Therapeutic B-cell depletion reverses progression of Alzheimer’s disease

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The function of B cells in Alzheimer’s disease (AD) is not fully understood. While immunoglobulins that target amyloid beta (Aβ) may interfere with plaque formation and hence progression of the disease, B cells may contribute beyond merely producing immunoglobulins. Here we show that AD is associated with accumulation of activated B cells in circulation, and with infiltration of B cells into the brain parenchyma, resulting in immunoglobulin deposits around Aβ plaques. Using three different murine transgenic models, we provide counterintuitive evidence that the AD progression requires B cells. Despite expression of the AD-fostering transgenes, the loss of B cells alone is sufficient to reduce Aβ plaque burden and disease-associated microglia. It reverses behavioral and memory deficits and restores TGFβ+ microglia, respectively. Moreover, therapeutic depletion of B cells at the onset of the disease retards AD progression in mice, suggesting that targeting B cells may also benefit AD patients.
Alzheimer’s disease (AD) is a progressive neurodegenerative disease that mostly affects elderly people. It is associated with impaired clearance of toxic protein aggregates from the brain parenchyma, such as amyloid-β (Aβ) peptides of abnormally cleaved amyloid precursor protein (APP). Although resident microglial cells phagocytize extracellular Aβ plaques with the help of astrocytes and TGFβ (refs. 2, 23), chronic inflammation, and Aβ production dysregulate this process, causing proliferation and subsequent replacement of homeostatic microglia with disease-associated microglia (DAM)3. As in mice with genetic TGFβ deficiency, which suffer from microgliosis and neuronal death6, DAM further exacerbate neuroinflammation and neuronal degeneration in AD19, at least in part, through expression of proinflammatory cytokines and downregulation of phagocytosis of Aβ plaques18–20. Consistent with a positive association between AD risk and old age11, when systemic inflammation is increased19, disease progression also depends on peripheral inflammation and activation of innate immune cells20. The role of the adaptive immunity in AD, however, remains poorly understood, and is mostly linked to T cells exerting both beneficial and harmful functions. For example, in APP/PS1 mice expressing the K670N and M146V mutations on PS1 and the P301L in a model for early-onset AD (EOAD, harbors the Swedish APP and sex- and age-matched C57BL/6 (WT) and 3×TgAD mice, a pronounced AD-like symptom manifestation25. Compared with control mice, 3×TgAD mice significantly upregulated the frequency and numbers of B cells in the circulation and secondary lymphoid organs (Supplementary Fig. 1A and not depicted; gating strategy is in Supplementary Fig. 9A), in naive CD5+ B1a and CD5− B1b cells (CD11b+ or/and CD23− CD43+ CD19+ cells, respectively) markedly increased, and conventional B2 cells decreased two- to threefold in the spleen and cervical lymph nodes (cLN; Fig. 1A–C and Supplementary Fig. 1B–D). The 3×TgAD mice also contained higher amounts of activated B cells expressing IFNγ, IL6, IL10, and TGFβ in circulation than control mice (Fig. 1D–G), mostly within B1a cells and, at lesser extent B1b and follicular B cells (Supplementary Fig. 1E–H). The AD mouse B cells, particularly B1a cells, markedly upregulated 4-1BBL (Supplementary Fig. 1I, J), resembling pathogenic B1a cells of aged subjects22,23 and suggesting their potential involvement in AD. To test this possibility, we have generated B-cell-deficient 3×TgAD mice (termed 3×TgAD-BKO, Supplementary Fig. 2A) by crossing 3×TgAD mice with JHT-Tg mice, which lack functional B cells due to the immunoglobulin IgH locus deletion that terminates B-cell development at the pro-B cell stage26. When mice reached 50–60 weeks of age, they were evaluated for hippocampus-dependent cognitive behavior, using the Morris water maze (MMW) task. During the 5-day hidden platform training, both 3×TgAD and 3×TgAD-BKO mice showed comparable performance (not depicted). However, 24 h after the last training session, a probe trial conducted in the absence of the hidden platform, revealed that 3×TgAD-BKO mice spent a significantly longer time in the target quadrant than their 3×TgAD littermates (p = 0.017, n = 13–15, Fig. 2A), indicating a stronger spatial memory of the platform’s location. We also tested exploratory behavior anomalies using the open field arena (OFA, a commonly used assay in 3×TgAD mice27,28). While 3×TgAD mice exhibited reduced activity as compared with WT mice in the OFA, the impairment was no longer detectable in age-matched 3×TgAD-BKO littermates (left panel, one-way ANOVA F(2,29) = 21.68, p < 0.0001; WT vs 3×TgAD p < 0.0001, 3×TgAD vs 3×TgAD-BKO p < 0.0001, n = 10–11, Fig. 2B), implying that 3×TgAD-associated behavioral impairments required B cells.

