Altered Neuroinflammation and Behavior after Traumatic Brain Injury in a Mouse Model of Alzheimer's Disease

Olga Kokiko-Cochran,¹ Lena Ransohoff,¹ Mike Veenstra,¹ Sungho Lee,¹ Maha Saber,¹ Matt Sikora,¹ Ryan Teknipp,¹ Guixiang Xu,¹ Shane Bemiller,¹ Gina Wilson,² Samuel Crish,² Kiran Bhaskar,³ Yu-Shang Lee,¹ Richard M. Ransohoff,⁴ and Bruce T. Lamb^{1,5}

Abstract

Traumatic brain injury (TBI) has acute and chronic sequelae, including an increased risk for the development of Alzheimer's disease (AD). TBI-associated neuroinflammation is characterized by activation of brain-resident microglia and infiltration of monocytes; however, recent studies have implicated beta-amyloid as a major manipulator of the inflammatory response. To examine neuroinflammation after TBI and development of AD-like features, these studies examined the effects of TBI in the presence and absence of beta-amyloid. The R1.40 mouse model of cerebral amyloidosis was used, with a focus on time points well before robust AD pathologies. Unexpectedly, in R1.40 mice, the acute neuroinflammatory response to TBI was strikingly muted, with reduced numbers of CNS myeloid cells acquiring a macrophage phenotype and decreased expression of inflammatory cytokines. At chronic time points, macrophage activation substantially declined in non-Tg TBI mice; however, it was relatively unchanged in R1.40 TBI mice. The persistent inflammatory response coincided with significant tissue loss between 3 and 120 days post-injury in R1.40 TBI mice, which was not observed in non-Tg TBI mice. Surprisingly, inflammatory cytokine expression was enhanced in R1.40 mice compared with non-Tg mice, regardless of injury group. Although R1.40 TBI mice demonstrated task-specific deficits in cognition, overall functional recovery was similar to non-Tg TBI mice. These findings suggest that accumulating beta-amyloid leads to an altered post-injury macrophage response at acute and chronic time points. Together, these studies emphasize the role of post-injury neuroinflammation in regulating long-term sequelae after TBI and also support recent studies implicating beta-amyloid as an immunomodulator.

Key words: Alzheimer's disease; macrophage; neuroinflammation; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a major risk factor for neurodegenerative diseases, prominently including Alzheimer's disease (AD).^{1–5} Patients with single moderate to severe head trauma can display key neuropathological features of AD, including accumulation of amyloid precursor protein (APP) as well as extracellular deposition of the beta-amyloid (A β) peptide in senile plaques, intracellular aggregation of the microtubule associated protein tau in neurofibrillary tangles, marked activation of microglia, and production of inflammatory cytokines.^{6–9}

Current evidence indicates a clear biological link between TBI and AD pathology, but the molecular pathways underlying this relationship are incompletely understood. Although many experimental studies have aimed to characterize the relationship between

TBI and AD,¹⁰ the variety of TBI as well as AD animal models used has often elicited more questions than answers, ultimately confirming the complexity of the TBI-AD interaction.

It has recently become evident that neuroinflammation is directly implicated in AD pathogenesis. Most salient are studies of immune-system genes such as TREM-2 and CD33, reporting findings that variant genes carry impressive effects for risk of AD and other neurodegenerations.^{11–13} In support of neuroinflammatory pathophysiology for AD and TBI, activated microglia, reactive astrocytes, cytokines, chemokines, and complement components are elevated in both the AD and TBI-exposed brain.^{6,7,14–17} On the epidemiological level, nonsteroidal anti-inflammatory drugs decreased risk for AD, and slowed disease progression in both clinical and pre-clinical experiments.^{18,19} Mechanisms that link TBI induced neuroinflammation and neurodegeneration remain to be identified.

¹Department of Neurosciences, Cleveland Clinic, Cleveland, Ohio.

