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Annotating PDFs using Adobe Reader X

Version 1.2 June 28, 2012

1. Know your version

The screen images in this document were captured on a Windows PC running Adobe Reader X. Though some of the images may differ in appearance from your platform/version, basic functionality remains similar. At the time of this writing, Adobe Reader X v10.1 is freely available and can be downloaded from:
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eProof files are self-contained PDF documents for viewing on-screen and for printing. They contain all appropriate formatting and fonts to ensure correct rendering on-screen and when printing hardcopy. DJS sends eProofs that can be viewed, annotated, and printed using the free version of Acrobat Reader 7 (or greater). These eProofs are "enabled" with commenting rights, therefore they can be modified by using special markup tools in Acrobat Reader that are not normally available unless using the Standard or Professional version.

3. Comment & Markup toolbar functionality

A. Show the Comment & Markup toolbar

The Comment & Markup toolbar doesn't appear by default. Do one of the following:

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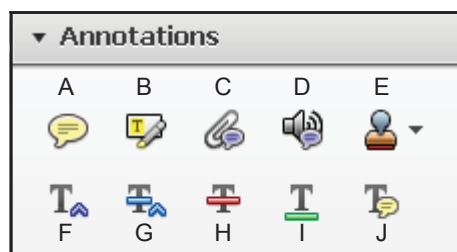
B. Select a commenting or markup tool from the Annotations window.

Note: After an initial comment is made, the tool changes back to the Select tool so that the comment can be moved, resized, or edited. (The Pencil, Highlight Text, and Line tools stay selected.)

C. Keep a commenting tool selected

Multiple comments can be added without reselecting the tool.
Select the tool to use (but don't use it yet).

- Right Click on the tool.
- Select Keep Tool Selected.



- A. Sticky Note tool
- B. Highlight Text tool
- C. Attach File tool
- D. Record Audio tool
- E. Stamp Tool
- F. Insert Text tool
- G. Replace Text tool
- H. Strikethrough Text tool
- I. Underline Text tool
- J. Add Note to Text tool

4. The Properties bar

The Properties bar can be used to format text and select options for individual tools.

To view the Properties bar, do one of the following:

- Right-click the toolbar area; choose Properties Bar.
- Select [Ctrl-E]



5. Using the comment and markup tools

To *insert*, *delete*, or *replace* text, use the corresponding tool. Select the tool, then select the text with the cursor (or simply position it) and begin typing. A pop-up note will appear based upon the modification (e.g., inserted text, replacement text, etc.). Use the Properties bar to format text in pop-up notes. A pop-up note can be minimized by selecting the  button inside it.

Pop-Up Text Properties

editorial

Still another activity by the highly promiscuous enzyme CYP3A4: 25-hydroxylation of cholesterol¹

Ulf Diczfalussy² and Ingemar Björkhem, *Editorial Board*
Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

Comment A: Please note that this article exists for test purposes only.

Comment D: put some awesome text here

Comment F: text in comment boxes can be colored; you can also change the color of the box itself

Sticky Note B: (Yellow)

Highlighted Text C: demonstrate 25-hydroxylase

Inserted Text D: In one study on the rate of 24

Crossed-out Text E: In one study on the rate of 24

Replaced Text F: A logical consequence of the fact that cholesterol is a substrate for CYP3A4 is that it may function as an inhibitor of the enzyme's activity.

- A. Sticky Note
- B. Attached file
- C. Highlighted text
- D. Inserted text
- E. Crossed-out (strike-through) text
- F. Replaced text

6. Inserting symbols or special characters

An 'insert symbol' feature is not available for annotations, and copying/pasting symbols or non-keyboard characters from Microsoft Word does not always work. Use angle brackets < > to indicate these special characters (e.g., <alpha>, <beta>).

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- Use the arrow keys to move the cursor beside the text to be edited.
- Hold down the shift key while simultaneously using arrow keys to select the block of text, if necessary.
- Insert, replace, or delete text, as needed.

8. Summary of main functions

Insert text - Use Insert Text tool (position cursor and begin typing)

Replace text - Use Replace Text tool (select text and begin typing)

Delete text - Use Strikethrough Text tool (select text and press delete key)

Highlight text - Use Highlight Text tool (select text)

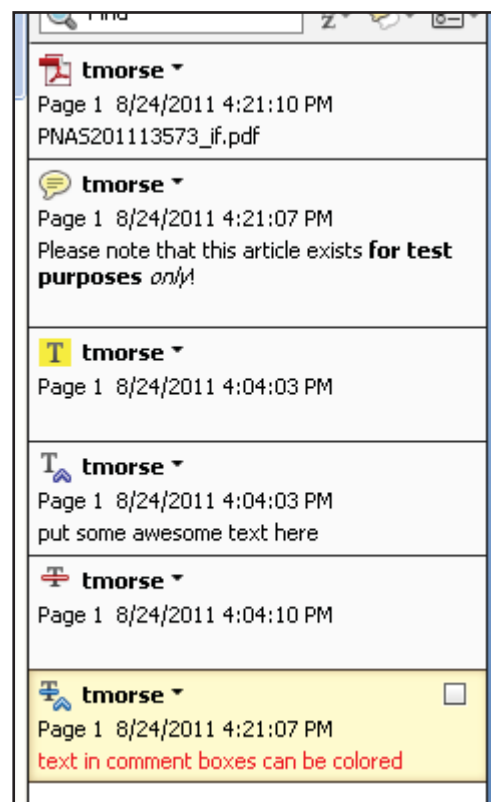
Attach a file - Use the Attach a File with Comment tool (select tool, position cursor and click mouse, select file)

9. Reviewing changes

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- Click the Comments button to reveal the comment tools
- Click the triangle next to Comments List (if not already visible)

Note: *Selecting a correction in the list will highlight the corresponding item in the document, and vice versa.*



Use the Comments list to review all changes

IFN- γ Production by Amyloid β -Specific Th1 Cells Promotes Microglial Activation and Increases Plaque Burden in a Mouse Model of Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by the presence of amyloid- β (A β)-containing plaques, neurofibrillary tangles, and neuronal loss in the brain. Inflammatory changes, typified by activated microglia, particularly adjacent to A β plaques, are also a characteristic of the disease, but it is unclear whether these contribute to the pathogenesis of AD or are a consequence of the progressive neurodegenerative processes. Furthermore, the factors that drive the inflammation and neurodegeneration remain poorly understood. CNS-infiltrating T cells play a pivotal role in the pathogenesis of multiple sclerosis, but their role in the progression of AD is still unclear. In this study, we examined the role of A β -specific T cells on A β accumulation in transgenic mice that overexpress amyloid precursor protein and presenilin 1 (APP/PS1). We found significant infiltration of T cells in the brains of APP/PS1 mice, and a proportion of these cells secreted IFN- γ or IL-17. A β -specific CD4⁺ T cells generated by immunization with A β and a TLR agonist and polarized in vitro to Th1-, Th2-, or IL-17-producing CD4⁺ T cells, were adoptively transferred to APP/PS1 mice at 6 to 7 mo of age. Assessment of animals 5 wk later revealed that Th1 cells, but not Th2 or IL-17-producing CD4⁺ T cells, increased microglial activation and A β deposition and that these changes were associated with impaired cognitive function. The effects of Th1 cells were attenuated by treatment of the APP/PS1 mice with an anti-IFN- γ Ab. Our study suggests that release of IFN- γ from infiltrating Th1 cells significantly accelerates markers of diseases in an animal model of AD. *The Journal of Immunology*, 2013, 190: 000–000.

A role for inflammation in the pathogenesis of Alzheimer's disease (AD) is suggested by epidemiological studies that have reported a decreased incidence of AD in patients treated with nonsteroidal anti-inflammatory drugs (1, 2); these findings are supported by evidence of preventative effects of these drugs in animal models of AD (3). Whereas the classical characteristics of AD are the presence of amyloid- β (A β) plaques and neurofibrillary tangles, together with selective neuronal loss, there is also evidence of innate immune activation in AD, with activation of microglia, the primary resident immune cell of the CNS. Activated microglia are found in the brain of AD patients with mild to moderate dementia (4) and in a significant proportion of cases with mild cognitive impairment (5). Microglia secrete

inflammatory cytokines like IL-1 β and TNF- α , which increase activity and expression of secretases (6, 7), contributing to A β deposition and the early pathogenic changes in AD (8). Inflammatory cytokines released from activated microglia are known to be potentially cytotoxic, but there is evidence indicating a positive effect of an inflammatory environment on A β clearance (9–11). Microglia demonstrate significant plasticity and also adopt other phenotypes that are associated with tissue repair (12). Furthermore, immune cells in the AD brain can have an alternative activated state as well as the classical proinflammatory phenotype (13). Cell-surface expression of MHC class II and costimulatory molecules is enhanced on activated microglia (14, 15), enabling them to act as APC. However, circulating cells, including T cells, are infrequently observed in the normal CNS, although there is a population of perivascular macrophages, distinct from microglia (16–18), and these cells may play an important anti-inflammatory function, perhaps mediated by a change in hypothalamic–pituitary–adrenal axis function (19).