To confirm this conclusion, we tested APP/PS1 mice, another model of EOAD, that exhibit earlier AD pathology compared with 3×TgAD mice29. Of note: subsequent experiments were therefore conducted in female and male, 20–35-week-old mice. Flow cytometry evaluation of circulating B cells revealed that APP/PS1 mice also markedly upregulated B cells expressing IL10 (Fig. 2C) as in 3×TgAD mice (Fig. 1F). However, APP/PS1 mice did not upregulate 4-1BBL+ B cells (presumably due to their relatively young age22,23) despite a significant increase of B1a cells in circulation and the cLN compared with control littermates (Supplementary Fig. 2B, C). We next generated B-cell-deficient APP/PS1-BKO mice by crossing APP/PS1 and JHT-Tg mice. Analysis of spatial learning using the MWM revealed that compared with age- and sex-matched APP/PS1 or WT littermates, B-cell deficiency significantly improved learning deficiency in APP/PS1 mice as evident by latency to reach the escape platform (t(2,33) = 8.288, p = 0.0012; WT vs APP/PS1 p = 0.0006, APP/PS1 vs APP/PS1-BKO p = 0.003, n = 12, Fig. 2D); and in the number of platform region crossings in a probe test conducted 24 h following learning (Kruskal–Wallis ANOVA p = 0.0004; WT vs APP/PS1 p = 0.0002; APP/PS1 vs APP/PS1-BKO p = 0.028, Fig. 2E). Consistent with the lack of exploratory behavior impairment in APP/PS1 mice20, no difference in exploratory behavior of our mice in the OFA (not depicted). Thus, the spatial learning impairments exhibited by APP/PS1 are B-cell dependent.

Because the memory impairments in EOAD are caused by accumulation of Aβ plaques and hippocampal microgliosis31, we...
conducted an immunofluorescent analysis for Aβ plaques (using the 6E10 Ab) and ionized calcium binding adaptor 1 (Iba, a microglial cell marker) in cryopreserved brain sections of APP/PS1-BKO (30 weeks old), 3×TgAD-BKO (60–70 weeks old) and age- and sex-matched control mice. Given that in this model the early intraneuronal Aβ deposition in the subiculum is linked to cognitive impairments32, and that the subiculum and hippocampal CA1 atrophy is increased in AD patients33, from hereon we primarily analyzed the subiculum. It showed a marked upregulation of Aβ plaques in APP/PS1 and 3×TgAD mice (as compared with healthy control mice), which was reversed in APP/PS1-BKO and 3×TgAD-BKO mice respectively (n = 4–7, Fig. 3A–C and Supplementary Fig. 3A–D). Analysis of frontal cortex and hippocampus in 3×TgAD-BKO mice also revealed that the increase of soluble Aβ42 and Aβ40 was reversed (n = 2–6, Fig. 3D and Supplementary Fig. 3E). Since both 3×TgAD and 3×TgAD-BKO mice express high levels of the APP transgene in hippocampal neurons (Fig. 3E), we concluded that the benefit of the B-cell deficiency was probably in reduced formation and/or increased clearance of Aβ peptides. Accordingly, large ameboid microglial cells (>5 μm²), which indicate dysfunctional overactivation34,35 and impaired clearance of Aβ plaques36, were significantly decreased in 3×TgAD-BKO mice to almost that of in WT mice (p < 0.05, compared with 3×TgAD, n = 3–9, Fig. 3F and Supplementary Fig. 3F, G). In contrast, activated microglia (regardless of their size) remained increased in APP/PS1 and