²Department of Pharmaceutical Science, Northeast Ohio Medical University, Rootstown, Ohio.

³Department of Molecular Genetics Microbiology and Neurology, University of New Mexico, Albuquerque New Mexico.

⁴Biogen Idec, Cambridge, Massachusetts.

⁵Department of Genetics and Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio.

The current study examined the acute and chronic macrophage response to TBI in the presence or absence of accumulating A β . Subsequent correlations were identified between macrophage alterations and long-term neurodegeneration and behavioral impairment. The Lamb laboratory has generated the R1.40 mouse model of cerebral amyloidosis, which contains a full genomic copy of the human *APP* gene with a familial AD mutation and develops age-related appearance of A β pathology at 12 months of age as well as earlier biochemical, neuropathological, and behavioral features of human AD.

Previous work from our group has demonstrated that microglial activation is directly involved in promoting the expression of cell cycle proteins in neuronal populations subject to degeneration in R1.40 mice, which is one of the earliest features of AD-like pathology in this model.^{20,21} R1.40 mice represented an ideal model for these studies, because TBI could be administered to young animals with accumulating A β but well before the presence of advanced AD-like pathologies—i.e. A β plaques, which are more closely associated with aging.

At 2 months of age, R1.40 and non-Tg mice were exposed to lateral fluid percussion injury (FPI) and examined at both acute (3 days post-injury [DPI]) and chronic (120 DPI; 6 months of age) time points. Surprisingly, the inflammatory reaction to TBI was markedly reduced at acute time points in R1.40 mice, which then displayed enhanced brain cavitation along with a unique pattern of cognitive impairment 4 months later. These results suggest that the specific character of the neuroinflammatory response to TBI affects subsequent neurodegeneration and cognitive function in a mouse model of AD.

One major implication of our findings is that the post-injury neuroinflammatory response is critical in mediating AD-like phenotypes. The presence of A β is not sufficient to worsen recovery after brain injury and, in fact, appears to offer protection from the acute post-injury macrophage response. These findings emphasize the role of neuroinflammation in altering long-term sequelae after TBI and support recent studies implicating the role of A β in altered neuroinflammatory responses.

Methods

Study design

The overall objective of this study was to characterize the macrophage response to TBI in the presence or absence of accumulating A β at both acute and chronic time points. Separate groups of R1.40 and non-Tg mice were used for acute and chronic studies. After surgical preparation, mice from each genotype were randomized to the TBI or sham group. Subsequent data analysis was performed by a separate investigator blinded to the experimental group. Power analyses were performed, and all sample sizes were selected based on a desire to reject the null hypothesis with a probability of 0.8. All subjects surviving to 3 DPI or 120 DPI were included in data analysis and no outliers were excluded.

Mice

To study the effects of TBI-induced inflammation in promoting AD-like phenotypes, we used the genomic-based R1.40 mouse model of cerebral amyloidosis, which contains the entire human *APP* gene with the K670N/M671L familial AD mutation. This model modestly overexpresses (2.5X) all the different isoforms of human APP under endogenous human APP regulatory elements. R1.40 mice exhibit A β deposition and memory impairments at advanced ages (12–19 mos), but also exhibit altered microglial activation and aberrant expression of cell cycle proteins within neurons (termed cell cycle events [CCEs]) as early as 6 months of age. Two-month old homozygous R1.40 mice maintained on the

C57BL/6J genetic background were used for all studies. Age- and sex-matched non-Tg C57BL/6J animals served as controls for all studies. Animals were housed at the Cleveland Clinic Biological Resources Unit, and all procedures were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic.

Surgical preparation and injury

The lateral FPI model of TBI was chosen for the current studies because FPI remains one of the most extensively used and well-characterized models of TBI. The microglial response after FPI has been well characterized, and evidence shows that there are multiple peaks of microglial activation occurring within the first week as well as several months post-injury corresponding with clinical pathology and behavioral deficits.^{22,23} Taken together, these data suggest that the FPI represents an ideal model to study the effects of TBI-induced macrophage activation in promoting AD-like phenotypes.