The blood–brain barrier plays a key role in protecting the brain, restricting the entry of pathogens and macromolecules. An intact blood–brain barrier is also important in restricting entry of circulating cells, and increased blood–brain barrier permeability, which is a characteristic of several neurodegenerative conditions including multiple sclerosis, AD, and Parkinson's disease (20–22), is associated with infiltration of circulating immune cells. Studies in multiple sclerosis and experimental allergic encephalomyelitis (EAE), a mouse model of multiple sclerosis, have shown that T cells, particularly IL-17-producing CD4⁺ T cells (Th17) cells, infiltrate the brain and spinal cord and are central to the pathogenesis of the disease (23). The role of Th1 cells in CNS inflammation associated with EAE is more controversial, with some studies suggesting that Th1 cells contribute to pathology and

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The online version of this article contains supplemental material.

Abbreviations used in this article: AD, Alzheimer's disease; A β , amyloid- β ; APP, amyloid precursor protein; dH₂O, distilled H₂O; EAE, experimental autoimmune encephalomyelitis; HBSS/FBS, HBSS containing 3% FBS; PS1, presenilin 1; RT, room temperature; Th17 cells, IL-17-producing CD4⁺ T cells; WT, wild-type.

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others suggesting a protective role for IFN- γ through inhibition of Th17 cells. As well as their role in demyelination, the interaction of T cells with microglia contributes to the inflammatory changes observed in EAE (24).

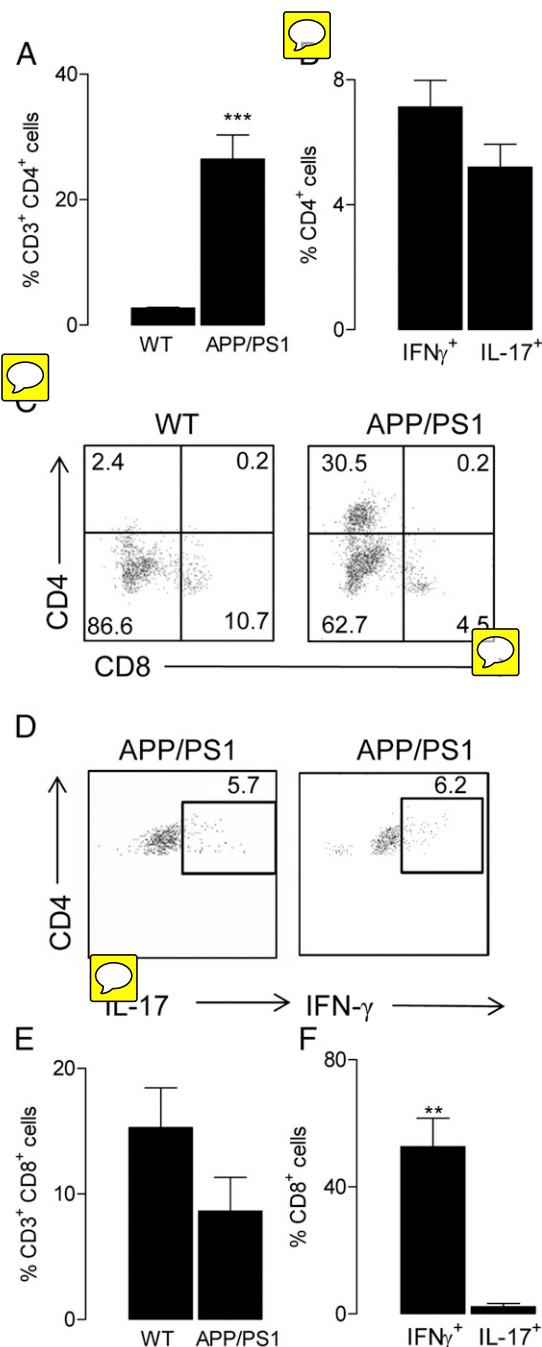


FIGURE 1. Th1 and Th17 infiltrate the brain of APP/PS1 mice. Mononuclear cells were prepared from the brain of APP/PS1 and WT mice, and cells were surface stained with Abs specific for CD3, CD4, CD8, intracellular IL-17, and IFN- γ , and flow cytometric analysis was performed. Mean frequency (A) and representative dot plots (B) of CD4⁺ and CD8⁺ cells in brain of WT and APP/PS1 mice. Mean frequency (C) and representative dot plots (D) of CD4⁺ cells stained positively for IFN- γ and IL-17 in brain of APP/PS1 mice. (E) Mean frequency of CD8⁺ in brain of tissue prepared from WT and APP/PS1 mice. (F) Mean frequency of CD8⁺ cells stained positively for IFN- γ and IL-17 in brain tissue prepared from APP/PS1 mice. ** p < 0.01, Student t test for independent means ($n \geq 4$), *** p < 0.001, Student t test for independent means. Representative of three experiments.

T cells are also present in the brain of patients with AD (25–28), and infiltration may result from increased expression of CXCR2 and MIP-1 α on the T cells (29). Although T cells, in particular Th2 or regulatory T cells, can have a protective role in the brain (30, 31), the entry of activated effector T cells, particularly Th1 or Th17 cells, into the brain in which inflammatory changes are ongoing, is likely to escalate the inflammatory cascade. Consistent with this is the finding that A β -induced release of inflammatory cytokines from glia was exacerbated by Th1 and Th17 cells (32), and this effect was attenuated by Th2 cells. Immunization with A β peptides, formulated with various adjuvants, is being evaluated both in preclinical models and in the clinic as a potential therapy for AD based on Ab-mediated reduction of A β plaque burden (33). However, a proportion of AD patients who received a vaccine containing A β peptide formulated with the adjuvant QS21 (AN1792) developed meningoencephalitis (34). It is possible that the generation of certain subtypes of A β -specific T cells may contribute to inflammatory pathology in AD.

In this study, we used a transgenic mouse model of AD that overexpresses amyloid precursor protein (APP) with the Swedish mutation and exon-9-deleted presenilin 1 (PS1; APP/PS1 mice) to

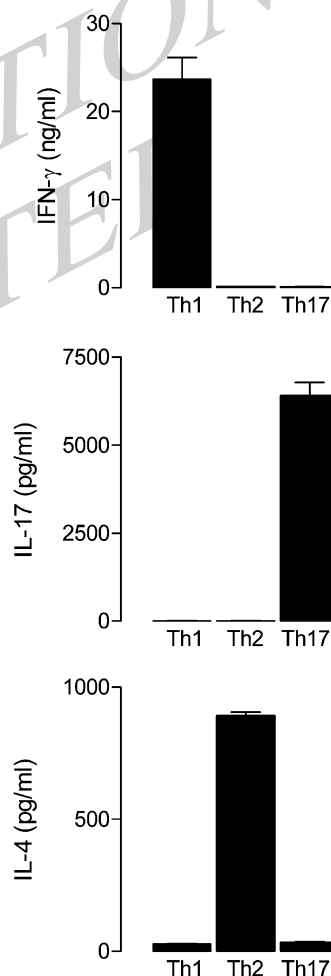


FIGURE 2. Cytokine production by in vitro polarized A β -specific T cells. Popliteal lymph nodes harvested from mice immunized with A β and CpG were cultured with A β _{1–42} in the presence of IL-12 to generate Th1 cells, dexamethasone, IL-4, and anti-IFN- γ to generate Th2 cells, or IL-23 and anti-IFN- γ to generate Th17 cells. IFN- γ , IL-4, and IL-17 concentrations were determined by ELISA on supernatants removed 3 d after stimulation with Ag and APC. Values are expressed as means $\pm$ SEM ($n = 4$); representative of four experiments.

determine whether A β -specific T cell subsets can modulate A β burden and affect microglial activation. A β -specific effector T cells were generated by immunization with A β and CpG, polarized *in vitro* to Th1, Th2, and Th17 cells, and adoptively transferred to 6- to 7-mo-old APP/PS1 mice. We found that A β -specific Th1 cells increased A β deposition and microglial activation in APP/PS1 mice and negatively impacted on spatial learning. Treatment of mice with anti-IFN- γ Ab ameliorated these changes, suggesting that release of IFN- γ from infiltrating Th1 cells accelerates the pathology in these animals.