![Figure 1](https://doi.org/10.1038/s41467-021-22479-4)  
**Fig. 1 Activated B cells were increased in 3×TgAD mice.** Compared with congenic, age- and sex-matched WT mice, B1a (CD5 CD11b CD19, A) and B1b cells (CD5 CD11b CD19, B) were increased, while B2 cells (CD5 CD11b CD19, C) were decreased in the cervical lymph nodes of 3×TgAD mice. AD also activated B cells, as they upregulated IFNγ (D), IL6 (E), IL10 (F), and TGFβ (G) in the peripheral blood of mice. Frequency (%) mean ± SEM is shown; each symbol is for a single mouse, n = 5–6 in A–C, n = 15 in D–G. Gating strategy is shown in Supplementary Fig. 9A. **p < 0.01; ***p < 0.001 in unpaired t test.
To understand this discrepancy, we compared the function of microglia in these two models. Brain myeloid cells were isolated and stimulated with phorbol 12-myristate 13-acetate and ionomycin (PMAi, which induces microglial cytokine expression and proliferation) for 4–6 h in the presence of monensin, and then surface markers and intracellular (IC) cytokines of microglia (CD45IntCD11b+) were analyzed using flow cytometry. The loss of B cells did not affect interleukin (IL)1β expression, which was markedly upregulated in both 3×TgAD and APP/PS1 (Supplementary Fig. 4A and Fig. 4A and not depicted; gating strategy is in Supplementary Fig. 9B). In contrast, we noted a marked decrease in TGFβ+ and IFNγ+ microglia (presumably quiescent and resting microglia) in APP/PS1 and 3×TgAD mice (Fig. 4B, C and Supplementary Fig. 4B–D). The TGFβ+ (at lesser extent IFNγ+) microglial cells were normalized to the levels of WT control in APP/PS1-KO and 3×TgAD-KO mice (Fig. 4B, C and Supplementary Fig. 4B–D). Brain and hippocampal RNA microarray analyses indicated that B-cell deficiency prevented loss of TGFβ1+ microglia in the hippocampus and brains of 3×TgAD mice, as its expression was upregulated (Fig. 4D) while DAM-related transcriptional signature genes, such as Igarx, Cst7, Clec7a, Mamdc2, and Saa3 (ref. 4) were downregulated in 3×TgAD-KBO mice (Supplementary Fig. 4E, Supplementary Data 1–3, and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165111). B-cell deficiency did not affect expression of IL1β nor other DAM genes, such as TNFα, Igf1, and Lilrb4 (Supplementary Fig. 4E and Supplementary Data 1–3). In sum, despite expression of AD-promoting transgenes, DAM accumulation, Aβ plaque deposition, and thus the disease progression required B cells in both 3×TgAD and APP/PS1 models of AD.

Next, we sought to test whether progression of EOAD can also be controlled by a therapeutic inactivation or depletion of B cells at the disease onset. To test this idea, 3×TgAD mice (60–70 weeks old, female) were intraperitoneally injected with anti-CD20/B220 antibody (which depletes B cells in the circulation) for 2 months. Control mice were treated with isotype-matched IgG. The anti-CD20/B220 Ab efficiently depleted B cells in the circulation (Supplementary Fig. 5A). Compared with control treated mice, the anti-CD20/B220 Ab-treated 3×TgAD showed a trend toward increased activity in the OFA (p = 0.07, 30 min, n = 5, Supplementary Fig. 5B). The treatment however significantly reduced the number of Aβ plaques in the subiculum of AD mice (p < 0.05, Fig. 4E, F), but did not affect the number of large (>50 µm²) Iba1+ microglial cells in the hippocampus (Supplementary Fig. 5C). To understand this discrepancy, we repeated anti-CD20/B220 Ab treatment in a different cohort of female 60–70-week-old 3×TgAD mice for 2 months (n = 6–7) and then performed a flow cytometry analysis.
evaluation of microglial cytokines in the perfused brains. As noted above, in 3×TgAD-BKO mice, B-cell depletion significantly reversed the decrease of TGFβ+ and IFNγ+ microglia in 3×TgAD mice to almost the levels in WT mice (both in terms of frequency and numbers, p < 0.05, Fig. 4C, G and Supplementary Fig. 5D, E) while not affecting IL1β+ microglia, which was comparably upregulated in aged 3×TgAD and WT mice (Supplementary Fig. 5F).

