plasticity, and neuronal survival in an...
Reduced Aβ and Increased Inflammation in PS Mutant Mice

A well-established role of presenilins is the control of APP processing via their function as components of the γ-secretase complex that generates Aβ from APP CTFs. Aβ peptides and particularly Aβ42 are known to have neurotoxic properties at increased levels, which could be relevant to the deficits observed in PS cDKO mice. Our data, however, argue against this idea because the levels of Aβ40 and Aβ42 are significantly reduced in the PS cDKO cerebral cortex. The degree of reduction was found to be similar to that in PS1 cKO mice, confirming that PS1 is responsible for the majority of Aβ production in vivo. The small amount of residual Aβ likely reflects the presence of PS1 in glial cells and interneurons lacking Cre expression. To verify this hypothesis, we further examined the levels of Aβ40 and Aβ42 in NPC-PS cDKO mice, which lack presenilins in all cells in the nervous system. Aβ40 was under the detection limits of the assay, confirming that its generation is totally controlled by presenilins. Interestingly, we could still detect Aβ42, raising the possibility that additional enzymatic activities other than presenilin-controlled γ-secretase may also be responsible for production of Aβ42, as has been suggested previously by studies in cultured cells (20, 21). Although the phenotypes in PS cDKO mice could theoretically be caused by...

Fig. 3. Inflammatory responses in the neocortex and hippocampus of PS cDKO mice. Immunohistochemistry for GFAP, cathepsin S, complement component C1q, and CD45 on sagittal brain sections from control and PS cDKO mice at 6–7 months of age reveals high increases in immunoreactivity in the PS cDKO brain. Light microscopy pictures of the neocortex are shown in panel A and of the CA1 region of the hippocampus in panel B. Staining for GFAP labels activated astrocytes throughout the neocortex and hippocampus of PS cDKO mice, whereas in the control sections only background staining is present in the neocortex and a few labeled astrocytes are present in the hippocampus. High cathepsin S immunoreactivity is observed in both the neocortex and hippocampus of PS cDKO mice, whereas virtually no staining is seen in the control sections. Cathepsin S is present in a punctate pattern, reflecting its lysosomal distribution. Complement component C1q is up-regulated only in the hippocampus of PS cDKO mice, whereas no staining is observed in the neocortex. For both cathepsin S and complement component C1q, immunoreactivity in the PS cDKO hippocampus is defined in the stratum oriens and stratum radiatum of the hippocampus proper, as shown for the CA1 region in panel B, as well as in the lacunosum moleculare layer and the dentate gyrus molecular layer. Strongly activated microglia labeled with CD45 are present in the hippocampus of PS cDKO mice as well as in the neocortex, mainly at the lower layers. SO, stratum oriens; Py, pyramidal cell layer; SR, stratum radiatum. Scale bar, 100 μm.
the reduction in Aβ peptides, this is unlikely for the following reasons. First, the levels of Aβ40 and Aβ42 are reduced to similar extents in PS cDKO and PS1 cKO mice despite the severe impairments in the former and the absence of neurodegeneration and synaptic plasticity deficits in the latter (10, 11). Second, PS cDKO mice exhibit much more severe phenotypes than APP-null mice, in which Aβ peptides are absent (22). Our results therefore indicate that the neuropathology in PS cDKO mice is independent of Aβ generation.

Despite the similar Aβ levels in PS cDKO and PS1 cKO mice, the amount of CTFs was slightly higher in the PS cDKO cerebral cortex. The absence of correlation between reduced Aβ production and CTF accumulation has been reported previously in cultured cells expressing mutant forms of PS1, suggesting roles of PS1 in CTF degradation or APP trafficking (23–25). Our results suggest that, in the adult brain, PS2 has a more evident function in potential direct effects on CTF production and/or degradation than in the control of Aβ generation. CTF expression in transgenic mice results in impaired learning, long-term potentiation, and neuronal survival (26, 27), which could lead to the idea that the increase in CTF levels may contribute to the PS cDKO pathology. The deficits in CTF-expressing transgenic mice, though, are associated with increased Aβ immunoreactivity (27, 28), therefore rendering unclear whether CTF alone is responsible for the phenotypes observed. Injection of recombinant CTFs into mice has been reported to have neurotoxic properties (29), raising the possibility of harmful effects of the increased CTFs in PS cDKO mice. However, it is unknown whether the respective native fragment could exert the same effect in increased levels. Also, the concentration of recombinant CTFs in the brain after injection is likely to be much higher than the difference in CTF concentration between PS cDKO and PS1 cKO mice. Because PS1 cKO mice exhibit only a very mild phenotype (10) but display comparable CTF levels with PS cDKO mice, it is unlikely that the small increase in CTF production could account for the observed impairments.