Materials and Methods

Animals

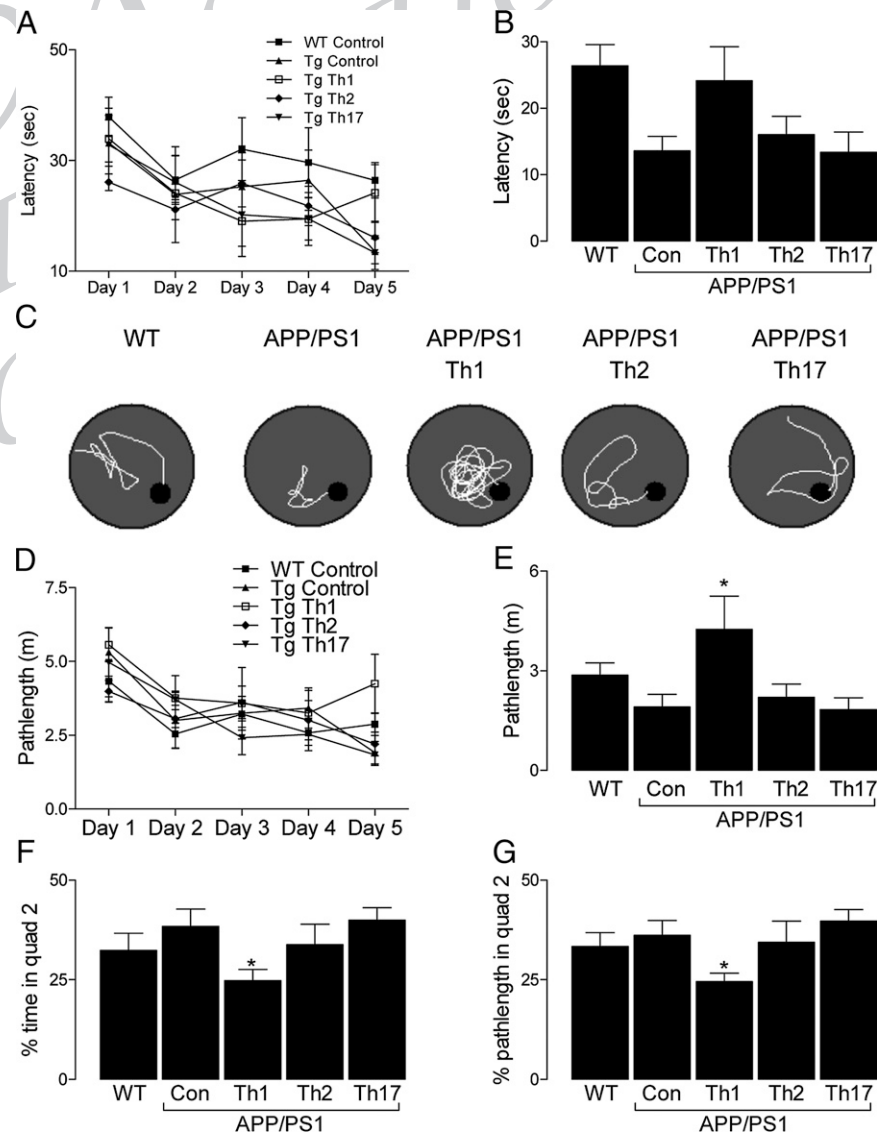
APP/PS1 mice and wild-type (WT) littermates (6 to 7 mo old) were obtained from The Jackson Laboratory and subsequently bred in a specific pathogen-free unit in the Bioresources Unit in Trinity College Dublin. GFP mice were a gift from Matthew Campbell, School of Genetics and Microbiology, Trinity College Dublin. Mice used were transgenic animals on a C57/Bl6J background expressing eGFP cDNA under the control of a chicken β -actin promoter and CMV enhancer. All mice were maintained in controlled conditions (temperature 22 to 23°C, 12-h light-dark cycle, and food and water *ad libitum*) under veterinary supervision, and experimentation was carried out under a license granted by the Minister for Health and Children (Ireland) and with the appropriate ethical approval.

Isolation and FACS analysis on mononuclear cell isolation from CNS tissue

APP/PS1 mice and nontransgenic littermates were anesthetized with sodium pentobarbital (40 μ l) and perfused intracardially with sterile ice-cold PBS (20 ml). The brain was removed and placed in HBSS (2 ml) containing 3% FBS (HBSS/FBS; Sigma-Aldrich). Tissue was dissociated through a sterile 70- μ m nylon mesh filter, washed with HBSS/FBS, and centrifuged at $170 \times g$ for 10 min at room temperature (RT). The supernatant was removed and the remaining pellet resuspended in HBSS/FBS (2 ml) containing collagenase D (1 mg/ml; Roche) and DNase I (10 μ g/ml; Sigma-Aldrich) and incubated for 1 h at 37°C. Cells were washed in HBSS/FBS and centrifuged at 1200 rpm for 5 min. Supernatants were removed, and cells were resuspended in 1.088 g/ml Percoll (9 ml; Sigma-Aldrich). This was underlaid with 1.122 g/ml Percoll (5 ml) and overlaid with 1.072 g/ml Percoll (9 ml) followed by 1.030 g/ml Percoll (9 ml) and finally PBS (9 ml). Percoll gradients were centrifuged at $1250 \times g$ for 45 min at 18°C. Mononuclear cells were removed from between the 1.088/1.072 and 1.072/1.030 g/ml interfaces, washed twice in HBSS/FBS, and counted.

Mononuclear cells prepared from CNS tissue were prepared for intracellular staining using a cell permeabilization kit (DakoCytomation). Cells were centrifuged at 1,200 rpm for 5 min before stimulation with X-Vivo media (200 μ l) containing PMA (10 ng/ml; Sigma-Aldrich), ionomycin (1 μ g/ml; Sigma-Aldrich), and brefeldin A (5 μ g/ml; Sigma-Aldrich) for 5 h. Following stimulation, cells were centrifuged at 1200 rpm for 5 min and resuspended. Low-affinity IgG receptors (Fc γ RIII) were blocked by incubating cells in FACS buffer (50 μ l/sample) containing CD16/CD32

FIGURE 3. Spatial learning is impaired in APP/PS1 mice that received A β -specific Th1 cells. A β -specific Th1, Th2, and Th17 cells, generated as described in Fig. 2, were injected *i.v.* into APP/PS1 mice at 6 to 7 mo of age. Two weeks after injection, cognitive function was analyzed in the Morris Water Maze test. Training commenced after 1 d of habituation and continued for 5 consecutive d on which the mice underwent four 1-min trials with an intertrial interval of 5 min. The day after the final day of training, the platform was removed, and mice were given a single 60-s probe trial. The percentage of time each animal spent swimming in the quadrant previously containing the platform was measured. Path length was also assessed. **(A)** The latency to reach the platform of all groups. **(B)** Mean latency on d 5 of training. **(C)** Sample paths for individual mice in each treatment group. **(D)** and **(E)** The path length taken to reach the platform. **(F)** and **(G)** In the probe test, the percentage of the total time and distance (i.e., path length) each animal spent swimming in the quadrant was measured. * $p < 0.05$, ANOVA; $n \geq 5$; representative of two experiments. Con, control; Tg, transgenic.



Fc γ RIII (1:100) for 10 min at RT. Cells were incubated in 50 μ l/sample FACS buffer containing the appropriate FACS Abs for 15 min at RT and fixed in IntraStain Reagent A (50 μ l/sample; DakoCytomation) for 15 min at RT. Cells were washed twice with FACS buffer and centrifuged at 1200 rpm for 5 min, permeabilized with IntraStain Reagent B (50 μ l/sample; DakoCytomation) plus intracellular Abs for 15 min at RT in the dark, washed twice in FACS buffer, and centrifuged at 1200 rpm for 5 min. Immunofluorescence analysis was performed on a DakoCytomation Cyan, data acquired using Summit software (DakoCytomation), and the results analyzed using FlowJo software (Tree Star).

Generation of A β -specific T cell lines and in vivo transfer

WT mice were immunized in the footpad with A β ₁₋₄₂ (75 μ g/mouse) and CpG (25 μ g/mouse) and boosted after 21 d. Mice were sacrificed 7 d later; the spleens and popliteal lymph nodes were harvested and restimulated with A β ₁₋₄₂ (25 μ g/ml) in the presence of IL-12 (10 ng/ml) to generate Th1 cells, dexamethasone (1×10^{-8} M), IL-4 (10 ng/ml), and anti-IFN- γ (5 μ g/ml) to generate Th2 cells, or IL-23 (10 ng/ml) and anti-IFN- γ (5 μ g/ml) to generate Th17 cells. After 4 d, IL-2 (5 ng/ml) was added to the Th1 and Th2 cell preparations, RPMI culture medium only was added to the Th17 cell cultures, and incubation continued for a further 7 d. Cells were washed and injected i.v. (15×10^6 cells/mouse in 300 μ l serum-free medium) into 6- to 7-mo-old APP/PS1 mice. Control animals received in 300 μ l serum-free medium alone. Behavior analysis was assessed 2 wk after T cell transfer. Samples of supernatant were assessed by ELISA (see below) for IFN- γ , IL-4, IL-10, IL-17, and IL-5 production.

In a separate series of experiments, 6- to 7-mo-old APP/PS1 and WT control mice were injected i.p. with anti-IFN- γ Ab (600 μ g) or a control Ab (β -galactosidase: 600 μ g; R&D Systems) and after 24 h were injected i.v. with Th1 cells (15×10^6 cells/mouse) as described above. Anti-IFN- γ or β -galactosidase Ab injections were repeated 3, 7, 10, 14, 17, 21, 24, 28, and 31 d after T cell transfer. Behavioral analysis was assessed 21 d after T cell transfer.

Tracking of A β -specific Th1 cells into the brain

A β -specific Th1 were generated from GFP mice immunized with A β ₁₋₄₂ and CpG, restimulated in vitro with A β ₁₋₄₂ and IL-12, and expanded with IL-2 as described above. Cells were washed and injected i.v. (15×10^6 cells/mouse) into 6- to 7-mo-old APP/PS1 mice or WT mice. Mice were sacrificed 14 d later and mononuclear cells prepared from CNS tissue. Cells were stimulated with PMA and ionomycin and stained for surface CD3, CD4, CD8, and intracellular IFN- γ . Immunofluorescence analysis was performed on a DakoCytomation Cyan as described above.