To validate these results, we used a third mouse model of EOAD, the 5×FAD mice that develop a more aggressive AD pathology by 40 weeks of age because of a large burden of Aβ plaques caused by additive effects of five familial AD mutations41. In this model, others have linked AD progression to a decrease in frequency of potentially beneficial B1a cells42. However, our cohorts of 5×FAD mice did not exhibit a decrease in B cells, including B1a and B1b cells (both in terms of number and frequency), as compared with control age-matched littermates (Supplementary Fig. 6A, B). The B cells and B1 cells in 5×FAD mice instead appeared to be activated, as they significantly upregulated expression of 4-1BBL (Supplementary Fig. 6C, D).

In concordance, IC cytokine staining of splenic cells revealed marked increase of IL10, TGFβ, and IFNγ in B cells
To test whether depletion of these activated B cells also ameliorates AD, 5×FAD mice (35–47 weeks old, female) were i.p. injected with anti-CD20/B220 Ab or control IgG (n = 7–12) for 2 months. Mice were then evaluated in the OFA for exploratory behavior and anxiety. While control IgG-treated 5×FAD mice showed reduced exploratory behavior compared with WT littermates, this effect was reversed in B-cell-depleted mice (F(2,25) = 5.25, p = 0.013; wt vs 5×FAD p = 0.03, 5×FAD+IgG vs 5×FAD + aCD20 p = 0.02, Fig. 5A). To confirm this result, we quantified Aβ plaques and activated microglia in the hippocampus of these mice. Compared with IgG treatment, B-cell depletion in 5×FAD mice significantly reduced the number of Aβ plaques (Fig. 5B, C) and the large-sized Iba1+ microglia (Fig. 5D). We repeated the 2-month B-cell depletion experiment in a different cohort of age-matched, female 5×FAD and WT mice (n = 5–12), and evaluated microglial cells using flow cytometry in perfused with saline brains. Similar to 3×TgAD and APP/PS1 mice, TGFβ+ and IL10+ microglia were significantly

(Supplementary Fig. 6E). To test whether depletion of these activated B cells also ameliorates AD, 5×FAD mice (35–47 weeks old, female) were i.p. injected with anti-CD20/B220 Ab or control IgG (n = 7–12) for 2 months. Mice were then evaluated in the OFA for exploratory behavior and anxiety. While control IgG-treated 5×FAD mice showed reduced exploratory behavior compared with WT littermates, this effect was reversed in B-cell-depleted mice (F(2,25) = 5.25, p = 0.013; wt vs 5×FAD p = 0.03, 5×FAD+IgG vs 5×FAD + aCD20 p = 0.02, Fig. 5A). To confirm this result, we quantified Aβ plaques and activated microglia in the hippocampus of these mice. Compared with IgG treatment, B-cell depletion in 5×FAD mice significantly reduced the number of Aβ plaques (Fig. 5B, C) and the large-sized Iba1+ microglia (Fig. 5D). We repeated the 2-month B-cell depletion experiment in a different cohort of age-matched, female 5×FAD and WT mice (n = 5–12), and evaluated microglial cells using flow cytometry in perfused with saline brains. Similar to 3×TgAD and APP/PS1 mice, TGFβ+ and IL10+ microglia were significantly

