

Mutant presenilin-1 deregulated peripheral immunity exacerbates Alzheimer-like pathology

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Abstract

Mutations in the presenilin-1 (PS1) gene are independent causes of familial Alzheimer's disease (AD). AD patients have dysregulated immunity, and PS1 mutant mice exhibit abnormal systemic immune responses. To test whether immune function abnormality caused by a mutant human PS1 gene (mhPS1) could modify AD-like pathology, we reconstituted immune systems of AD model mice carrying a mutant human amyloid precursor protein gene (mhAPP; Tg2576 mice) or both mhAPP and mhPS1 genes (PSAPP mice) with allogeneic bone marrow cells. Here, we report a marked reduction in amyloid- β (A β) levels, β -amyloid plaques and brain inflammatory responses in PSAPP mice following strain-matched wild-type PS1 bone marrow reconstitution. These effects occurred with immune switching from pro-inflammatory T helper (Th) 1 to anti-inflammatory Th2 immune responses in the periphery and in the brain, which likely instructed microglia to phagocytose and clear A β in an *ex vivo* assay. Conversely, Tg2576 mice displayed accelerated AD-like pathology when reconstituted with mhPS1 bone marrow. These data show that haematopoietic cells bearing the mhPS1 transgene exacerbate AD-like pathology, suggesting a novel therapeutic strategy for AD based on targeting PS1 in peripheral immune cells.

Keywords: Alzheimer's disease • A β plaques • bone marrow cells • immune • microglia

Introduction

Mutations in the presenilin-1 (PS1) gene have been implicated as causative agents in 18–50% of autosomal dominant cases of early-onset Alzheimer's disease (AD). Moreover, polymorphic PS1

alleles have also been suggested to be genetic risk factors in late-onset AD cases [1]. It is widely accepted that PS1 mutations contribute to AD pathogenesis by promoting amyloid- β (A β) production [2]. Yet, little attention has been paid to PS1 immunoregulation as a possible factor in AD pathoetiology, despite evidence that PS1 is known to play regulatory roles in differentiation and function of immune cells including microglia [3–5]. In support of this, PS1 dysfunction is associated with an inability to process complex antigens for presentation to T and B cells, a process that is required for adaptive immunity [6, 7]. Additionally, PS1 dysfunction profoundly inhibits A β phagocytosis and clearance from the brain [8, 9].

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There are a number of transgenic mouse models of AD that have been constructed with mutant human amyloid precursor protein (mhAPP) with or without mutant human PS1 alleles [10]. At least five transgenic mouse lines exist that bear mhAPP mutations known to be causative of early onset familial AD: PDAPP, Tg2576, APP23, TgCRND8 and J20. Each of these lines has unique genetic characteristics (*i.e.* different APP mutations, different promoters and different mouse genetic backgrounds), yielding different transgene expression levels and varying severities of AD-like pathology. Given that it is well characterized and widely used in the field as a model of AD-like pathology, we utilized the Tg2576 mouse model developed by Hsiao and coworkers, which overproduces A β peptides and develops β -amyloid plaques and other AD-like pathological features with age [11].

The other major class of mutations that cause familial AD reside in the PS1 gene, the protein product of which is an integral part of the γ -secretase complex that cleaves APP to give rise to A β peptides [12, 13]. Mice that are singly transgenic for mutant human PS1 gene (mhPS1) (M146L) preferentially generate more of the highly amyloidogenic, potentially pathogenic A β ₁₋₄₂ peptide, but do not manifest β -amyloid plaques [14]. Similarly, mice bearing a deletion of PS1 exon 9 (Δ E9) demonstrate increased levels of endogenous A β peptides but do not manifest cerebral β -amyloid plaques unless a mhAPP allele is co-expressed [15, 16]. Thus, these different PS1 mutations all seem to promote a toxic gain of PS1 function [17].

We investigated the putative contribution of altered PS1 immune function to AD-like pathology in Tg2576 or PSAPP AD mouse models. The former bears a mhAPP ('Swedish' 670/671 double mutation) [11], while the latter expresses both mhAPP and mhPS1 (Δ E9) transgenes (designated PSAPP) [9, 16]. Both of these transgenic lines manifest age-dependent AD-like pathology including brain inflammatory reactions and cerebral amyloidosis characterized by elevated A β ₁₋₄₀, ₄₂ levels and β -amyloid plaques [9, 11, 16]. We utilized head-sparing irradiation followed by adoptive transfer of bone marrow cells (BMCs) to selectively exchange host for donor immune cells in these AD model mice. Specifically, we first subjected PSAPP and Tg2576 mice to a lethal dose (9.5 Gy) of head-sparing irradiation to deplete haematopoietic (immune progenitor) cells while maintaining blood-brain barrier integrity and central nervous system (CNS) health [18]. We then isolated BMCs from mice carrying (*i*) wild-type PS1, (*ii*) mhPS1 (M146V) alone or (*iii*) mhPS1 (Δ E9) plus mhAPP transgenes (designated PSAPP) and subsequently generated chimeric mice bearing these donor-derived peripheral immune cells.

Strikingly, chimeric PSAPP mice bearing wild-type PS1 BMCs demonstrated mitigation of cerebral A β levels/ β -amyloid plaques and elevated brain-to-blood efflux of A β . Additionally, chimeric Tg2576 mice with mhPS1 haematopoietic cells manifested accelerated β -amyloid pathology. Taken together, these results suggest that genetic or acquired dysfunction in PS1 plays a previously unappreciated immunomodulatory role in AD pathogenesis. Restoration of physiologic PS1 immune function may be a viable therapeutic strategy for AD.

Materials and methods

Reagents

Mouse antibodies against A β (clones 6E10 and 4G8) were obtained from Covance Research Products (Emeryville, CA, USA). An A β ELISA was developed, characterized and routinely utilized in our laboratory [19]. The DuoSet™ mouse tumour necrosis factor- α (TNF- α) ELISA kit was obtained from R&D systems (Minneapolis, MN, USA). Capture and detection antibodies for mouse interleukin-1 β (IL-1 β) ELISA were obtained from eBioscience (San Diego, CA, USA).

Mice

Double transgenic 'Swedish' APP_{K670N/M671L} (APP^{swe}) + PS1 Δ E9 B6C3-Tg 85Dbo/J strain (PSAPP mice) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). These transgenic mice develop β -amyloid deposits in the brain by 6–7 months of age [16]. Singly transgenic mhAPP (APP^{swe}) Tg2576 mice were purchased from Taconic (Germantown, NY, USA), and develop brain β -amyloid deposits by 10–12 months of age [11]. Singly transgenic mhPS1 (M146V) mice were kindly provided by Gary Arendash. Because these animals do not develop frank β -amyloid deposits in their brains, we used them as donors (but not recipients) in allogeneic bone marrow transplantation experiments. All mice were genotyped for the presence of mhAPP and mhPS1 transgenes as described elsewhere [9]. Animals were housed and maintained in the College of Medicine Animal Facility at the University of South Florida (USF), and all experiments were conducted in compliance with protocols approved by the USF Institution Animal Care and Use Committee.