Behavioral analysis

Gait was analyzed in WT and APP/PS1 mice using the footprint test to assess stride length, hind- and front limb base widths. Muscular strength and coordination were assessed using the inverted screen and wire-hang tests. Two days later, 2 wk after administration of A β -specific T cells, mice were tested for spatial memory in the Morris water maze. The pool (1.2 m diameter; 0.6 m high; 0.24 m water depth; 0.15 m platform diameter placed in the northwest quadrant of the pool; 0.13 m from the edge of the pool) was sited in a well-lit room (22 to 23°C), and distinct visual cues were placed on the curtains that encircled the pool. Training commenced after 1 d of habitation and continued for 5 consecutive d on which the mice underwent four 1-min trials with an intertrial interval of 5 min. Each trial ended when mice located the platform or after 60 s when mice that failed to locate the platform were led to it; animals remained on the platform for 20 s. The day after the final day of training, the platform was removed, and mice were given a single 60-s probe trial. The percentage of time each animal spent swimming in the quadrant previously containing the platform was measured. Path length was also assessed.

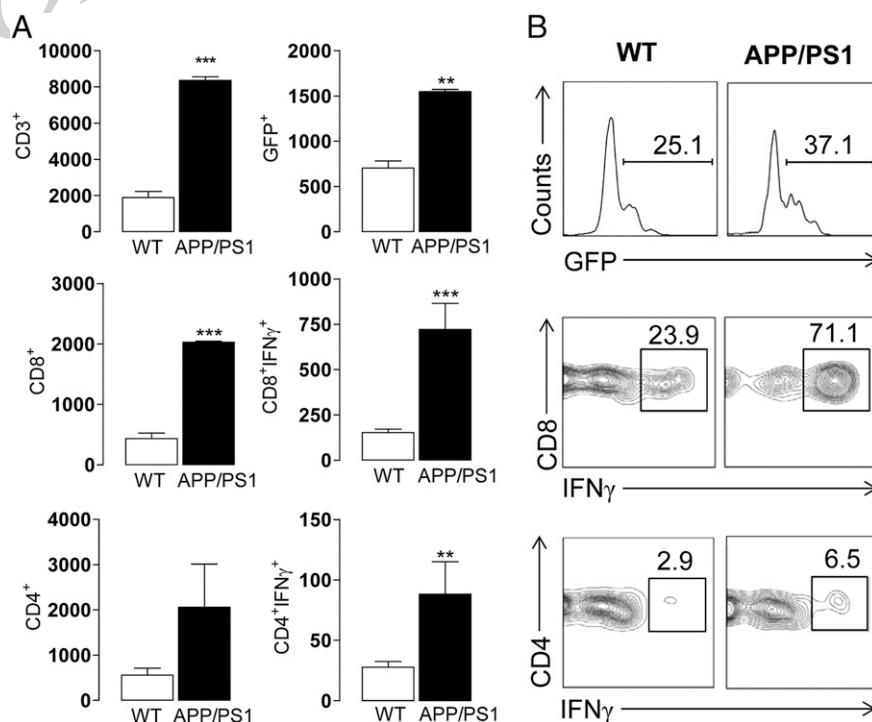
Preparation of tissue

In the first study, in which the effect of transfer of Th1, Th2, and Th17 cells was assessed, mice were killed 24 h after the last behavioral analysis. In the second study, in which the effect of anti-IFN- γ Ab was assessed, mice were killed 34 d after the first injection of Ab. They were anesthetized with sodium pentobarbital (40 μ l; Euthatal; Merial Animal Health) and perfused intracardially with ice-cold PBS (20 ml). The brains were rapidly removed, and one half of the brain was stored for later extraction and analysis of A β . The second half of the brain, which was used for immunohistochemical analysis, was placed onto cork discs, coated with optimum cooling temperature compound (Sakura Tissue-Tek), snap-frozen in prechilled isopropanol, and stored at -80°C. Before sectioning, the tissue was allowed to equilibrate to -20°C for 2 h. Sagittal sections (10- μ m thick) were prepared using a cryostat (Leica, Meyer, U.K.), mounted on gelatin-coated (Flukaerland) glass slides, allowed to dry for 20 min, and stored at -20°C for later immunohistochemical analysis.

Detection of A β

Snap-frozen cortical tissue was homogenized in five volumes (w/v) of homogenizing buffer (SDS/NaCl in distilled H₂O [dH₂O] with proteases) and centrifuged (15,000 rpm, 40 min, 4°C). The supernatant samples were removed to extract SDS-soluble A β , and the pellets were kept for extraction of insoluble A β . Supernatants were equalized (3 mg/ml) with homogenizing buffer using a BCA protein assay, and samples were neutralized by the addition of 10% (w/v) 0.5 M Tris-HCl (pH 6.8). Samples

FIGURE 4. Transferred A β -specific Th1 cells migrate into the brain of APP/PS1 mice. A β -specific Th1 cells, generated from GFP mice were injected i.v. into 6- to 7-mo-old WT or APP/PS1 mice. Two weeks after injection, mice were sacrificed, and mononuclear cells were prepared from the brain. Cells were surface-stained with Abs specific for CD3, CD4, and CD8 and intracellularly stained for IFN- γ , and flow cytometric analysis was performed to quantify GFP-expressing and IFN- γ -secreting T cells in the brain. (A) Results are mean absolute number of the indicated cells in the brain. (B) Sample FACS plots of GFP⁺ T cells (gated on CD3), IFN- γ ⁺CD8⁺, and IFN- γ ⁺CD4⁺ cells; number represent percentage positive. Data in (A) represent mean $\pm$ SEM from four animals per experimental group from one experiment; data in (B) representative of four mice. *** p < 0.01, **** p < 0.001, Student t test for independent means.



were stored at -20°C for later detection of soluble A β . Pellets were incubated in guanidine buffer (50 μl ; 5 M guanidine-HCl/50 mM Tris-HCl, pH 8; Sigma-Aldrich) for 4 h on ice. Samples were centrifuged (15,000 rpm, 30 min, 4°C), and the supernatant samples were equalized (0.3 mg/ml) with guanidine buffer and stored at -20°C for later detection of insoluble A β using MSD 96-well multi-spot 4G8 A β triple ultra-sensitive assay kits according to the manufacturer's instructions (Meso Scale Discovery). Standards (A β_{1-38} , 0–3,000 pg/ml; A β_{1-40} , 0–10,000 pg/ml; A β_{1-42} , 0–3,000 pg/ml) and samples were added to the 96-well plates, incubated (2 h, RT), washed, and read in a Sector Imager plate reader (Meso Scale Discovery) immediately after addition of the MSD read buffer. A β concentrations were calculated with reference to the standard curves and expressed as picograms per milliliter.

Immunohistochemistry

Cryostat sections were assessed for A β plaque deposition by staining with Congo red. Sections, equilibrated to RT, were fixed in ice-cold methanol for 5 min, washed in PBS, and incubated at room temperature for 20 min in an alkaline solution prepared by adding NaOH (2 ml; 1 M) to saturated NaCl (200 ml; 80% ethanol in dH_2O). Thereafter, sections were incubated in filtered Congo red solution (0.2% Congo red dye in the same alkaline solution) for 30 min, rinsed in dH_2O , incubated in methyl green solution (1% in dH_2O) for 30 s, washed, and dehydrated by dipping in 80, 95, and then 100% ethanol. Sections were dried, incubated in 100% xylene (3×5 min), mounted onto slides using dibutyl phthalate in xylene (RA Lamb), and allowed to dry overnight.

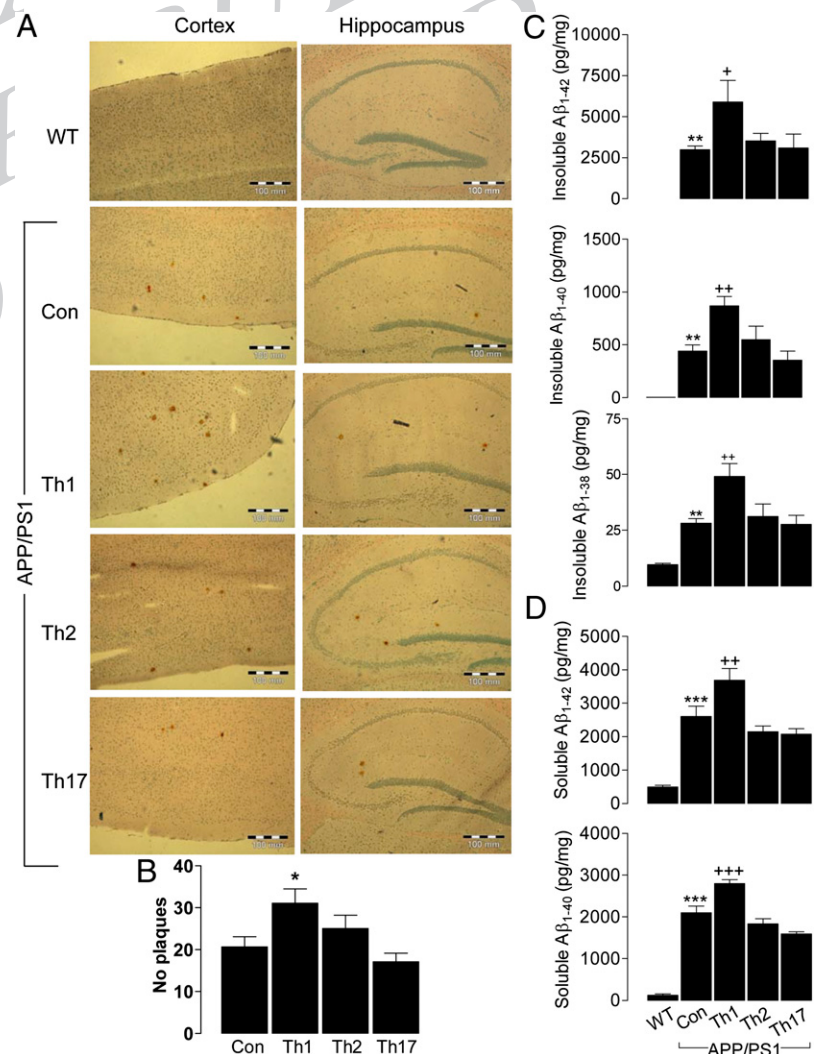
To assess CD11b, sections were fixed in an acetone/ethanol mixture (1:1) for 5–10 min, and endogenous peroxidase activity was blocked by incubating in 0.3% H_2O_2 in PBS for 5 min. Sections were washed, blocked in 10% rabbit serum (Vector Laboratories), incubated overnight at 25°C in rat anti-CD11b Ab (1:100 in 5% rabbit serum in PBS; clone 5C6; Serotec),