Briefly, at 2 months of age, all mice were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic frame. Bupivacaine (0.25%, 50 μ L) was administered subcutaneously before midline incision. A 3.0 mm craniotomy was trephined over the right parietal cortex midway between bregma and lambda leaving the dura intact. A modified Leur-Loc syringe (3.0 mm inside diameter) was placed over the exposed dura and held in place by cyanoacrylate adhesive.

Animals recovered on a heating pad before being returned to their home cage. Twenty-four hours after surgical preparation, all mice were anesthetized with the same combination of ketamine and xylazine and connected to the FPI device. Animals in the injured group received a moderate level FPI ($M = 1.0$ atmospheres of extracranial pressure). Animals in the sham group were connected to the injury device; however, no fluid pulse was delivered. After FPI or sham injury, the syringe and adhesive were removed, and the incision was sutured. Mice were sacrificed at either 3 DPI or 130 DPI (1 week after completion of spatial memory testing in the water maze). This resulted in a two (TBI, sham) by two (R1.40, non-Tg) factorial design with four experimental groups (Table 1).

TABLE 1. ANIMAL NUMBERS PER EXPERIMENT

Post-injury time point	Experiments	Non-Tg		R1.40	
		Sham	TBI	TBI	Sham
3 DPI	Immunohistochemistry	6	6	6	6
	Flow cytometry	3	6	7	4
	Western immunoblotting	5	6	6	5
	A β ELISA	4	4	4	4
	Multiplex assay	6	6	6	6
	TOTAL	15	18	19	16
120 DPI	Immunohistochemistry	6	6	6	6
	Western immunoblotting	6	6	6	6
	A β ELISA	5	6	6	6
	Multiplex assay	6	6	6	6
	Behavioral testing	13	14	12	13
	TOTAL	13	14	12	13

After traumatic brain injury (TBI) or sham injury, separate groups of mice were aged to 3 or 120 days post-injury (DPI). Brain tissue from the same subjects were used for Western immunoblotting, enzyme-linked immunosorbent assay (ELISA), and multiplex assay at 3 DPI as indicated by gray shading. At 120 DPI, half of the mice that completed behavioral testing were used for immunohistochemistry and half of the mice were used for biochemistry. Brain tissue from the same subjects was used for Western immunoblotting, ELISA, and multiplex assay at 120 DPI, as indicated by gray shading. Notably, a few extra samples were included per group at 120 DPI for behavioral testing.

Primary antibodies

Inflammatory markers. Rabbit polyclonal antibody against ionized calcium binding adaptor molecule 1 (Iba1; Wako); rat monoclonal anti-CD45 (Serotec); mouse monoclonal anti-F4/80 (Serotec); rat monoclonal anti-CD68 (Abcam); sheep polyclonal anti-TREM2 (R&D Systems); rabbit polyclonal anti-glial fibrillary acidic protein (GFAP, Sigma).

APP antibodies. Mouse monoclonal anti-human A β (clone 6E10; Signet Labs); rabbit polyclonal anti-APP (clone CT15; kindly provided by Edward H. Koo).

Microtubule associated protein tau antibodies. Mouse monoclonal antibody against AT8 (pSer202+Thr205; Pierce), AT180 (pThr231; Pierce), and Tau5 (Invitrogen).