(Fig. 4 B-cell deficiency at least in part reversed the DAM phenotype. The results of flow cytometric quantification of IL1β+ (A) and TGFβ+ (B, C) microglia (CD11b+CD45int) in the brains of indicated mice (n = 11–12) are shown. Genetic B-cell deficiency in APP/PS1 mice (APP/PS1-BKO, B) or transient depletion of circulating B cells (aCD20/B220, C) at the onset of AD (70–79 weeks of age 3×TgAD mice) reversed the AD-associated decrease of TGFβ+ microglia. mRNA microarray analyses of hippocampi and brains (without hippocampus) of 3×TgAD mice revealed that the B-cell deficiency (3×TgAD-BKO) upregulates expression of TGFβ1, but not TGFβ2 and TGFβ3 (D, n = 3). Therapeutic depletion of B cells (aCD20/B220) at the onset of AD ameliorated AD (E–G), as it markedly decreased Aβ plaques in the subiculum (quantification and representative images are shown in E and F, respectively; Aβ plaque (green) and Iba1+ microglia (red, F), n = 6–8; independently reproduced twice). B-cell depletion reversed the reduction of IFNγ+ microglia in 3×TgAD mice (G, n = 5–7). Mean ± SEM is shown; each symbol is for a single mouse. Gating strategy is shown in Supplementary Fig. 9B. *p < 0.05; ***p < 0.001 in one-way ANOVA (A–C, G) or unpaired t test (F).
decreased in 5×FAD mouse brains as compared with that of WT mice \( (p < 0.001, \text{Fig. 5E, F}) \). B-cell depletion reversed the decrease of TGF\( \beta^{+} \) and IL10\( ^{+} \) microglia in 5×FAD mice (Fig. 5E, F). As in other models, the treatment did not decrease hippocampal IL1\( \beta^{+} \) microglia (Supplementary Fig. 7A, B), presumably to support clearance of A\( \beta \) plaques. Collectively, we concluded that depletion of B cells can retard progression of AD even if applied at the onset of the disease.

We then wondered whether B cells promote AD by infiltrating the brain parenchyma. Since the low numbers of B cells (<1% of CD45\( ^{+} \) cells, not depicted) detected in flowcytometric analyses cannot discriminate between cells residing inside or outside of the parenchyma, we performed immune fluorescent staining of perfused and cryopreserved brain sections of mice. Unlike control age-matched WT mouse brains, we clearly detected a significant increase of B cells in the parenchyma of frontal cortex and
hippocampus of 5×FAD mice (Fig. 5G and Supplementary Fig. 7C–E). Importantly, this increase of B cells was almost completely lost in B-cell-depleted 5×FAD mice (Fig. 5G and Supplementary Fig. 7D, E), implying that the cells originated in the circulation. We also stained the brain sections for the presence of IgG. While WT mouse brains were almost devoid of immunoglobulin (Fig. 5H, I), readily detectable and numerous IgG foci were present in the hippocampus parenchyma of 5×FAD mice often colocalized with microglia and Aβ plaques (Fig. 5H, I). This brain IgG increase was lost in B-cell-depleted 5×FAD mice (Fig. 5H, I). However, the depletion did not affect levels of total IgG nor minuscule amounts of Aβ-specific antibody in the circulation (Supplementary Fig. 8A–C), implying that B cells in the brain parenchyma produced IgG and presumably promoted AD.

Discussion

Taken together, we provide counterintuitive evidence for a “dark” side of B cells—they exacerbate manifestation of AD-like symptoms in addition to producing potentially beneficial Aβ plaque-reducing immunoglobulins47–21 and expressing AD-ameliorating cytokines42. Although the exacerbation in Rag-deficient APP and 5×FAD mice is linked to the loss of protective B cells and T cells16,21, our data revealed that the genetic loss of B cells alone or their transient depletion at the onset of AD improves the disease symptoms of three different mouse models. Unlike a recent report that linked AD progression to the reduction of anti-inflammatory B1a cells in 5×FAD mice42, the numbers of B1a and B1b cells in PB, spleen, and cLN were either unaffected (in 5×FAD mice even when followed for 4, 7, and 12 months) or upregulated (in 3xTgA and APP/PS1 mice). However, regardless of their numbers, we recently reported that the function of B1a cells is not static and is rather controlled by the inflammatory milieu. In the aged hosts, B1a cells lose their anti-inflammatory activity and activate pathogenic functions, such as becoming 4-1BBL+ B1a cells (termed 4BL cells) that induce cytolytic granzyme-B+ CD8+ T cells and promote insulin resistance23,44. In concordance, B1 cells (as well B2 cells, in some models) in AD mice also acquired an inflamed phenotype, as they upregulated expression of cytokines, such as IFNγ, IL6, TNFα, and IL10, and/or upregulated TGFβ and 4-1BBL. Although age-associated B cells (CD21−CD23−CD19+) also accumulate in aging, we did not detect their involvement in our three types of mice with AD.