washed, and incubated for 2 h at RT in biotinylated rabbit anti-rat IgG (1:200 in 5% rabbit serum in PBS; Vector Laboratories). Sections were washed, incubated in Vectastain Elite ABC reagent (2 drops of A/B in 5 ml PBS; Vector Laboratories) for 1 h at RT, washed, and developed using the substrate 3, 3'-diaminobenzidine-enhanced liquid substrate system tetrahydrochloride (one drop solution B in 1 ml solution A) for ~ 10 min until the color developed and counterstained with 1% methyl green for 10 min. Samples were dehydrated by dipping in graded ethanol (70, 80, 95, and 100%) and incubating in xylene (VWR International). Sections were mounted with dibutyl phthalate in xylene, dried overnight, and stored at RT. The sections were examined using an Olympus lx51 light microscope (Olympus, Tokyo, Japan), and micrographs were taken using an Olympus UCMAD3 (Olympus) at $\times 40$ magnification. Data were quantified using the Immunoratio plugin (<http://imtmicroscope.uta.fi/immunoratio/>) available for the ImageJ software package (National Institutes of Health) (35). Colocalization of A β and CD11b was examined by confocal microscopy. Frozen brain sections brought to RT, fixed in ice-cold methanol, washed, permeabilized in 0.1% Triton (Sigma-Aldrich) in PHEM buffer, and washed. Nonspecific binding was blocked by incubating sections in 10% normal goat serum (2 h, RT), and sections were incubated overnight with pan-A β Ab (1:1000; Calbiochem) and rat anti-CD11b Ab (1:100, clone 5C6, AbD; Serotec) in 5% normal goat serum in PHEM buffer. Sections were washed, incubated in secondary Ab ALEXA 488 (1:4000; Invitrogen) and Alexa 546 (1:1000; Invitrogen; 90 min, RT), washed, mounted, and analyzed using confocal microscopy (Axioplan 2; Zeiss).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad). Data were analyzed using Student *t* test, two-way ANOVA, or one-way ANOVA followed by Newman-Keuls post hoc test. Data are expressed as means with SEM and deemed statistically significant when $p < 0.05$.

FIGURE 5. Transfer of A β -specific Th1 cells enhanced A β deposition in brains of APP/PS1 mice. APP/PS1 mice were injected with A β -specific Th1, Th2, or Th17 cells as described in Fig. 3. (A) Cryostat sections were stained with Congo red to assess A β -containing plaques in hippocampus and cortex; the mean number of plaques was recorded (B). (C) The concentrations of insoluble A β_{1-42} , A β_{1-40} , and A β_{1-38} in the cortical tissue were quantified by ELISA. (D) The concentrations of soluble A β_{1-42} and A β_{1-40} in cortical tissue were quantified by ELISA. * $p < 0.05$, ANOVA. ** $p < 0.01$, ANOVA, APP/PS1 versus WT. + $p < 0.05$; ++ $p < 0.01$, ANOVA versus control untreated APP/PS1 mice ($n = 5$ to 6); representative of two experiments. Con, control.



Results

Th1 and Th17 cell are present in the periphery and infiltrate the brains of APP/PS1 mice

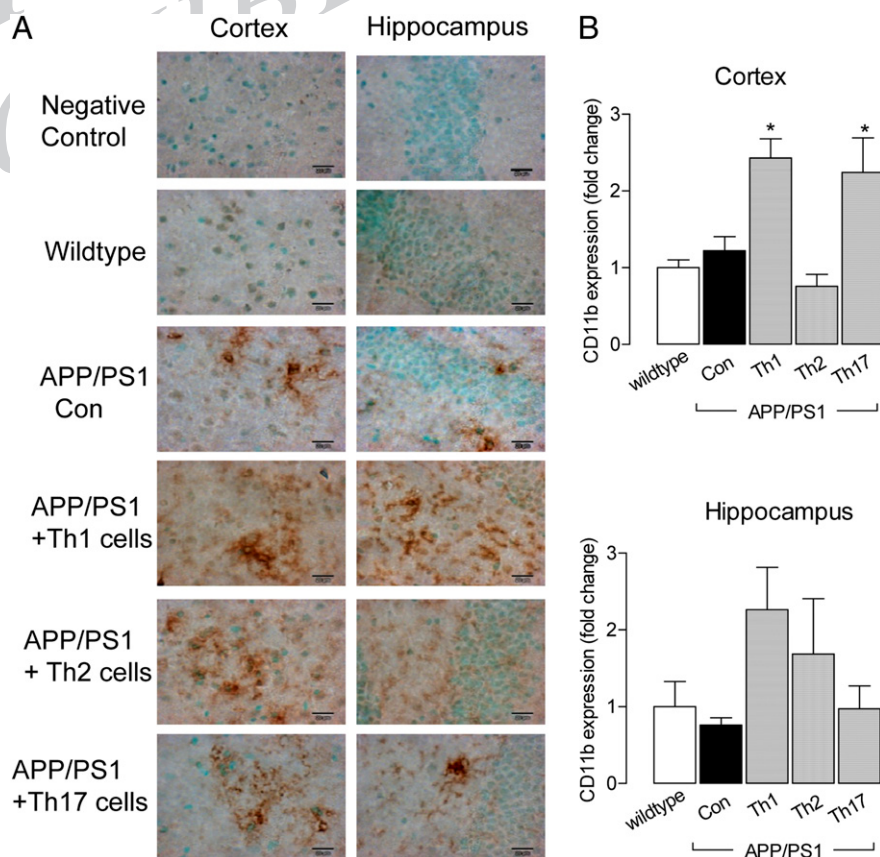
We used flow cytometry to assess the presence of T cells in the brain of WT and APP/PS1 mice. We found that there were very few CD3⁺CD4⁺ cells in the brain of WT mice but a significantly greater number in brain tissue prepared from APP/PS1 mice ($***p < 0.001$; Student *t* test for independent means; Fig. 1A). Intracellular staining revealed that a proportion of CD4⁺ cells stained positively for IFN- γ and also for IL-17 (Fig. 1B–D). There was no genotype-related difference in the number of CD3⁺CD8⁺ cells in the brain (Fig. 1E), although intracellular staining indicated that a greater proportion of these cells stained positively for IFN- γ compared with IL-17 ($**p < 0.01$; Student *t* test for independent means; Fig. 1F).

A β -specific Th1 cells impair cognitive function in APP/PS1 mice

Having demonstrated the presence of Th1 and Th17 cells in the brain of APP/PS1 mice, we set out to evaluate the effect of administration of A β -specific T cells on cognitive function, A β accumulation, and microglial activation in 6- to 7-mo-old APP/PS1 mice in which early pathological changes have been reported (36). To amplify A β -specific T cells, WT mice were immunized twice (0, 21 d) with A β and CpG, an adjuvant known to promote Th1 and Th17 responses. Short-term A β -specific Th1, Th2, and Th17 cell lines were generated by restimulation with Ag and APC in the presence of polarizing mixture described in the *Materials and Methods* section. This protocol resulted in the generation of highly polarized populations of Th1, Th2, and Th17 cells; Th1 cells produced high levels of IFN- γ and low IL-4 and IL-17, Th2 cells secreted high levels of IL-4 and low IL-17 and IFN- γ , and Th17 cells produced high levels of IL-17 and no IL-4 or IFN- γ .

(Fig. 2). After one round of Ag-stimulation, surviving T cells were washed and injected i.v. (15×10^6 cells/mouse) into 6- to 7-mo-old APP/PS1 or WT mice. Mice were tested for spatial memory in the Morris water maze 2 wk after administration of A β -specific T cells. The latency to reach the platform decreased over the 5-d training period but changes were similar in WT mice and control-treated APP/PS1 mice or APP/PS1 mice that received T cells (Fig. 3A), and no treatment effect was observed on day 5 of training (Fig. 3B). The path length taken to reach the platform decreased with training, except in APP/PS1 mice, which received Th1 cells (Fig. 3D) as shown by the representative traces obtained on day 5 (Fig. 3C). The mean path length on day 5 was significantly increased in these mice compared with untreated APP/PS1 mice ($*p < 0.05$; ANOVA; Fig. 3E). In contrast, transfer of Th1 cells into WT mice had no significant effect on path length taken to reach the platform or mean path length on day 5 (Supplemental Fig. 1). The day after the final day of training, the platform was removed, and mice underwent a single 60-s probe trial. The percentage of the total time and distance (i.e., path length) each animal spent swimming in the quadrant that previously contained the platform was significantly decreased in APP/PS1 mice that received Th1 cells compared with untreated APP/PS1 mice ($*p < 0.05$; ANOVA; $n \geq 5$; Fig. 3F, 3G). Therefore, Th1 cell transfer induces a deficit in spatial learning in APP/PS1 mice at an age at which such deficits are not generally observed. Importantly, no motor deficits were observed in these animals; stride length, hind limb base width, and front limb base width were similar in all groups of mice, and, on the hangwire task, there were no differences in the latency to fall between groups (data not shown). These findings suggest that transfer of Th1, but not Th2 or Th17 cells, around the time of onset of A β plaque formation impairs cognitive function in APP/PS1 mice.