Bone marrow transplantation and generation of chimeric mice

For PSAPP mouse experiments, blood was withdrawn before irradiation and reconstitution at 6 months of age to obtain baseline values. Four-to-six hours after irradiation, PSAPP mice were reconstituted with BMCs, and all PSAPP mice were killed at 10 months of age. For Tg2576 mouse experiments, blood was withdrawn at 12 months of age before irradiation and reconstitution to obtain baseline values. Four-to-six hours after irradiation, Tg2576 mice were reconstituted with BMCs, and all Tg2576 mice were killed at 16 months of age. Mice were first subjected to a lethal dose of linear X-ray radiation (9.5 Gy). Irradiation was delivered in a head-sparing method where the head was placed outside the irradiation field and also protected with a lead 'cap' to prevent head irradiation and associated CNS injury [18]. Mice were then reconstituted *via* tail vein injection with phosphate-buffered saline (PBS; control) or BMCs (5×10^6 cells) isolated from 1-month-old strain-matched male (*i*) wild-type PS1, (*ii*) mhPS1 (M146V) transgenic or (*iii*) PSAPP transgenic mice. As shown in Table 1, this yielded four groups of chimeric mice ($n = 8$ females per group): (*i*) PSAPP mice receiving sham treatment (PSAPP/PBS), (*ii*) PSAPP mice receiving BMCs from PSAPP mice (PSAPP/PSAPP-BMC), (*iii*) PSAPP mice receiving mutant PS1 BMCs (PSAPP/PS1M146V-BMC) or (*iv*) PSAPP mice receiving wild-type BMCs (PSAPP/wild-type-BMC). It is important to note that the PSAPP/PBS group received tail vein injection of PBS but did not receive lethal irradiation, as this would likely result in mortality prior to the conclusion of experiments in the absence of some form of bone marrow

Table 1

Experimental design	PSAPP/PBS	PSAPP/PASAPP-BMC	PSAPP/PS1M146V-BMC	PSAPP/wild-type-BMC
Head-sparring irradiation	No	Yes	Yes	Yes
Treatment				
PBS	+			
PSAPP-BMC (5×10^6)		+		
PS1M146V-BMC (5×10^6)			+	
Wild-type-BMC (5×10^6)				+

Recipients: the BMC transfer. Start, 6 months of age; end, 10 months of age.

Donors: 1 month of age.

transplantation. In addition, in order to determine protein expression of endogenous mouse and mhPS1 proteins (including M146V and $\Delta E9$ mutants) from BMCs, we performed Western blot analysis on cell lysates prepared from mhPS1 (M146V) and PSAPP mouse-derived BMCs. Results showed expression of both endogenous murine and mhPS1 proteins (data not shown).

Bone marrow reconstituted mice were maintained under specific pathogen-free conditions and continuously given antibiotic in drinking water after transplantation. Further, a peripheral blood sample was collected (100 μ l) from all bone marrow transplanted mice on a monthly basis, and the plasma was stored at -80°C . Four months after bone marrow reconstitution, all animals were killed for analyses of A β levels/ β -amyloid load in the brain according to previously described methods [20].

PCR

To verify efficiency of irradiation and BMC reconstitution, we assayed blood samples from lethally irradiated and BMC reconstituted mice. Blood samples were taken from each mouse on a monthly basis and DNA was purified according to the manufacturer's recommendation (DNeasy Blood & Tissue Kit: Qiagen, Valencia, CA, USA). DNA abundance was assessed spectrophotometrically. PCR was performed on genomic DNA using a commercially available kit (HotStar Taq Master Mix, Qiagen) and a Bio-Rad iCycler thermocycler (Richmond, CA, USA) according to the manufacturer's instructions. Mouse primer pairs consisted of the following DNA sequences: PS1 $_{\Delta E9}$ sense, 5'-AAT AGA GAA CGG CAG GAG CA-3'; antisense, 5'-GCC ATG AGG GCA CTA ATC AT-3'; PS1 (M146V) sense, 5'-GCAGGCTGACATCCCAAGGG-3'; antisense, 5'-GCCTCAACCTGGAGATGG GGC-3'; β -actin sense, 5'-AAT TCG GAT GGC TAC GTA CAT GGC TG-3'; antisense, 5'-AGC TTG CTG TAT TGG CCT CCA TCG TA-3'. The primers yielded PCR products of 608, 553 and 800 bp, respectively. Amplicons were electrophoresed on 1% ethidium bromide-stained agarose gels, and visualized by ultraviolet transillumination as described previously [21]. The optimum number of cycles was determined experimentally for each gene product, and signal intensities of target gene products were normalized to β -actin amplified under identical conditions and expressed as a percentage of the control value. All densitometric analyses were carried out using the Fluor-S Multimager with Quantity One software (Bio-Rad).

Tissue preparation

Mice were killed with isofluorane and transcardially perfused with ice-cold physiological saline. Brains were rapidly isolated and quartered using a mouse brain slicer. The first and second anterior quarters were homogenized for ELISA analysis, and the third and fourth posterior quarters were used for cryostat sectioning. Posterior brain quarters were post-fixed in 4% paraformaldehyde in physiological saline at 4°C overnight, and then cryoprotected through a graded series of sucrose solutions (10%, 20% and 30% sucrose, each at 4°C overnight). Brains were sectioned in a coronal plane at 25 μ m thickness on a freezing stage using a sliding microtome. Sections were then stored in Dulbecco's phosphate-buffered saline (DPBS, pH 7.4) with 100 mM sodium azide at 4°C .

Immunohistochemistry

A β immunohistochemical staining was performed with six 25 μ m free floating sections spaced 200 μ m apart through each anatomic region of interest (hippocampus and neocortex) as previously described [22, 23]. Briefly, sections were blocked for endogenous peroxidases with 10% methanol and 0.3% hydrogen peroxide in DPBS. This was followed by an additional wash with DPBS, and then sections were permeabilized (100 mM lysine, 0.2% Triton X-100, 4% normal serum in DPBS). Sections were then incubated overnight with anti-human A β antibody (4G8, directed against amino acids 17-26 of A β peptide). The following day, sections were washed and signal was revealed using the VECTASTAIN[®] Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) coupled with the diaminobenzidine substrate. β -amyloid plaques positive for 4G8 were visualized under bright field using an Olympus BX-51 microscope (Olympus, Tokyo, Japan). A β burden was determined by quantitative image analysis. Briefly, low-magnification images (original magnification = 5 \times) containing cingulate cortex, hippocampus and entorhinal cortex were captured from coronal brain sections and a threshold optical density was obtained that discriminated staining from background. Manual editing of each field was used to eliminate artefacts. Data are reported as percentage of immunolabelled area captured from cingulate cortex, hippocampus and entorhinal cortex (positive pixels divided by total pixels captured). Quantitative image analysis was performed by a single examiner (T.M.) blinded to sample identities.