Consistent with a recent RNA-seq report that revealed presence of mature B cells in the brains of AD mice4, our data indicate that AD increases B cells in the brain, and their IgG in the cortex and hippocampus parenchyma, which was often colocalized with Aβ plaques and activated microglia. As in multiple sclerosis and cognitive dysfunction following stroke45–47, B cells in the brain presumably produce immunoglobulins and proinflammatory factors exacerbating AD-promoting neuroinflammation. First, transfer of IgG from mouse brains to human brains is an inefficient process, as only 0.0017% of intravenously injected immunoglobulin reaches the hippocampus of WT and AD mice. Second, consistent with presumed ability of the intra-blood–brain-barrier sites of AD patients to synthesize immunoglobulin49, transient depletion of B cells in 5×FAD mice significantly decreased brain B cells and IgG without affecting Aβ-specific and nonspecific antibody levels in sera. Although immunoglobulin is thought to activate and promote microglial uptake of Aβ plaques21, the Aβ–IgG complex in CSF is thought to negatively affect the cognitive status of AD patients20. Our data also indicate that the loss of B cells, thus IgG, in the brain significantly retards the development of AD. Although the mechanism of this process is a topic of a different study, we think that brain IgG (or its immune complex) alone or in concert with B-cell cytokines exacerbates neuroinflammation in AD. To do this, the brain IgG presumably targets chronically stressed DAMs (and other brain cells) through their upregulated Fc receptors and complement11–32, as recently shown for myelin–IgG immune complexes from the brain of people with multiple sclerosis, which break immune tolerance of human microglia to microbial stimuli and cause harmful neuroinflammation via FcyRI and FcγRIIa54. This in turn leads to downregulation of TGFβ+ microglia, i.e., reduction in survival of resting M0 microglia and Aβ plaque clearance30,52. Similar decrease in expression of TGFβ in DAM and increase in signals of mature B cells is also noted in recent brain RNA-seq results of AD mice. Conversely, the loss of B cells increased TGFβ+ microglia as well downregulated expression of Trem2, Clec7a, and Il10 in the hippocampus, i.e., replacement of DAM with microglia that eliminate Aβ oligomers and other neurotoxic debris8–10, reduced Aβ plaques and improved behavioral impairments of our AD mice. It is tempting to speculate that B cells similarly participate in FQAD in humans, as the severity of the disease in 3- and 5×FAD mice is correlated with accumulation of double-negative memory CCR6+ B cells in the circulation. Moreover, elderly humans accumulate pathogenic 4-1BBL+TNFα+ B cells that induce antigen-specific CD8+ T cells and promote insulin resistance22,23, two events linked to AD57,58.

We therefore propose that the inactivation of B cells can also benefit humans with AD, as therapeutic B-cell removal even at the onset of the disease reversed manifestation of AD in mice.

Methods

Mice. The animal protocols were approved and permission was granted to perform animal experiments by the ACUC committee of the National Institute on Aging (ASP 321-LMB1-2022) under the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, 1985). AD transgenic mice (5–8 weeks of age, females) were bred, aged, and housed in the same, specific pathogen-free environment at the National Institute on Aging (NIA). Female C57BL/6 mice (Stock # 000664) were purchased from Jackson Laboratory (Bar Harbor, ME) and congenic 3xTgA mice (triple transgenic with three human genes associated with familial AD, B6;129-Psen1tm1Mpm Tg(APPswe,tauP301L)1 Lfa/Mmjax)24,25, APP/PS1 3×TgAD mice (triple transgenic with three human genes associated with familial AD, B6;129-Psen1tm1Mpm Tg(APPswe,tauP301L)1 Lfa/Mmjax)24,25, APP/PS1 mice (B6.Cg-Tg(APPswe,Sen1D9E9)538SwaJ). 5×FAD mice express mutant human APP and PSEN1 genes (B6.Cg-Tg(APPswe,Sen1D9E9)538SwaJ). 3×FAD mice express mutant human APP and PSEN1 genes (B6.Cg-Tg(APPswe,Sen1D9E9)538SwaJ). 5×FAD mice express mutant human APP and PSEN1 genes (B6.Cg-Tg(APPswe,Sen1D9E9)538SwaJ), and Tg15250 mice (B6.129P2-Jab1tm1Wjt/J). The mice were acclimated to their environment for 4–7 days before the experiment, and were maintained at NIA, Baltimore, MD. Transient B-cell depletion was performed as previously reported22,24, such as mice were injected intraperitoneally (i.p., three times six for 2–3 months) with anti-CD20 Ab (Clone 2D11, Genetech, 100 μg/mouse) and anti-B220 Ab (150 μg/mouse, TIB-146, BioXCell).