FIGURE 6. Transfer of A β -specific Th1 cells increase CD11b immunoreactivity in hippocampus and cortex of APP/PS1 mice. APP/PS1 mice were injected with A β -specific Th1, Th2, or Th17 cells as described in Fig. 3. (A) Microglial activation was assessed by CD11b immunoreactivity. (B) Data are means $\pm$ SEM. $*p < 0.05$, ANOVA versus control ($n = 3$ –5) representative of two experiments. Con, control.



We tracked the migration of transferred T cells into the CNS using A β -specific Th1 cells generated from GFP mice immunized with A β and CpG and polarized with IL-12. We found a higher

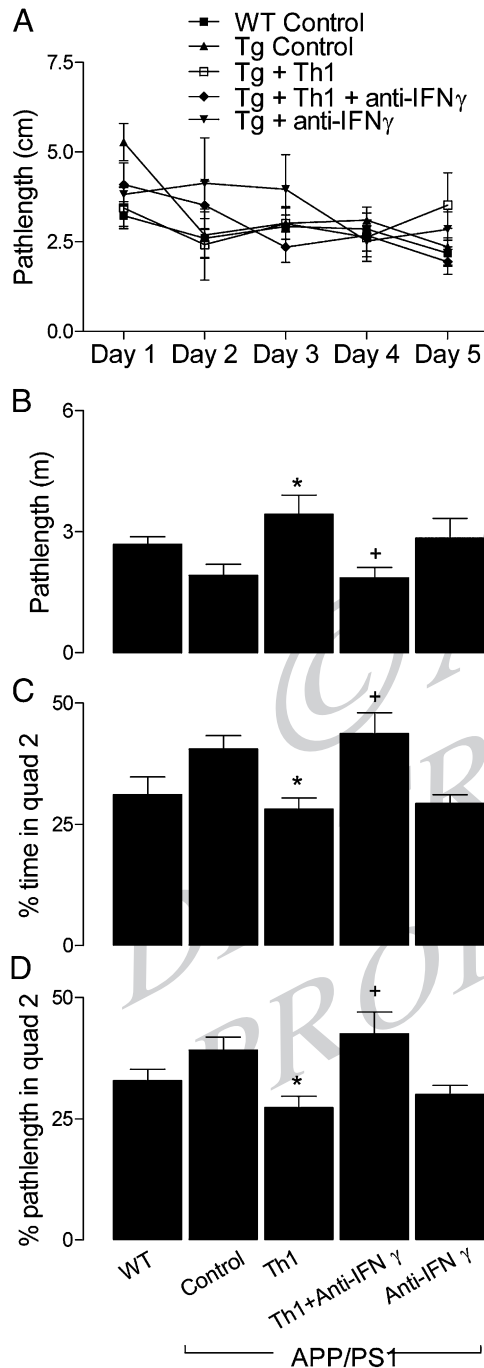


FIGURE 7. Anti-IFN- γ Ab attenuated the effect of Th1 cells on behavioral deficits. APP/PS1 mice were injected with A β -specific Th1 cells as described in Fig. 3, and mice were treated with anti-IFN- γ Ab or anti- β -galactosidase as a control Ab before and after injection of the cells. Three weeks after injection, cognitive function was analyzed in the Morris Water Maze test as described in Fig. 3. (A) The path length taken to reach the platform. (B) Mean path length on day 5 of training. (C and D) In the probe test, the time and path length in the quadrant that previously contained the platform (expressed as a percentage of the total) was measured. Data represent mean $\pm$ SEM from four to five animals per experimental group from two experiments. * p < 0.05, ANOVA, APP/PS1+Th1 cells versus control APP/PS1 mice, ⁺ p < 0.05, ANOVA, APP/PS1 + Th1 cells versus APP/PS1 plus Th1 cells plus anti-IFN- γ Ab. Tg, transgenic.

proportion of CD3⁺ T cells in the brain of APP/PS1, compared with WT, mice after transfer of A β -specific Th1 cells (Fig. 4). Furthermore, we detected GFP⁺ cells in the brain 14 d following transfer of Th1 cells, and this was significantly greater in APP/PS1 mice. Finally, we found that CD8⁺ as well as CD4⁺ cells infiltrated the brain, and a significant number of these secreted IFN- γ (Fig. 4). These findings suggested that at least a proportion of A β -specific Th1 cells migrate into the brain following systemic delivery, and this is more pronounced in APP/PS1 when compared with WT mice. In addition, IFN- γ -secreting CD8 T cells are detected in higher numbers in the brains of APP/PS1 compared with WT mice.

A β -specific Th1 cells enhance A β plaque burden and enhance microglial activation in APP/PS1 mice

A β deposition has been reported in the brain of APP/PS1 mice as early as 6 mo of age (37). Cryostat sections prepared from the 6- to 7-mo-old APP/PS1 mice used in this study confirm the presence of A β -containing plaques in cortex and hippocampus. Adoptive transfer of A β -specific Th1 cells markedly increased A β load, particularly in cortex, whereas transfer of Th2 or Th17 cells had little effect (Fig. 5A). Mean plaque number was significantly increased in sections prepared from mice that received Th1 cells (* p < 0.05; ANOVA; Fig. 5B). Insoluble A β ₁₋₃₈, A β ₁₋₄₀, and A β ₁₋₄₂ were all significantly increased in tissue prepared from APP/PS1, compared with WT, mice (* p < 0.05; ** p < 0.01; ANOVA; Fig. 5C). Injection of Th1 cells induced a further increase in the

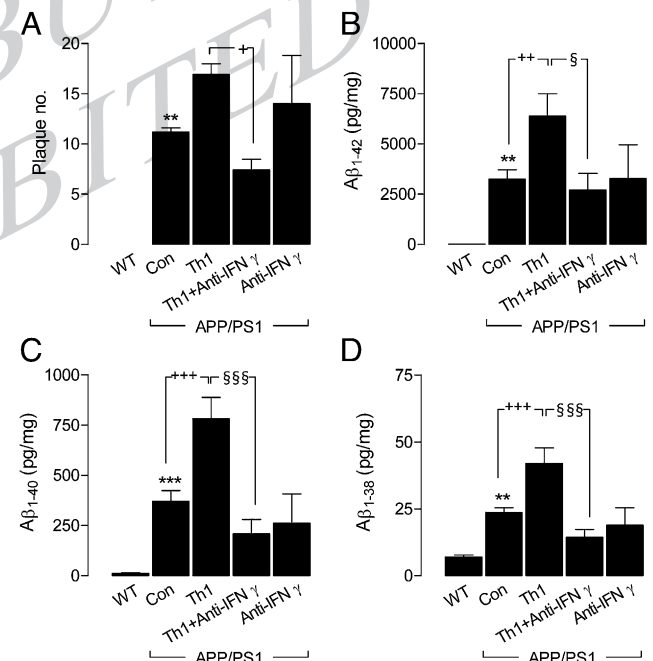


FIGURE 8. Anti-IFN- γ Ab attenuated the effect of Th1 cells on A β accumulation. APP/PS1 mice were injected with A β -specific Th1 cells and treated with anti-IFN- γ Ab or a control Ab as described in Fig. 6. Mice were sacrificed 5 wk after cell transfer. Cryostat sections were stained with Congo red to assess A β -containing plaques in hippocampus and cortex; the mean number of plaques was recorded (A), and the concentrations of insoluble A β ₁₋₄₂ (B), A β ₁₋₄₀ (C), and A β ₁₋₃₈ (D) were quantified by ELISA in brain tissue prepared from APP/PS1 and WT mice. Data represent mean $\pm$ SEM from four to five animals per experimental group from two experiments. ** p < 0.01, *** p < 0.001, ANOVA; (n = 4–6); ⁺ p < 0.05, ⁺⁺ p < 0.01, ⁺⁺⁺ p < 0.001, ANOVA; control APP/PS1 mice versus APP/PS1 mice that received Th1 cells; [§] p < 0.05; ^{§§§} p < 0.001, ANOVA, APP/PS1 + Th1 cells versus APP/PS1 + Th1 cells + anti-IFN- γ Ab. Con, control.

concentration of the three A β species ($^+p < 0.05$; $^{++}p < 0.01$, ANOVA, versus control APP/PS1 mice). Furthermore, soluble A β_{1-40} and A β_{1-42} were also significantly increased in tissue prepared from APP/PS1 following transfer of Th1 cells (Fig. 5D), although soluble A β_{1-38} was unchanged between treatment groups (data not shown). Neither Th2 nor Th17 cells exerted any significant effect on soluble or insoluble A β .