ELISA

For TNF- α and IL-1 β ELISAs, brains were isolated under sterile conditions on ice and placed in ice-cold lysis buffer. Brains were then sonicated on ice for approximately 3 min., allowed to stand for 15 min. at 4°C and centrifuged at 15,000 rpm for 15 min. Supernatants were then collected, and diluted at 1:4 in lysis buffer for TNF- α (R&D systems) and IL-1 β (eBioscience) ELISAs in strict accordance with the manufacturer's instructions. The BCA Protein Assay (Pierce, Rockford, IL, USA) was performed to measure protein concentration from each brain assayed prior to quantification of cytokines by ELISA to allow for sample normalization.

For A β ELISA, brain homogenates were prepared with lysis buffer as described above. Quantification of total A β species (including A β _{1-40, 42}) was performed according to published methods [23]. Briefly, 6E10 (capture antibody) was coated at 2 μ g/ml in PBS in 96-well immunoassay plates overnight at 4°C. The plates were washed with 0.05% Tween-20 in PBS five times and blocked with blocking buffer (PBS with 1% w/v bovine serum albumin and 5% v/v horse serum) for 2 hrs at room temperature. Total soluble A β species (including A β _{1-40, 42}) was detected in blood plasma or in brain homogenates prepared with lysis buffer described above at 1:4 or 1:10 dilutions, respectively. Detergent-insoluble total A β species (A β _{1-40, 42}) were detected in brain by a second extraction step of pellets from lysis buffer treatment in 5 M guanidine buffer, followed by a 1:10 dilution in lysis buffer [24]. Samples or A β standards were added to the plates and incubated overnight at 4°C. Following three washes, biotinylated antibody 4G8 (0.5 μ g/ml in PBS with 1% v/v bovine serum albumin) was added to the plates and incubated for 2 hrs at room temperature. After five washes, streptavidin-horseradish peroxidase (1:200 dilution in PBS with 1% v/v bovine serum albumin) was added to the 96-well plates for 30 min. at room temperature. Tetramethylbenzidine substrate was added to the plates and incubated for 15 min. at room temperature. Finally, 50 μ l of stop solution (2N H₂SO₄) was added to plate wells. The optical density of each well was immediately determined by a microplate reader with an emission wavelength of 450 nm.

Microglial phagocytosis assay

Primary cultures of murine microglia were derived from PBS, PSAPP-BMC, PSM146V-BMC or wild-type-BMC-reconstituted individual PSAPP mice as previously described [25, 26]. For fluorometric analysis of fluorescein isothiocyanate (FITC)-A β ₁₋₄₂, primary murine microglia were seeded at 1×10^5 cells/well ($n = 6$ for each condition) in 24-well tissue-culture plates containing 0.5 ml of complete RPMI 1640 medium. Cells were treated for 60 min. with 'aged' A β ₁₋₄₂ conjugated with FITC (Invitrogen, Carlsbad, CA, USA) [26]. Microglia were rinsed three times in A β -free complete medium, and media were exchanged with fresh A β -free complete medium for 10 min. both to allow for removal of non-incorporated A β and to promote concentration of the A β into phagolysosomes. Extracellular and cell-associated FITC-A β were quantified using an MSF reader (SpectraMax®, Molecular Devices, Sunnyvale, CA, USA) with an emission wavelength of 538 nm and an excitation wavelength of 485 nm. A standard curve from 0 to 500 nM of FITC-A β was run for each plate, and all samples fell within the linear range of the standard curve. Total cellular proteins were quantified using the Bio-Rad protein assay. The mean fluorescence values for each sample were determined by fluorometric analysis. Relative fold change values were calculated as the mean fluorescence value for each experimental sample over control. In this manner, both extracellular and cell-associated FITC-A β were quantified. To determine the extent to which cell death might have influenced phagocytic activity in the various

treatment groups, we performed LDH release assay, and no significant cell death was detected over the 3 hrs time frame in any of the treatment groups ($P > 0.05$).

Statistical analysis

All data were normally distributed; therefore, in instances of single mean comparisons, Levene's test for equality of the variance followed by t-test for independent samples was used to assess significance. In instances of multiple mean comparisons, ANOVA was used, followed by *post hoc* comparison using Bonferroni's method. α levels were set at 0.05 for all analyses. The statistical package for the social sciences release 10.0.5 (SPSS, Chicago, IL, USA) was used for all data analysis.

Results

Generation of chimeric PSAPP mice and cytokine profile switching in the periphery and in the CNS

In order to evaluate the effects of genetically manipulated immune cells on AD-like pathology, we began by establishing four groups of chimeric AD model mice. One group of PSAPP mice was irradiated and chimerically reconstituted with mhPS1 (M146V) BMCs. These animals demonstrated presence of the 553 bp mhPS1 (M146V) amplicon in DNA extracted from blood samples only following reconstitution (Fig. 1 and Table 2), whereas another group of PSAPP mice irradiated and then reconstituted with PSAPP BMCs demonstrated retention of the 608 bp mhPS1 Δ E9 PCR amplification product. Chimeric immune system reconstitution of PSAPP mice with wild-type BMCs led to a complete loss of 608 bp mhPS1 Δ E9 PCR amplicon, demonstrating successful replacement of PSAPP haematopoietic cells with wild-type PS1-expressing cells. As expected, sham treatment of PSAPP mice with a tail vein injection of PBS resulted in retention of the 608 bp mhPS1 Δ E9 PCR amplification product.

To evaluate the effects of immune system reconstitution with genetic variants of the PS1 and APP genes in PSAPP mice, plasma and brain cytokine concentrations were determined. We observed significantly higher levels of 'anti-inflammatory' T lymphocyte helper type 2 (Th2) cytokines including transforming growth factor-beta1 (TGF- β 1), granulocyte macrophage-colony stimulating factor, IL-4 and IL-10 in the plasma of PSAPP mice reconstituted with wild-type PS1 BMCs compared with all other groups evaluated (** $P < 0.001$; Fig 2A). These mice also demonstrated decreased levels of 'pro-inflammatory' cytokines that are often associated with Th1-type immune responses including TNF- α and interferon- γ (IFN- γ) compared with all other groups ($^{##}P < 0.001$; Fig. 2A).

To determine whether altered peripheral levels of these cytokines might impact on the CNS, we quantified levels of the prototypical Th2 cytokines (IL-4 and IL-10) in brain homogenates

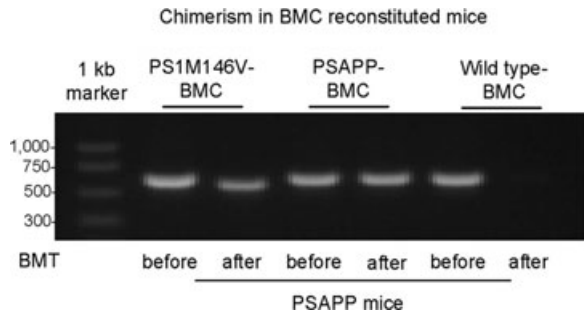


Fig. 1 Characterization of chimerism in PSAPP mice reconstituted with BMCs. Peripheral blood samples were collected from mice before and after adoptive transfer of BMCs. Peripheral lymphocytes were prepared from blood samples for PCR analysis before or after bone marrow transplantation. The PCR analysis detects DNA for recipient-derived mhPS1 (608 bp) or donor-derived platelet-derived growth factor promoter gene (which detects mhPS1 M146V; 553 bp) in peripheral lymphocytes from three groups of mice as indicated.