Other antibodies. Mouse monoclonal antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Cell Signaling)

Western immunoblotting

At 3 DPI or 120 DPI, half of the mice in each group ($n = 5-6$ per group) were deeply anesthetized with tribromoethanol and transcardially perfused with ice-cold 0.1 mol/L sodium phosphate buffer. Brain tissue was removed after perfusion and sagittally bisected into ipsilateral and contralateral hemispheres. Microdissection was used to separate cortices. Ipsilateral cortical lysates were prepared by homogenizing in Tissue-Protein Extraction Reagent (T-PER, 78510; Pierce) with protease (P8340 Sigma-Aldrich) and phosphatase (P5726; Sigma-Aldrich) inhibitor cocktails. Brain protein (20 μ g) was run on a 4–12% Novex NuPage gel (Invitrogen) and transferred to a polyvinylidene difluoride (PVDF) membrane. The Western blots were then blocked with 5% milk and incubated overnight at 4°C in primary antibodies (CT15 1:5000; 6E10 1:5000; GAPDH 1:20,000; AT8 1:5000; AT180 1:2000; Tau5 1:5000).

After incubation, blots were washed with phosphate buffered saline (PBS) and incubated with respective secondary antibodies. ECL reagent (NEL101001EA; Perkin Elmer) was used to develop the APP blots, and AlphaEaseFC™ Software (Alpha Innotech Corporation) was used to quantify immunoreactive bands. Tau blots were probed with goat anti-mouse secondary antibodies, IRDye (Thermo SAS-10176) for 1 h at a concentration of 1:10,000, and signal was measured and quantified using Odyssey® infrared imaging system (Li-Cor).

Immunohistochemistry

Half of the mice in each group ($n = 5-6$ per group) were deeply anesthetized with tribromoethanol and transcardially perfused with ice-cold 0.1 mol/L sodium phosphate buffer at 3 DPI and 120 DPI. Whole brain samples were removed from the cranium and drop-fixed in 4% paraformaldehyde in PBS and stored at 4°C. The brains were subsequently cryoprotected in 30% sucrose/PBS and then embedded in optimal cutting temperature (OCT) compound. Free floating, 30 μ m coronal sections were cut with a cryostat and processed for neuropathological analysis.

The following dilutions were used for primary antibodies: Iba1 1:500; CD45 1:500; F4/80 1:1000; CD68 1:500; Trem2: 1:50; CT15 1:500; all incubated at 4°C overnight followed by secondary antibodies conjugated to biotin (Vector Laboratories; 1:200). Sections were incubated with Avidin/Biotinylated enzyme complex (ABC reagent, Vector Laboratories; for immunohistochemistry) reagent for 1 h at room temperature followed by 3–3'-diaminobenzidine (DAB; Vector Laboratories) until a brown reaction was observed. Sections stained with GFAP (1:500) and AT8 (1:500) were incubated at 4°C

overnight followed by Alexa Fluor 488 and 546 secondary antibodies (Life Technologies).

Isolation of microglia and monocytes and flow cytometry

At 3 DPI, mice from each experimental group were deeply anesthetized with tribromoethanol and transcardially perfused with 1x Hank balanced salt solution (HBSS). Brain tissue was removed and sagittally bisected. Microglia and monocytes were subsequently isolated using an optimized method as described previously.²⁴ The ipsilateral (injured) hemisphere was collected in 4 mL of HBSS and dispersed with a plunger digestion cocktail (9.5 mL 1x HBSS, 50 μ L collagenase D, 10 μ L N-p-tosyl-L-lysine chloromethylketone (TLCK), 50 μ L DNase I, 25 mg dispase, 100 μ L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)) to wash the cells.

After a 15-min incubation at room temperature, samples were centrifuged at 1800 revolutions per minute (rpm) for 7 min at 18°C. The pellets were resuspended in a HBSS wash to remove the digestion cocktail and centrifuged again at 1800 rpm for 7 min at 18°C. After removal of the supernatant, pellets were resuspended in 10 mL of 30% isotonic (ISP) Percoll. The ISP/cell suspension was overlaid on 2 mL of 70% ISP and centrifuged at 800 rcf for 30 min at 18°C. After centrifugation, 2 mL of the 70%/30% interphase was collected in a clean conical tube with 10 mL of HBSS. Samples were centrifuged at 2200 rpm for 7 min at 4°C. Finally, the cells were resuspended in FACS buffer (PBS containing BSA and sodium azide)/FC block for 5 min at room temperature.