Sections prepared from WT and APP/PS1 mice were assessed for CD11b immunoreactivity as a measure of microglial activation. Immunoreactivity was negligible in sections of hippocampus and cortex prepared from WT mice (Fig. 6), whereas CD11b staining was observed in both areas in some but not all APP/PS1 mice. Quantification of the data indicated that CD11b expression was markedly increased in APP/PS1 mice that received Th1 cells, and the increase was significant in the case of the cortex ($^*p < 0.05$; ANOVA), where Th17 cells exerted a similar effect.

Neutralization of IFN- γ attenuates the effect of Th1 cells on behavioral deficits

Having shown a specific effect of Th1 cells on spatial memory and A β accumulation, we assessed the role of the key Th1 cytokine, IFN- γ , by treating APP/PS1 mice with a neutralizing anti-IFN- γ Ab prior to, and following, Th1 cell transfer. There was no significant effect of treatment on latency to reach the platform (data not shown), confirming the data shown in Fig. 3. However, we found that the path length taken to reach the platform decreased with training in all groups except in APP/PS1 mice, which received Th1 cells (Fig. 7A), and analysis of the mean data indicates that path length was significantly increased in this group compared with APP/PS1 mice that did not receive Th1 cells ($^*p < 0.05$; ANOVA; Fig. 7B). Administration of anti-IFN- γ Ab significantly attenuated the Th1 cell-induced effect ($^+p < 0.05$, ANOVA, versus APP/PS1 mice that received Th1 cells). In the probe test, treatment with Th1 cells decreased the percentage of the total time and distance each animal spent swimming in the quadrant that

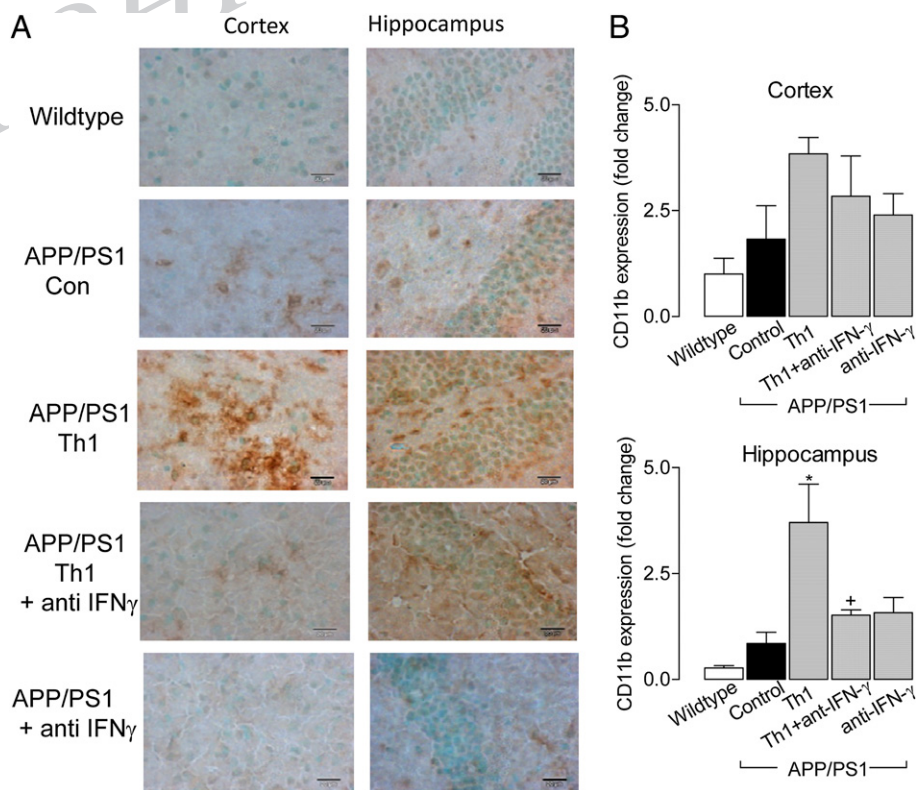
previously contained the platform ($^*p < 0.05$; ANOVA; Fig. 7C, 7D), confirming the findings presented in Fig. 3. Treatment with anti-IFN- γ significantly reversed the effect of Th1 cells ($^+p < 0.05$, ANOVA, versus APP/PS1 mice that received Th1 cells).

Neutralization of IFN- γ attenuates the effect of Th1 cells on A β plaque burden

Anti-IFN- γ Ab attenuated the effects of Th1 cells on plaque number and concentration of insoluble A β_{1-38} , A β_{1-40} , and A β_{1-42} in tissue prepared from APP/PS1 mice (Fig. 8). These measures were increased in tissue prepared from APP/PS1 mice compared with WT mice ($^{**}p < 0.01$, $^{***}p < 0.001$, ANOVA; Fig. 8A–D), and these were significantly increased by administration of Th1 cells ($^+p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$, ANOVA, control APP/PS1 mice versus APP/PS1 mice that received Th1 cells). The increase in A β_{1-38} , A β_{1-40} , and A β_{1-42} induced by Th1 cells was attenuated when mice were treated with anti-IFN- γ Ab ($^{\S}p < 0.05$, $^{\S\S}p < 0.001$, ANOVA, APP/PS1 mice that received Th1 cells versus Ab-treated APP/PS1 mice that received Th1 cells).

CD11b immunoreactivity was negligible in sections prepared from hippocampus and cortex of WT mice (Fig. 9), whereas some staining was observed in both areas in APP/PS1 mice. This was greater in APP/PS1 mice that received Th1 cells, but this effect was ameliorated to some degree in sections prepared from APP/PS1 mice that received Th1 cells and anti-IFN- γ Ab. Immunoreactivity was similar in sections prepared from control APP/PS1 mice and APP/PS1 mice, which received anti-IFN- γ Ab. Analysis of staining using confocal microscopy indicated that CD11b immunoreactivity (green staining; Fig. 10) was colocalized with A β deposition (red staining) in hippocampus and cortex. As shown in Figs. 6 and 9, A β accumulation was increased in sections prepared from APP/PS1 mice, which received Th1 cells, and this effect was attenuated by anti-IFN- γ Ab treatment (Fig. 10). These findings demonstrate that the impact of Th1 cells on A β plaque burden and microglial activation was mediated through IFN- γ .

FIGURE 9. Anti-IFN- γ Ab attenuated the effect of Th1 cells on CD11b immunoreactivity. APP/PS1 mice were injected with A β -specific Th1 cells and treated with anti-IFN- γ Ab or a control Ab as described in Fig. 6. Mice were sacrificed 5 wk after T cell transfer. (A) Microglial activation was assessed by CD11b immunoreactivity in the cortex and hippocampus. (B) Data are means $\pm$ SEM. Data represent mean $\pm$ SEM from four to seven animals per experimental group from two experiments. $^*p < 0.05$, ANOVA, versus control, $^+p < 0.05$, ANOVA, versus Th1. Con, control.



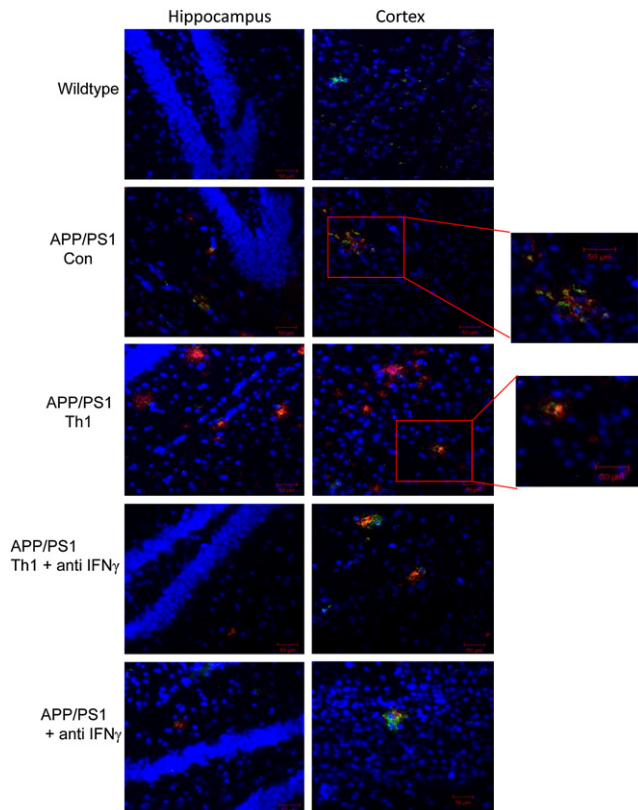


FIGURE 10. Anti-IFN- γ Ab attenuated the effect of Th1 cells on CD11b immunoreactivity. APP/PS1 mice were injected with A β -specific Th1 cells and treated with anti-IFN- γ Ab or a control Ab as described in Fig. 6. Mice were sacrificed 5 wk after T cell transfer. Microglial activation and A β deposition was assessed by confocal microscopy using CD11b and pan-A β Abs. Data are representative of five mice per experimental group from two experiments. Con, control.

Discussion

The significant finding of this study is that adoptive transfer of Th1 cells increases A β accumulation and microglial activation in the brain of 6- to 7-mo-old APP/PS1 mice and impairs performance in a Morris water maze; these effects are attenuated by treatment of mice with anti-IFN- γ Ab.