Table 2 Characterization of BMC reconstituted mice

	Recipient-derived PS1 mutant gene (608 bp) and donor-derived PDGF promoter gene (553 bp)	
	Before	After
PSAPP/PBS	608 bp	608 bp
PSAPP/PSAPP-BMC	608 bp	608 bp
PSAPP/PS1M146V-BMC	608 bp	553 bp
PSAPP/Wild type-BMC	608 bp	ND

from all four groups of chimeric mice. Data are presented as pg of each cytokine/mg of total cellular protein. Strikingly, both cytokines were markedly increased in PSAPP/wild-type BMC-reconstituted mouse brain homogenates compared with the three other chimeric mouse groups (** $P < 0.001$; Fig. 2B).

Wild-type PS1 bone marrow reconstitution mitigates cerebral amyloidosis and elevates plasma A β in PSAPP mice

We sought to determine whether wild-type PS1 bone marrow reconstitution in PSAPP mice might impact cerebral amyloidosis. Results show that chimeric reconstitution utilizing wild-type PS1 BMCs, but not PBS, mhPS1 (M146V) BMCs, or PSAPP BMCs led to a statistically significant 46% reduction in cerebral β -amyloid burden (** $P < 0.001$) (Fig. 3A and B). Biochemical analyses further supported this as demonstrated by significantly reduced detergent-soluble ($\downarrow 52\%$) and -insoluble ($\downarrow 38\%$) total A β species

(including A $\beta_{1-40, 42}$) in brain homogenates (** $P < 0.001$ for each comparison) (Fig. 3C). Interestingly, we did not note a significant difference on β -amyloid deposits or biochemical analyses of detergent-soluble or -insoluble total A β species in brain homogenates when comparing three groups of important controls, PSAPP/PBS, PSAPP/PSAPP-BMC, or PSAPP/PSAPP M146V-BMC groups to each other ($P > 0.5$), demonstrating that 1) head-sparing irradiation or 2) expression of a mhPS1 transgene by peripheral immune cells does not have a significant impact on cerebral amyloidosis in PSAPP mice.

DeMattos and colleagues [27] have previously put forth the 'peripheral sink hypothesis', which describes a mechanism by which A β is cleared from the brain to the blood. To determine whether immune reconstitution of PSAPP mice with wild-type BMCs promoted brain-to-blood efflux of A β , we drew blood from mice at 8 and 10 months after reconstitution. Interestingly, at 8 and 10 months following reconstitution, we noted significantly increased plasma levels of A β species (** $P < 0.001$) (Fig. 3D). These data support the notion that wild-type PS1 bone marrow reconstitution increases clearance of A β from the brain to the blood in the PSAPP mouse model of AD.

Increased microglial CD11b expression and enhanced A β phagocytosis *ex vivo* after wild-type PS1 bone marrow reconstitution

It has been reported that stimulation of microglial CD11b expression results in increased A β phagocytic activity [26] and more generally leads to promotion of microglial phagocytic responses [28]. Given that wild-type PS1 bone marrow reconstitution of PSAPP mice resulted in significantly reduced cerebral β -amyloid pathology, we wished to examine whether this effect was mediated at least in part by enhanced microglial phagocytosis of A β . We first examined microglial CD11b in mice by immunofluorescence and found it to be increased in CD11b⁺ microglial cells in wild-type PS1 reconstituted PSAPP animals compared to mice that received PSAPP-derived haematopoietic cells (Fig. 4A), mhPS1 (M146V)-derived BMCs, or PBS (data not shown).

To directly determine if microglia from wild-type PS1 bone marrow reconstituted mice had an altered A β phagocytosis profile, we prepared primary cultures of adult microglia as previously described [26] from mice infused with PBS or BMCs derived from PSAPP, mhPS1 (M146V), or PS1 wild-type mice. These cells were then subjected to an A β phagocytosis assay using native or A β antibody-opsonized [known to increase uptake of A β by microglia [26] fluorescently-tagged A β_{1-42} (FITC-A β_{1-42}) according to our previously described methods [26]. As shown in Fig. 4B, when measuring FITC A β_{1-42} in cell supernatants or lysates, one-way ANOVA followed by *post hoc* comparison revealed a significant increase in A β uptake by microglia derived from PS1 wild-type BMCs compared with cells cultured from PSAPP BMC-reconstituted PSAPP mice (** $P < 0.001$). This difference remained in the presence of IgG antibody directed against A β (2.5 μ g/ml) [26],