Gene expression profile studies are becoming increasingly valuable as an approach to delineate alterations at the molecular level that are associated with genetic manipulations and to attribute specific phenotypes to groups of functionally related genes. Here we show, by oligonucleotide microarray analysis, that differential expression of distinct groups of genes accompanies the pathology in PS cDKO mice. Most notably, genes that are involved in inflammatory responses are up-regulated in the PS cDKO cerebral cortex. We further confirmed by qRT-PCR, Western blot, and immunohistochemical analyses that the levels of GFAP are elevated in the cerebral cortex of PS cDKO mice. Increased expression of GFAP has been suggested to be among the earliest and most sensitive features of neuronal toxicity (30). Reactive astrocytes, of which GFAP is a marker, are present in Aβ plaques in the brain of AD patients (31–33). However, it has been reported that astrogliosis in the AD brain, as measured by GFAP levels, increases independently of Aβ accumulation but correlates with the duration of the disease, suggesting that it is not a response to Aβ but likely a reaction to neuronal and synaptic loss and tangle formation (17). PS cDKO mice exhibit reduced levels of Aβ in the cerebral cortex (Fig. 1) but develop synaptic loss and progressive neurodegeneration (11). Therefore, our finding that GFAP is up-regulated in PS cDKO mice is consistent with the idea that synaptic and neuronal loss can result in astrogliosis independently of Aβ.

The complement system, a significant part of the immune system used to target toxic substances, is involved in inflammatory responses in the brain of AD and Down syndrome patients (18). We show by a variety of different techniques that the initial part of the classical complement pathway, complement component C1q, is increased in PS cDKO mice. Interestingly, complement activation in PS cDKO mice occurs only in the hippocampus, with complete absence in the neocortex. This pattern presents the first morphological phenotype caused by loss of PS function in vivo that is clearly distinct between the neocortex and the hippocampus, although PS inactivation occurs to the same extent and under a similar time course in both regions (10, 11). The functional significance of this disparity and its possible correlation with other phenotypes constitute interesting subjects of potential future studies. C1q immunoreactivity in PS cDKO mice appears in a diffuse pattern likely representing localization at the neuropil, which at the age of the mice used for the analysis (6 months) is in the process of degeneration, as indicated by reduced dendritic and synaptic density (11). The characteristics of C1q staining in PS cDKO mice are in strong contrast with the pattern reported in transgenic mice carrying pathogenic mutations of APP and PS1, in which C1q is colocalized with fibrillar Aβ plaques and plaque-associated microglia (34). This difference could be expected based on the absence of amyloid plaques in PS cDKO mice, and it is interesting that two mouse lines with different PS mutations both exhibit activation of the complement system, but in highly divergent patterns.

Our results show that cathepsin S, a lysosomal cysteine protease important for normal major histocompatibility class II-dependent immunity (35), is up-regulated in the neocortex and the hippocampus of PS cDKO mice. Cathepsin S is secreted from microglia and macrophages in response to stimulation with inflammatory mediators, and its expression is strongly elevated after entorhinal cortex lesion of adult rat brain (36). Interestingly, cathepsin S immunoreactivity has been found to be increased in the brain of AD and Down syndrome patients (19). A more thorough comparison between the mechanisms that lead to cathepsin S up-regulation in PS cDKO mice and those that lead to its up-regulation in human disease or other animal models remains to be addressed in the future.

Activated microglia are mediators of inflammatory responses in several neuropathological conditions and are important targets for research in AD inflammation (31). Our immunohistochemical analysis using the microglial marker CD45 showed that strong microglial activation occurs in the brain of PS cDKO mice, most prominently in the hippocampal formation. When activated, microglia are capable of producing a variety of inflammatory molecules such as cytokines and reactive oxygen intermediates (31). Given the widespread inflammatory reaction observed in the brain of PS cDKO mice, as evidenced by strong astrocytic and microglial activations and increased levels of complement component C1q and cathepsin S, it is likely that a vast array of additional molecular mediators of inflammation are also present at high levels in the PS cDKO brain.

In summary, our results investigate how APP processing is affected by loss of presenilin function in the adult brain and characterize at the molecular, biochemical, and morphological level several inflammatory responses in the cerebral cortex of PS cDKO mice. These findings identify additional molecular events that underlie the observed phenotypes and provide further evidence that PS cDKO mice are a valuable model system of neuropathology associated with progressive neurodegeneration.

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