It has been recognized for some time that T cells can infiltrate the brain (38, 39). T cell infiltration is significantly enhanced under pathological conditions (for example, in multiple sclerosis and EAE), and this is due, at least to some extent, to an increase in blood-brain barrier permeability (23, 40). In this study, we report that there is a significant increase in the number of CD3⁺CD4⁺ cells in the brain of APP/PS1 mice compared with WT mice and that a proportion of these are Th1 and Th17 cells. Consistent with this is the observation that significant numbers of peripheral T cells are present in the postmortem brain of AD patients compared with the relatively low numbers of cells in other degenerative dementia cases and, importantly, that these cells are clustered in areas of the brain in which pathology is more marked, such as the hippocampus and limbic regions (25). However, the role of T cells in the pathogenesis of AD is not clear, with circumstantial evidence of both host protective and damaging roles for A β -specific T cells. Peripheral T cells specific for A β _{1–40} have been detected in healthy individuals, but were absent in patients with AD (41), possibly suggesting that A β _{1–40}-specific T cells may prevent the development of A β plaques. It has also been reported that Th1 cells directed against A β _{1–42} are present in young individuals but decline with age and are lost in patients with

AD, in whom IL-10-producing regulatory T cells predominate (42).

Vaccine studies in mouse models have shown that immunization with A β ₄₂ in CFA prevented the development of A β plaques and reduced the development of AD-like neuropathology (33, 43). The protection was associated with Ab and could be mimicked by passive transfer of A β -specific Abs (44). There is also evidence from a clinical trial that active immunization with A β ₄₂, formulated with the adjuvant QS21 (AN 1792), can reduce plaque burden in AD patients (45), though a number of patients developed meningoencephalitis, and the trial was halted. Although the cause of the meningoencephalitis is not clear, it has been suggested that it could result from the induction of inflammatory T cell responses (46). Interestingly, QS-21, the adjuvant used in AD vaccine, has been shown to promote Th1 responses to coadministered foreign Ag in mice (47). Our findings are consistent with a pathogenic role for Th1 cells, at least in a mouse model of AD.

To evaluate the impact of different T cell subtypes on plaque burden in the brain, we adoptively transferred A β -specific Th1, Th2, and Th17 cells into 6- to 7-mo-old APP/PS1 mice. Consistent with previous findings (36), we found that there was some A β accumulation in the brain of the 6-mo-old APP/PS1 mice. This was accompanied by increased concentrations of A β _{1–42}, A β _{1–40}, and A β _{1–38} in cortical tissue. However, transfer of Th1 cells increased deposition of A β (determined by Congo red staining) and markedly increased cortical A β concentration. This suggests that A β -specific Th1 cells may play a role in the development of A β plaques in the brain. This was confirmed by treatment of mice with a neutralizing anti-IFN- γ Ab, which attenuated the effect of Th1 cells on A β accumulation. In contrast with the effect of Th1 cells, transfer of Th17 cells, which have been associated with pathology in EAE and other autoimmune/inflammatory diseases, and Th2 cells, which have a more anti-inflammatory function in other diseases, did not enhance A β accumulation in the brain. These findings are consistent with our earlier report that A β -specific Th1 cells enhance proinflammatory cytokine production and MHC class II and costimulatory molecule expression by A β -stimulated microglia, whereas A β -specific Th2 cells suppress cytokine production by glial cells (32).

Under resting conditions, microglia are maintained in a quiescent state in the brain because of the presence of neuroimmune regulatory molecules that enable the interaction with other cells, low concentrations of stimulatory factors such as IFN- γ and other inflammatory cytokines, and the presence of minimal numbers of immune cells like T cells (13). However, microglial activation occurs following any insult, and an activated state is a characteristic of most, if not all, neurodegenerative diseases in which these cells can assume the role of APC. Modest microglial activation was observed in the hippocampus and cortex of 6- to 7-mo-old APP/PS1 mice but transfer of Th1 cells markedly increased activation. This is consistent with our previous findings that showed that A β -specific Th1 cells increased microglial activation in vitro (32). In parallel with its effect on A β accumulation, treatment of mice with anti-IFN- γ Ab attenuated the effect of Th1 cells on microglial activation. It is well established that IFN- γ is among the potent activators of microglia (15, 48) and synergizes with A β to increase expression of cell-surface markers of activation and production of inflammatory cytokines (15, 49). Chakrabarty et al. (50) reported that viral delivery of IFN- γ gene promotes microglial activation and clearance of A β . We observed that Th1 cells also promoted microglial activation but that this was associated with an increase in A β plaques. We do not have a definitive explanation for the discrepancy in these studies other than the dif-

ferences in the experimental approaches: virally-delivered IFN- γ , which had effects, such as basal ganglia calcification, in WT as well as Tg mice, compared with i.v. injected A β -specific Th1 cells, in which the effects were largely confined to Tg mice. One interpretation of the data, as suggested in this study, is that anti-IFN- γ prevents Th1 cell-induced activation of microglia, but it is possible that the Ab treatment affects infiltration of cells, perhaps by altering chemotaxis or exerting an effect on blood-brain barrier permeability.

Our studies with Th1 cells expressing GFP demonstrated that at least a proportion of the transferred Th1 cells did migrate from the periphery into the CNS. Interestingly, IFN- γ -secreting CD8 as well as CD4 T cells were detected in the brain following i.v. injection of A β -specific Th1 cells. This is consistent with studies in the EAE model that have demonstrated that Th1 cells preferentially infiltrate the CNS and facilitate recruitment of other inflammatory T cells (51). Interestingly, the migration of T cells into the brain and subsequent behavioral deficits was significantly more pronounced following transfer of A β -specific Th1 cells into APP/PS1 when compared with WT mice. This may reflect the higher A β burden in the APP/PS1 mice and might suggest local Ag stimulation of IFN- γ -secreting T cells, which were at a significantly higher frequency in the brains of APP/PS1 compared with WT mice.

Previous studies from this laboratory have shown that A β -specific Th1 cells enhanced A β -induced activation of microglia (32). Furthermore, the increase in microglial activation in APP/PS1 mice was accompanied by increased expression of inflammatory cytokines, including TNF- α and IL-1 β (52). Interestingly, TNF- α and IL-1 β have been shown to increase activity and/or expression of γ - and β -secretases (6, 7), which leads to A β deposition. Although activated microglia may phagocytose and remove A β aggregates (50), IL-1 β -expressing microglia are associated with A β plaques and neurofibrillary tangles in the brain of AD patients, where they correlate with progressive neuronal damage (53). Furthermore, IL-1 β can promote synthesis of the APP in endothelial cells (54). It has also been reported that IFN- γ -induced activation of microglia enhanced processing of APP and suppressed A β clearance (55). We found that Th1 cells, which increase IL-1 β expression by microglia (32), enhanced soluble and insoluble A β concentrations in the brains of APP/PS1 mice. However, it must be acknowledged that A β potently activates microglia in vitro and in vivo (56, 57), and therefore, the possibility of a feedback loop, leading to persistent microglial activation and A β accumulation with the subsequent pathogenic consequences, is well recognized.

Although there was significant A β accumulation in the brain of 6- to 7-mo-old APP/PS1 mice, we found no evidence of genotype-related changes during the training phase in the Morris water maze or during the probe test, contrasting with previous reports that indicated a deficit in slightly older (8-mo-old) APP/PS1 mice (58, 59). It has been suggested that cognitive deficits correlate with insoluble A β in Tg2576 mice (60) and APP/PS1 mice (61), but this view is not supported by the present findings. However, we report that transfer of Th1 cells doubled the concentration of insoluble A β in brain tissue, and this was associated with deterioration in cognitive function in the probe test; this raises the possibility that a threshold concentration of A β must be reached before an impact on spatial learning is exerted. In contrast to the effect of Th1 cells on APP/PS1 mice, transfer of Th2 cells or Th17 cells exerted no effect in the spatial learning task or on either plaque number or A β accumulation. The present findings are at variance with an earlier report that indicated that adoptive transfer of a mixed T cell preparation improved performance of 10-mo-old

APP/PS1 mice in a radial arm maze task (62). Although no effect on insoluble A β or A β plaque numbers was observed, the authors suggested that microglia or monocytes were stimulated to clear A β because the distribution of A β -immunoreactive cells in hippocampus of mice that received T cells was similar to the distribution of MHC class II-positive cells. More recent data from this group suggested that the beneficial effects on behavior may be Th2 cell-mediated because the effect was evident when T cells had been incubated in vitro in the presence of IL-2 and IL-4 (31). We have recently reported that the A β -induced microglial activation in vitro is attenuated by Th2 cells (32), and the current study found that Th2 cells, unlike Th1 cells, did not enhance plaque burden in vivo.

Although beneficial effects of T cells in the brain have also been observed (63), the evidence presented in this study indicates that Th1 cells, but not Th2 or Th17 cells, contribute to A β accumulation and development of a functional deficit in APP/PS1 mice during the early stages of development of pathology. In this model, the effects appear to be mediated by IFN- γ and are associated with enhanced microglial activation, which may trigger inflammatory changes that propagate a damaging cascade of events and further development of pathology.

Acknowledgments

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Disclosures

K.H.G.M. is a co-founder and shareholder in Opsona Therapeutics and Tri-Mod Therapeutics Ltd, startup companies involved in the development of immunotherapeutics. The other authors have no conflicts of interest.

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