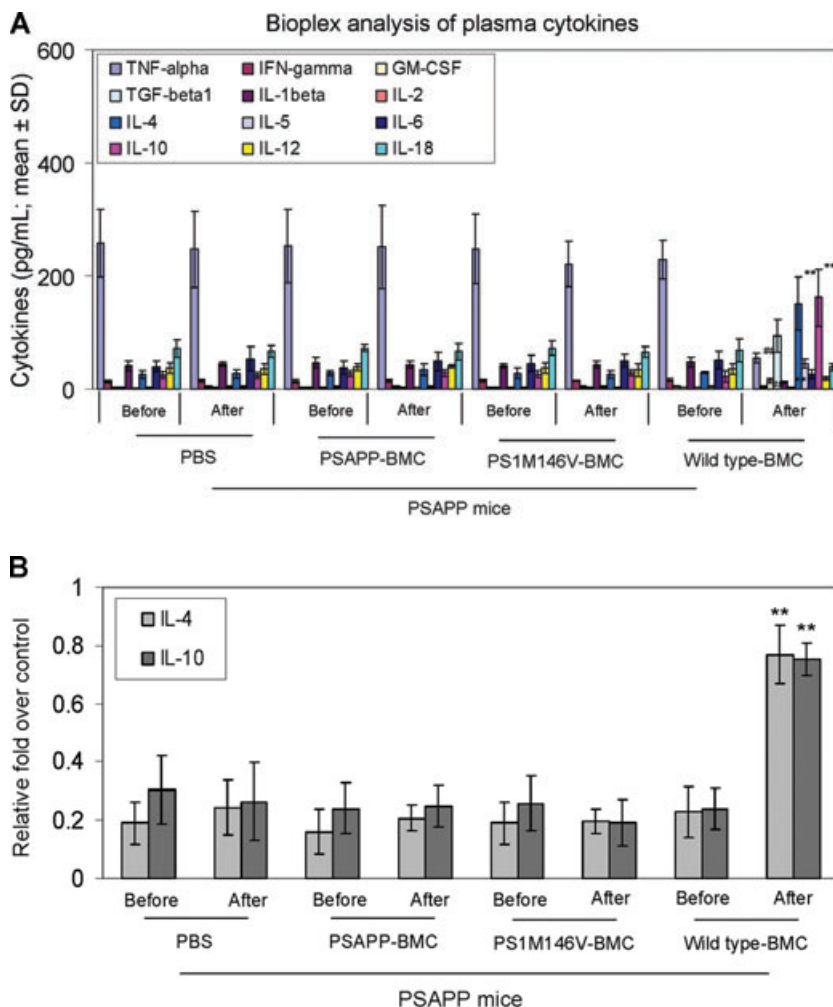


Fig. 2 Effects of bone marrow reconstitution on immune function in PSAPP mice. For all PSAPP mouse experiments, blood was withdrawn before irradiation and reconstitution at 6 months of age to obtain baseline values. Four to six hours after irradiation, mice were reconstituted with BMCs, and all PSAPP mice were killed at 10 months of age. At the time of killing (10 months of age), blood was withdrawn from the heart, and plasma cytokine levels were analysed by the Bioplex protein multi-array system, which utilizes Luminex-based technology [47]. **(A)** Plasma IL-4, IL-10 and TGF- β_1 are increased in wild-type-BMC-reconstituted PSAPP mice. A t-test for independent samples showed significant differences between wild-type-BMC- and PBS-, PSAPP- or PS1M146V-infused PSAPP mice for IL-4, IL-10 and TGF- β_1 (** $P < 0.001$). **(B)** Cytokine levels were analysed in brain homogenates from identical animals. Data are presented as pg of each cytokine/mg of total cellular protein. A t-test for independent samples showed significant differences between PSAPP/wild-type BMC-infused and PBS-, PSAPP- or PS1M146V-infused PSAPP mice for both IL-4 and IL-10 (** $P < 0.001$).

wherein we noted significantly enhanced A β uptake by microglia isolated from adult PSAPP mice reconstituted with wild-type-BMCs compared with microglia derived from syngeneic PSAPP BMC-reconstituted mice ($^{##}P < 0.001$). In addition, we observed significantly increased microglial phagocytosis of A β_{1-42} when comparing cells isolated from PSAPP/wild-type-BMC-reconstituted mice with those from PSAPP/PBS or PSAPP/PS1M146V-BMC mice ($P < 0.001$; data not shown).

It has been demonstrated that the pro-inflammatory cytokines TNF- α and IL-1 β inhibit A β phagocytosis by microglia, and this effect can be reversed by the anti-inflammatory Th2 cytokines IL-4 and IL-10 [29]. Having shown an increase in the Th2 cytokines IL-4 and IL-10 in the periphery and in the brain after wild-type PS1 BMCs reconstitution of PSAPP mice, we measured levels of the Th1-associated cytokines TNF- α and IL-1 β in brains of PSAPP mice that underwent reconstitution with various groups of genetically modified BMCs. As shown in Fig. 4C, both pro-inflammatory cytokines revealed a statistically significant reduction between

32% and 46% in PSAPP mice reconstituted with wild-type PS1 BMCs (** $P < 0.001$). Taken together, these data show that peripheral immune system reconstitution with wild-type PS1 BMCs causes a shift from Th1 to Th2 cytokine responses in the brain. Given that we found reduced Th1-associated pro-inflammatory cytokines and increased Th2 cytokines both in the periphery and in the brain, we suggest that this cytokine shift instructs microglia to phagocytose and clear brain A β .

Tg2576 mice reconstituted with PS1 mutant bone marrow have accelerated cerebral amyloidosis

Given our results showing that reconstitution of PSAPP mice with BMCs bearing wild-type PS1 alleles brings about reduced cerebral amyloidosis, we asked whether the converse may be true. Specifically, to explore whether mhPS1 expression by peripheral immune cells might impact on cerebral amyloidosis, we established