The following antibodies were used for cell surface staining of isolated microglia and monocytes: anti-mouse CD11b-PE (eBioscience, 1:100) anti-mouse CD45-APC (BioLegend, 1:500), anti-mouse F4/80 (BioLegend, 1:100). Flow cytometry was performed with a BD LSRFortessa (BD Bioscience) and analyzed using FlowJo software (version 9.3.1, TreeStar, Inc. Ashland, OR). All data were normalized to R1.40 sham for analysis.

A β enzyme-linked immunosorbent assay (ELISA)

The level of A β 1-40 in ipsilateral cortical brain extracts from brain injured and sham R1.40 and non-Tg mice were determined by ELISA (Life Technologies). Homogenized brain tissue (also used for Western blots) was mixed gently at room temperature for 3–4 h, diluted five-fold with Biosource sample/standard diluent containing 1xprotease inhibitors (Calbiochem). Samples were further diluted five-fold with sample/standard diluent (containing AEBSEF [Sigma] protease inhibitor at 1 mM), and the ELISA was performed as prescribed by the manufacturer. The plate was read using a fluorometric plate reader (Perkin/Elmer). Sample values were compared to a standard curve, and pmole/g brain values calculated.

Multiplex assay

Ipsilateral cortical brain extracts were used for multiplex cytokine analysis. Samples were diluted 1:10, and total protein content was assessed in tissue samples using the bicinichronic acid (BCA) assay, following the manufacturer's instructions (Thermo Fisher, Cat# 23225), and cytokine levels were all normalized to total protein concentration. Multiplex analyses were conducted in Greiner, flat-bottom 96-well plates (Greiner Bio-One, Ref# 655096) according to the manufacturer's instructions using reagents provided with the kit (Invitrogen Mouse 20-plex Cytokine Panel, Cat# LMC0006M). In brief, 25 μ L of antibody-coupled bead solution was pipetted into each well. Samples were diluted 1:4 in assay diluent to a total volume of 100 μ L, with each well containing approximately 200–300 μ g of total sample protein. One hundred microliters of reconstituted standard were serially diluted (1:3) and added to appropriate wells. Twenty-five microliters of T-Per in

75 μ L of assay diluent were used as a blank solution. Finally, 50 μ L of incubation buffer were added to all wells. Plates were kept light-protected during an overnight incubation (4°C, 16–18 h) on a titer plate shaker (Thermo Scientific, model #4625Q) at 600 rpm.

After sample incubation, plates were washed with a mild detergent solution using a magnetic 96-well separator (Invitrogen Cat# A14179) three times (1 min per wash). One hundred microliters of horseradish peroxidase-conjugated detection antibody were added to each well and incubated at room temperature for 1 h (shaking at 600 rpm). Plates were washed three times and 100 μ L of streptavidin-*r*pe were added to each well. After the 30 min streptavidin-R-phycoerythrin incubation, plates were again washed three times followed by a final addition of 125 μ L wash solution to all wells. After 3 min of vigorous shaking, plates were read on a Luminex Magpix unit (Life Technologies), and initial analyses were performed by Xponent software and results exported into Microsoft Excel for further processing. Sample size was set to 50 μ L, and minimum count was set to 100 events/bead region.

Lesion analysis

Sections transversing the lesion cavity from –0.20 to –3.50 mm from bregma were selected and imaged using Quickscan via Pathscan Enabler IV. Image J was used to create a stack of images from each brain sample. Image Stacks were imported to Reconstruct software²⁵ for volumetric analysis and three-dimensional (3D) reconstruction. First, the entire coronal section was outlined so that ipsilateral and contralateral hemispheres were similar in size. Then the lesion cavity was outlined, and a percentage ratio to the entire coronal section was calculated and compared across groups. The ipsilateral hippocampus from –0.20 to –3.50 mm from bregma was also outlined, and a percentage ratio to the remaining tissue was calculated and compared across groups (9 sections, 330 μ m apart). A subset of sections was stained with cresyl violet, but because no discernible differences were detected between brain injured groups, final lesion analysis was completed on Iba1 stained sections.