Tissues and blood processing. Single-cell suspension of spleen and cLN was prepared using a 70 μm cell strainer (BD Falcon, Bedford, MA). Blood was collected in tube with 2 mg/ml of Na-heparin (Sigma). Spleen and blood cell suspensions were treated with ACK buffer to remove red blood cells. The brains were dissociated with Adult Brain Dissociation kit for mouse and rat (MiltenyiBiotec, Auburn, CA) using GentleMACS™ Dissociator (MiltenyiBiotec), following the manufacturer’s instruction.

Flow cytometry (FACS). Antibodies (Ab, see Supplementary Data 4) to mouse CD19, CD5, CD11b, CD43, 4-1BBL, IFNγ, IL10, IL16, CD11b, and CD45 and their isotype-matched control Ab were purchased from Biolegend, eBioscience, BD Bioscience, and R&D Systems, unless specified. For IC cytokine staining, cells were stimulated with 50 ng/ml PMA (Tocris Bioscience) and 500 ng/ml ionomycin (Tocris Bioscience) for 1–2 h, followed by Golgi stop for 3–4 h using 10 μmol/l of Monensin or Brefeldin A (eBioscience); and then stained following manufacturer’s instruction for IC fixation and permeabilization (eBioscience). Concentration of antibody used in FACS staining was 1 μg per 10^6 cells. Data were analyzed on FACS Canto II (BD) or CytoFLEX (Beckman Coulter, Inc.) using FlowJo software (Tree Star, Inc.) or CytExpert software (Beckman Coulter, Inc.).

Brain immune fluorescent staining. Mice were perfused with PBS for 20–30 min after euthanasia with CO2, and the brains were removed and washed with PBS, and then brain half was fixed by 4% PFA in PBS. After 24-h fixation, the brain was washed with 10% PBS buffer containing 30% sucrose in 15 min until the brain sank to the bottom. Next, the brains were embedded in OCT compound, frozen on dry ice and stored in −80 °C before cryosection. A total of 30-μm thick coronal
sections containing hippocampus were collected with 240 μm interval to get eight sets and two to six slices of each mouse were stained for each immunofluorescence staining. For immunofluorescence staining, we adopted free-floating staining method: after two washes with PBS, the brain slices were incubated in 0.3 M glycine buffer for 30 min at room temperature (RT), and then blocked and permeabilized with IF buffer (2% donkey serum, 2% BSA, and 0.1% Triton X-100 in PBS) for 30–60 min at RT. Brain slices were incubated with designated Abs or their isotype control immuno-globulin, followed, over night before secondary incubations at RT. After three washes with PBS, brain slices were incubated with fluorochrome-conjugated secondary Abs from Abcam (Donkey anti-mouse IgG H&L-Alexa Fluor 488; Donkey anti-rabbit IgG H&L-Alexa Fluor 568; Donkey anti-rat IgG H&L-Alexa Fluor 647); at RT for 2 h. After three washes with PBS, slides were mounted with ProLong® Gold/Diamond Antifade Mountant with DAPI (Invitrogen). For IgG staining, brain slices were incubated with Iba1 antibody for 2 h at RT followed by wash with PBS, and then incubated with Alexa Fluor 488 labeled F(ab′2)/Goat anti-Mouse IgG (H + L) Antibody (cat # A-11017) and Donkey anti-rabbit IgG H&L-Alexa Fluor 568. The information of the first antibodies used is as below: anti-β-Amyloid, 1-16 Antibody (clone 6E10, cat # 803002), Recombinant Anti-Iba-1 antibody (cat # ab178486), Mouse IL1 β /L1-1F2 Antibody (cat # AF-401-NA), Anti-CD45R (B220) antibody (cat # ab64110), ZO-1 Polyclonal Antibody (cat # 40-2300), and Mouse Laminin α4 Antibody (clone 6C3, cat # ab2315). Images were acquired with a Zeiss LSM 710 confocal microscope equipped with an ×20/0.8 and a ×20 Plan-Apochromat dry objective lenses (Carl Zeiss). For Aβ plaque and microglia quantification, subcubic region for 3xTgAD model, or dentate gyrus region for APP/PS1 model, or whole hippocampus region for 5xFAD model were respectively identified in the coronal brain slices and imaged. For brain IL1β or IL1β staining, subcubic region was imaged. For brain B220+ B cell quantification, the whole brain was observed and images for B220+ B cells were captured. The quantification of all images was performed with ImageJ software. For Aβ and microglia quantification, the image of up to six slices per mouse were acquired and quantified in the subcubic region (3xTgAD model), DG region (APP/PS1 model), or whole hippocampus (5xFAD model). Briefly, brain channel image was converted into 8-bit image and proper threshold was chosen and fixed for all the images in the same experiment, then “analyze particles” function was used to quantify the number of area for each E10 Ig and Iba1+ microglia. The representative image for microglia quantification was shown in Supplementary Fig. 3G. For B220+ B cell quantification, the whole brain region was carefully observed and the images of B220+ B cells were acquired, and the location of B cells was distinguished by ZO-1 or laminin α4 staining or bright field.