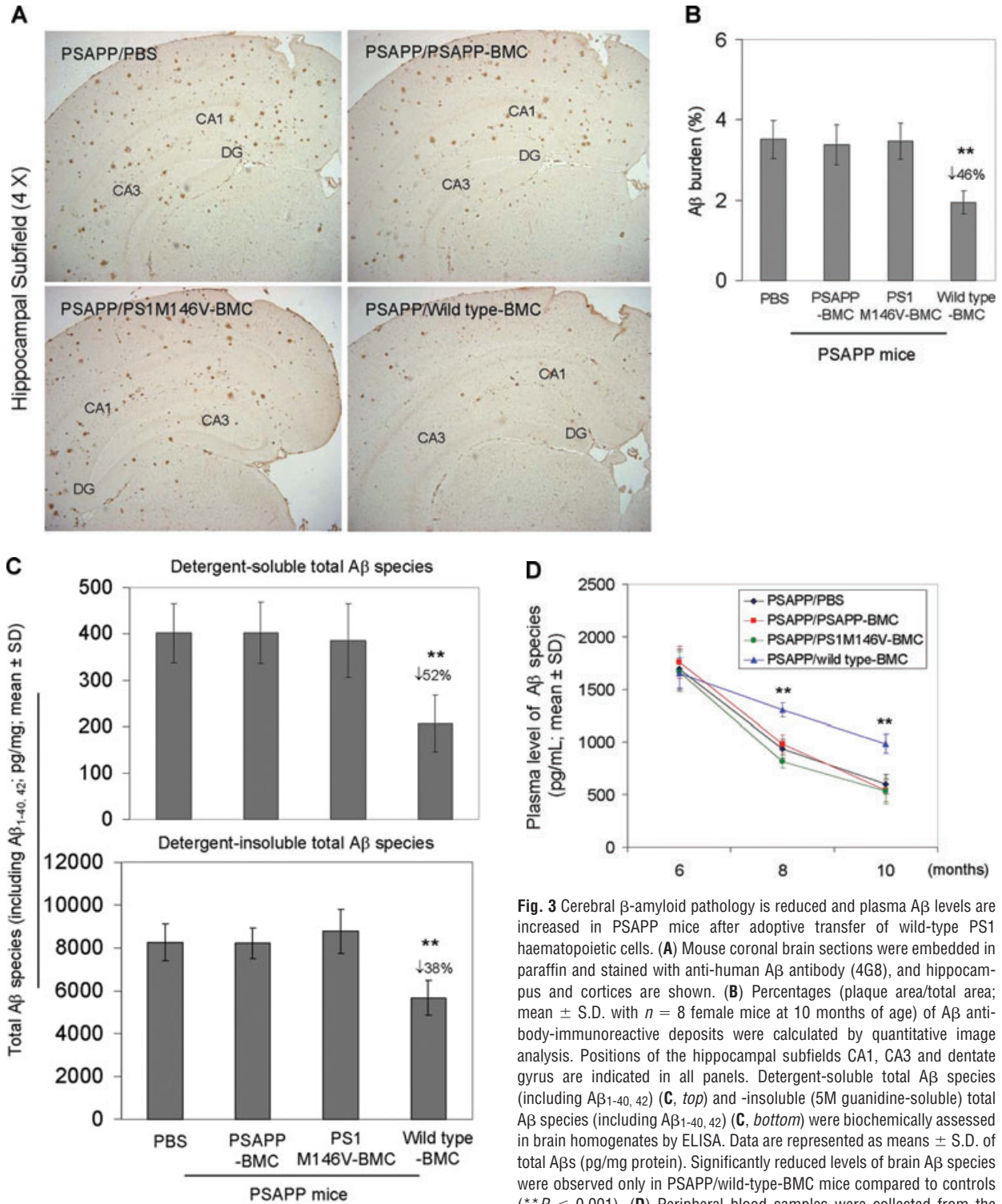


Fig. 3 Cerebral β -amyloid pathology is reduced and plasma A β levels are increased in PSAPP mice after adoptive transfer of wild-type PS1 haematopoietic cells. **(A)** Mouse coronal brain sections were embedded in paraffin and stained with anti-human A β antibody (4G8), and hippocampus and cortices are shown. **(B)** Percentages (plaque area/total area; mean \pm S.D. with $n = 8$ female mice at 10 months of age) of A β antibody-immunoreactive deposits were calculated by quantitative image analysis. Positions of the hippocampal subfields CA1, CA3 and dentate gyrus are indicated in all panels. Detergent-soluble total A β species (including A $\beta_{1-40, 42}$) **(C, top)** and -insoluble (5M guanidine-soluble) total A β species (including A $\beta_{1-40, 42}$) **(C, bottom)** were biochemically assessed in brain homogenates by ELISA. Data are represented as means \pm S.D. of total A β s (pg/mg protein). Significantly reduced levels of brain A β species were observed only in PSAPP/wild-type-BMC mice compared to controls (** $P < 0.001$). **(D)** Peripheral blood samples were collected from the

mice bi-monthly throughout the 4-month reconstitution experiment with BMCs. Plasma total soluble A β species (including A $\beta_{1-40, 42}$) were measured by ELISA. Data are represented as means \pm S.D. of total A β species. Significantly increased levels of plasma total soluble A β species were observed only in PSAPP/wild-type-BMC mice at 6 and 10 months compared to controls (** $P < 0.001$).

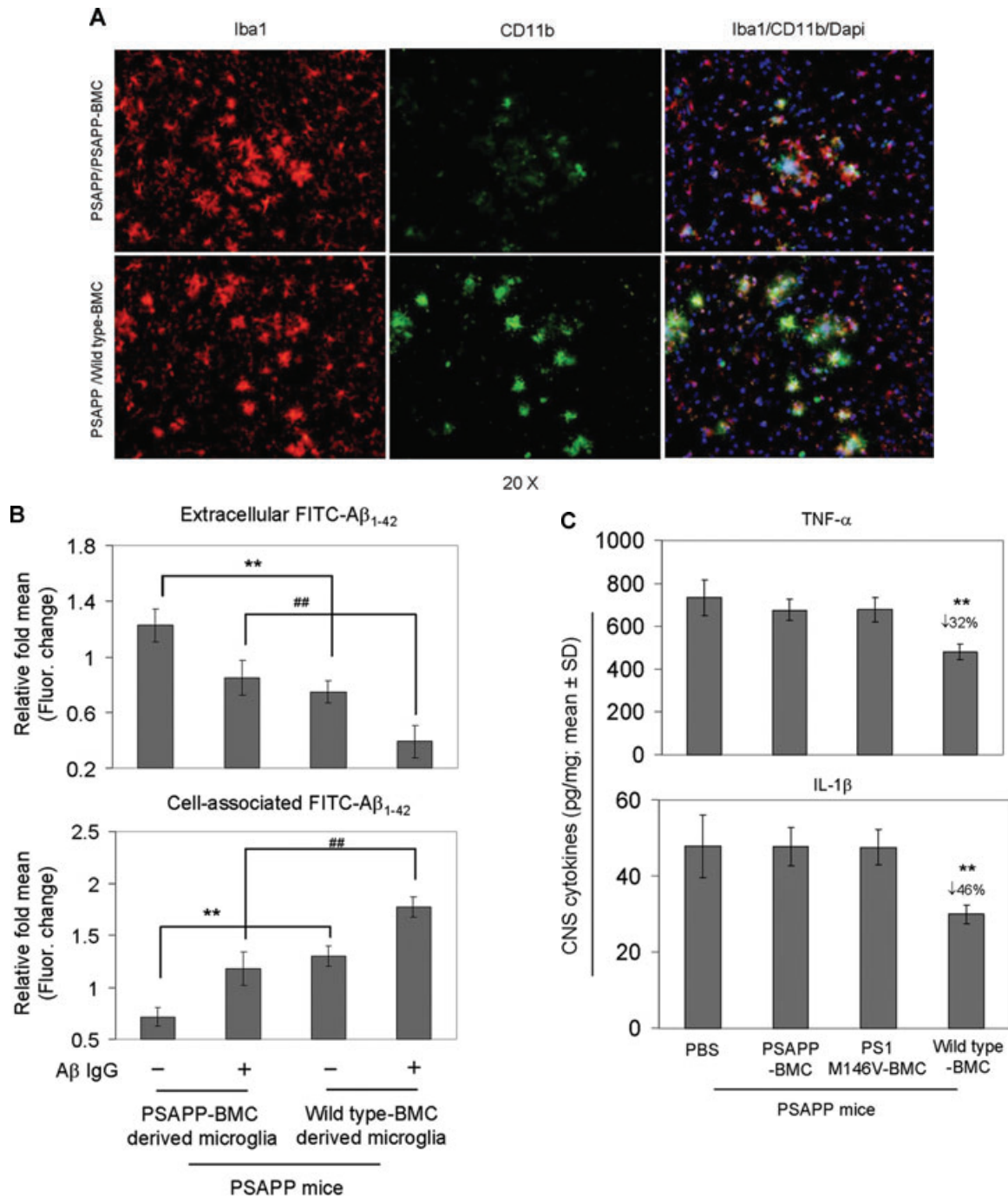


Fig. 4 PS1 wild-type BMCs increase microglial CD11b expression, promote microglial A β phagocytosis, and reduce T helper1 (Th1) cytokines in the CNS. **(A)** CD11b immunofluorescence is shown from brains of PSAPP mice reconstituted with PS1 wild-type BMCs (wild-type-BMC) or syngeneic PSAPP BMCs (PSAPP-BMC). **(B)** Microglial phagocytosis assay results are shown for extracellular and cell-associated FITC-A β_{1-42} , which was detected using a fluorometer. Data are represented as the relative fold of mean fluorescence (\pm S.D.) over control for each sample ($n = 4$ for each condition presented). Primary microglial cells from adult PSAPP/PSAPP-BMC or PSAPP/wild-type-BMC mice are shown in **B** (** or ## $P < 0.001$). **(C)** CNS abundance of cytokines was measured by ELISA. Data are represented as mean \pm S.D. of each cytokine. Significantly decreased levels of TNF- α or IL-1 β were observed in PSAPP/wild-type-BMC mice compared to controls (** $P < 0.001$).

four groups of Tg2576 mice (singly transgenic for 'Swedish' mhAPP) with chimeric immune systems as follows: (i) Tg2576 mice receiving sham treatment (Tg2576/PBS), (ii) Tg2576 mice receiving syngeneic BMCs from Tg2576 mice (Tg2576/Tg2576-BMC), (iii) Tg2576 mice receiving mhPS1 BMCs (Tg2576/PS1M146V-BMC) or (iv) Tg2576 mice receiving wild-type BMCs (Tg2576/wild-type-BMC). Strikingly, analysis of β -amyloid pathology revealed a statistically significant enhancement in Tg2576 mice reconstituted with PS1M146V-BMCs (Fig. 5A and B [$**P < 0.001$]). In addition, biochemical analyses revealed that mhPS1 (M146V) BMC reconstituted Tg2576 mice demonstrated significantly increased detergent-soluble and -insoluble A β in brain homogenates as compared with PBS, Tg2576, or wild-type PS1 chimeric Tg2576 mouse groups (Fig. 5C) ($**P < 0.001$). Interestingly, we did not note a difference on β -amyloid deposits or biochemical analyses of detergent-soluble or -insoluble A β in brain homogenates when comparing Tg2576/PBS, Tg2576/Tg2576-BMC, or Tg2576/wild-type-BMC groups to each other, demonstrating that (i) head-sparing irradiation or (ii) expression of a mhAPP transgene by peripheral immune cells does not have a significant impact on cerebral amyloidosis in Tg2576 mice. Furthermore, we assayed brain Th1 and Th2-associated cytokines, including TNF- α , IL-1 β , IL-4 and IL-10, in these mice by probing brain homogenates with cytokine ELISAs. Complementary to our findings in PSAPP mice reconstituted with wild-type PS1 BMCs, results from Tg2576 mice reconstituted with mutant PS1 BMCs showed a skewness toward pro-inflammatory Th1-associated cytokines (including TNF- α and IL-1 β) in the brain (data not shown).

Discussion

In this report, we have investigated the putative role of PS1 in the peripheral immune system on AD-like pathology. Recent evidence suggesting little or no peripheral immune cell infiltration into brains of mice subjected to head-sparing irradiation has enabled us to independently manipulate the genetic composition of haematopoietic cells without radiation-induced injury to the CNS known to be caused by 'full-body' irradiation [18, 30]. Thus, by employing host and donor animals with differing genetic makeups, a chimeric animal can be engineered. We utilized this technology to reconstitute immune systems expressing select variants of PS1 and APP genes, mutations in which are well established as independent causes of familial AD. Based on studies of impaired immune system function [3, 5, 6] and increased AD-like pathology in animals bearing mhPS1 transgenes [31–35], we suggested that mhPS1 expression by peripheral immune cells of PSAPP mice might exacerbate AD-like pathology by promoting a pro-inflammatory Th1-like cytokine response. Conversely, we predicted that reconstitution of the haematopoietic compartment in PSAPP mice with PS1 wild-type BMCs might promote processes involved in clearing cerebral amyloidosis, thereby providing resolution to this hallmark AD-like pathology. Results presented here can be inter-

preted as supporting both hypotheses. Interestingly, in terms of the latter, we noted enhancement of two types of A β clearance machinery: (i) brain-to-blood efflux of A β and (ii) increased microglial uptake of A β in an *ex vivo* assay. Both of these A β clearance mechanisms occurred in concert with a relative increase in pro-inflammatory Th1-associated cytokines and a reduction in anti-inflammatory Th2 immune responses.

In concert with PS1-mediated alteration of cerebral amyloidosis in AD mouse models, we observed that abundance of TNF- α and IL-1 β was inversely associated with both brain-to-blood efflux of A β and with microglial phagocytosis of A β . Conversely, prototypical Th2 cytokines IL-4 and IL-10 directly correlated with these beneficial A β clearance pathways. These findings dovetail with a previous report showing that TNF- α and IL-1 β directly impair microglial phagocytosis of fibrillar A β in culture, and that this effect could be reversed in the presence of the Th2 cytokines IL-4 or IL-10 [29].

Although TNF- α and IL-1 β are innate immune cytokines that are often associated with pro-inflammatory Th1 immune responses, it is noteworthy that these cytokines are multi-functional molecules with numerous activities in the brain under both physiological and pathophysiological conditions. For example, it has been demonstrated that central blockade of IL-1 transmission in transgenic mice with brain-directed overexpression of human soluble IL-1 receptor antagonist leads to an atrophic brain phenotype, with modified levels of APP and PS1 expression [36]. This report highlights the dual role of IL-1 β as both a stimulus for neuroinjury when produced at high levels and as a neurotopic factor under more physiological conditions, and it is well known that TNF- α can behave in a similar dual fashion. Thus, pharmacotherapeutics aimed at reducing bioactivity of these pro-inflammatory innate cytokines would need to be carefully titrated to avoid unwanted adverse events.

In a recent report, the Th1/Th2 fate selection choices of naïve CD4⁺ T cells lacking γ -secretase, RBP-J (the key downstream Notch transcriptional regulator), or both were not impaired [37]. Based on their results, Ong and coworkers concluded that PS1 and Notch may act as genetic modifiers of T helper cell differentiation, but may not be obligatory factors for this process. In terms of the present work, we suggest that Δ E9 mutant PS1 promotes more efficient Th1 skewing, but we wish to clarify that PS1 mutation is likely not obligatorily required for Th1 differentiation. Given that Th1/Th2 fate determination is paramount to enable proper functioning of adaptive immunity, it is becoming clear that a whole host of molecules have likely evolved to referee this process.

Yet, dysfunction/imbalance in Th immunity has been demonstrated in the elderly and in AD patients. Recent studies have indicated skewness from Th2 to Th1 immunity with aging (the greatest risk factor for AD) [38], and a similar imbalance in favour of Th1 immunity has been noted in AD patients [39]. In fact, the term 'inflammaging' has been coined by Franceschi and colleagues [40] to describe the reduction in anti-inflammatory immune responses and shift in favour of pro-inflammatory Th1 immunity that occurs with aging and even more so with AD [39].