Measurement of soluble Aβ42/40 peptides. Cerebral cortex and hippocampus were separated from PBS-perfused mouse brains, and stored at –80 °C. Soluble and insoluble protein fractions were purified using a modification of previously published protocol for Aβ peptides61. Briefly, tissues were mechanically homogenized in in TBS-Trition 1% (120 mM NaCl, 50 mM Tris, pH 8.0, 150 mg/ml tissue buffer), including protease inhibitor cocktail (1:100, P2714, Sigma, St. Louis, MO), then incubated on ice for 30 min following centrifugation for 120 min at 17,000 × g at 4 °C. Supernatant, containing TBS-Trition-soluble fraction of mouse Aβ40–42 and Aβ42–42 was removed and stored at –80 °C. Pellet containing protein, called insoluble Aβ was resuspended in 70% formic acid and incubated on ice for 30 min, followed by centrifugation at the same conditions as mentioned above. Formic acid-soluble supernatant was neutralized and washed using 1 M Tris (pH = 11, 20-time the volume of the formic acid) and stored at –20 °C. Total protein concentration was determined using the BCA method (cat # 23225, Thermo Scientific, Waltham, MA). TBS-S and formic acid-soluble levels Aβ40 and Aβ42 in the cortex were measured using sandwich-ELISA protocol in 96-well polystyrene microplates (655061, Greinerbio-one, Monroe, NC) were coated with 4–8 μg/ml Aβ42–42 peptide (AnaSpec, cat # A5-24224) and quantified using HRP-conjugated goat anti-mouse IgA, IgG, or IgM antibodies. For serum immunoglobulin ELISA, IgG Isotyping Mouse Uncoated ELISA Kit (Invitrogen, Cat. No. # 88-50630-88) was used, following the manufacturer’s instruction.

Statistical analysis. All statistical analyses were performed with GraphPad Prism (Prism 8; Graph Pad Software, Inc.). Normality tests were conducted to decide whether the data are from normally distributed population. Unpaired t test or one-way ANOVA, for two groups or three groups, respectively, was used if the data are considered to be normally distributed, otherwise Mann–Whitney test or Kruskal–Wallis test was used for two groups or three groups, respectively. Due to changes in variance across days of invisible platform testing in MMW, as is typical in this task61, each day was individually analyzed using one-way ANOVA. Bonferroni post hoc tests were conducted when appropriate. The results were presented as the mean with each individual data point or in bar graph ± SEM. A p value <0.05 was considered significant (*p < 0.05, **p < 0.01, and ***p < 0.001).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data associated with this study can be found in the paper or supplementary materials. The microarray data are deposited at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165111. Source data are provided with this paper.

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Author contributions

K.K., X.W., E.Ra., and M.B. performed the research and collected and analyzed data; T.I., M.D., and R.M. performed experiments; F.G. provided bioinformatics support, E.Ro. and E.O. provided critical interpretation; and A.B. wrote the manuscript and conceived, designed, and supervised the study.

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Competing interests

The authors declare no competing interests.

Additional information

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