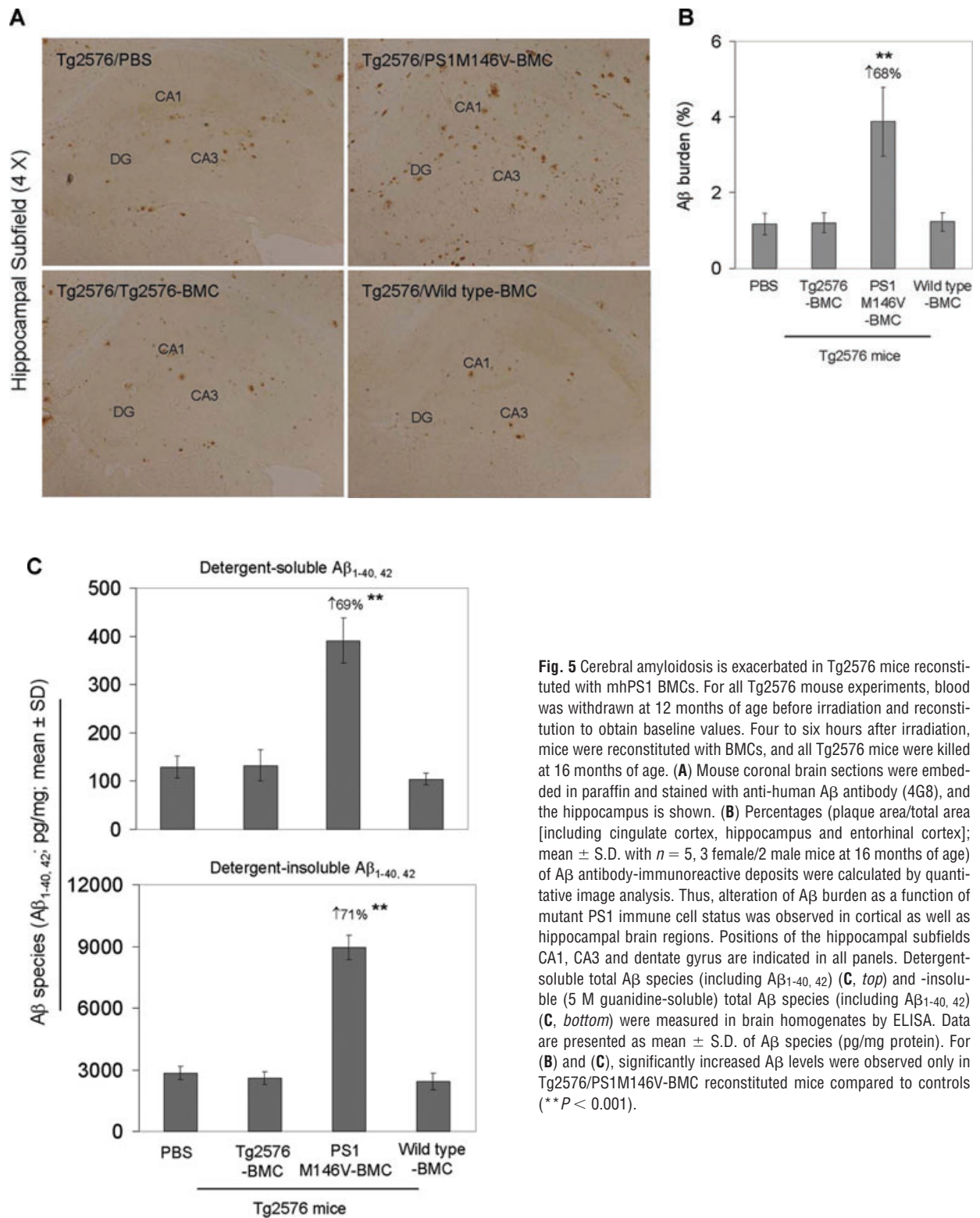


Fig. 5 Cerebral amyloidosis is exacerbated in Tg2576 mice reconstituted with mhPS1 BMCs. For all Tg2576 mouse experiments, blood was withdrawn at 12 months of age before irradiation and reconstitution to obtain baseline values. Four to six hours after irradiation, mice were reconstituted with BMCs, and all Tg2576 mice were killed at 16 months of age. **(A)** Mouse coronal brain sections were embedded in paraffin and stained with anti-human Aβ antibody (4G8), and the hippocampus is shown. **(B)** Percentages (plaque area/total area [including cingulate cortex, hippocampus and entorhinal cortex]; mean ± S.D. with $n = 5$, 3 female/2 male mice at 16 months of age) of Aβ antibody-immunoreactive deposits were calculated by quantitative image analysis. Thus, alteration of Aβ burden as a function of mutant PS1 immune cell status was observed in cortical as well as hippocampal brain regions. Positions of the hippocampal subfields CA1, CA3 and dentate gyrus are indicated in all panels. Detergent-soluble total Aβ species (including Aβ_{1-40,42}) **(C, top)** and -insoluble (5 M guanidine-soluble) total Aβ species (including Aβ_{1-40,42}) **(C, bottom)** were measured in brain homogenates by ELISA. Data are presented as mean ± S.D. of Aβ species (pg/mg protein). For **(B)** and **(C)**, significantly increased Aβ levels were observed only in Tg2576/PS1M146V-BMC reconstituted mice compared to controls (** $P < 0.001$).

In our present work, we show that the pro-inflammatory innate immune cytokines IL-1 β and TNF- α are reduced in the PSAPP mouse model following reconstitution with wild-type PS1 immune cells (Fig. 2A). Also in that figure, we noted corresponding increases in the anti-inflammatory cytokines IL-4 and IL-10 under the same condition. These results dovetail with cytokine data reported in AD patients *versus* controls. Specifically, Lombardi and colleagues reported that stimulated blood mononuclear cells from AD patients produced higher amounts of IL-1 and TNF- α compared with healthy persons [41], and Bruunsgaard and coworkers reported that high serum TNF- α concentrations were positively associated with AD and with other pro-inflammatory markers in centenarians [42]. Another study found increased TNF- α levels in patients with late-onset AD compared to non-demented controls [43]. Increased TNF- α levels were even found in individuals presenting with mild cognitive impairment [44], now widely recognized as a precursor to AD. Finally, in a recent unbiased screen for plasma signalling proteins, Ray and colleagues and Britschgi and Wyss-Coray identified 18 blood plasma signalling proteins that correctly classify AD with ~90% accuracy and predict conversion from mild cognitive impairment to AD. Strikingly, more than half of these plasma proteins were immune/inflammatory signalling proteins [45, 46]. Thus, it seems that AD patients do demonstrate dysregulation of systemic pro-inflammatory responses, and that such indices may serve as biomarkers for mild cognitive impairment and AD.

A notable limitation of these present studies involves differentiation of endogenous *versus* donor microglia, although we specifically selected and utilized a method proposed by Mildner and colleagues [18] that demonstrated that by utilizing linear head-sparing lethal X-ray irradiation, no donor monocytes

invaded into the brain, however we recognize the unlikely possibility of that small numbers of donor monocytes may have invaded into CNS tissues of the animals analysed in our studies.

In summary, we suggest that peripheral immune system dysfunction associated with an Δ E9 mhPS1 allele exacerbates cerebral amyloidosis by impacting brain A β clearance. Our study is limited in that not all familial AD mutations involve an exon 9 mutation in the PS1 gene. It is certainly possible that exon 9 deleted PS1 mutants behave phenotypically different compared to other familial AD PS1 mutants. In this regard, it is interesting to note the paradoxical nature of PS1 mutations that as many as one third of the 467 amino acids in the PS1 open reading frame are targets for disease-causing mutations, yet these clinical mutations all seem to lead to a similar toxic gain-of-function for PS1 [17]. Nonetheless, if results from these next-generation chimeric mouse models of AD are applicable to the human syndrome, then they open the possibility for a novel therapeutic approach aimed at restoring PS1 immune homeostasis.

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