Morphometric analysis of microglia and immunoreactivity

Morphometric analysis of microglial activation was performed on Iba1-stained sections (–1.94 from bregma). Images were acquired at 20X objective using Image-Pro Plus software (Media Cybernetics) as described previously.²⁶ Analyzed images were taken from the ipsilateral-cortex, both lateral and medial to the injury site, the ipsilateral-thalamus, and the contralateral-CA2 region from six animals per group. Form factor (FF) measures contour irregularity of a cell by the formula $(4 \times \pi \times \text{Ac})/\text{Pc}^2$, where Ac is the area and Pc is the perimeter of the cell. FF values are higher in activated microglia, which are characterized by large cell bodies with numerous, sparsely ramified processes. To quantify the percent area of immunostaining, stained images were taken at 20X objective and converted to gray scale after background subtraction in Adobe Photoshop. Adjusted gray scale images were then imported into Image J and converted to thresholded binary images for quantification.

Behavioral analysis

Rotarod. Balance and coordination was assessed with a standard rotarod test (Rotamex, Columbus Instruments). All animals received baseline training on the rotarod 24 h before surgical preparation. Post-injury motor assessment was completed at 1, 3, 6, 30, 60, and 90 DPI. Animals completed three 5-min trials on each testing day. Rod rotation increased from 4 rpm to 30 rpm during each trial. An average latency to fall was calculated for each testing day and compared between groups.

Y maze. The y maze was used to evaluate time dependent changes in post-injury spatial working memory. Animals completed one 5-min trial at 7, 30, 60, and 90 DPI. At the beginning of each trial, a mouse was placed in the center of the Y maze and allowed to freely explore for the entire trial. Total arm entries and spontaneous alternations between arms was recorded for each testing day and compared between groups.

Water maze. A standard version of the Morris water maze (MWM) was used to assess spatial learning and reference memory beginning at 120 DPI. A traditional version of memory testing was completed 120–125 DPI. Animals were placed in the pool at one of four start locations and given 60 sec to locate a submerged goal platform that remained in the same location throughout memory testing. Animals remained on the platform for 15 sec after 60 sec elapsed or after they located the platform on their own.

A single probe trial, in which the goal platform was removed, was completed at 126 DPI approximately 24 h after the last memory trial. After the probe trial, four visible platform trials were completed. During visible platform trials, animals were placed in the pool at one of four start locations and given 60 sec to locate a visible goal platform that was placed in a new location for each trial. Mice were sacrificed the following week at 130 DPI.

All trials were analyzed with EthoVision XT (Noldus) video tracking software. Two learning indices were used to evaluate within- and between-day learning in the water maze task.²⁷ The acquisition index measures within-day learning averaged across all training days, where t_F and t_L are the latency to reach the platform from the first and last trials of the day, respectively, and k is the number of days of training in the water maze: $([t_F d_1 - t_L d_1] + \dots + [t_F d_k - t_L d_k])/k$. The savings index measures between-day learning averaged across all training days, where the difference is calculated between the latency to reach the platform on last trial of a day and the first trial of the subsequent day: $([t_L d_1 - t_F d_2] + \dots + [t_L d_{k-1} - t_F d_k])/k$.

Statistical analysis

All data are presented as mean $\pm$ standard error of the mean (SEM). All statistical analysis was completed with SPSS Version 22 or GraphPad Prism 5. A mixed model factorial analysis of variance (ANOVA) was used to evaluate group differences. Student *t* test (two-tailed, unpaired) was used to evaluate time dependent changes in lesion size and Y maze. ANOVA with repeated measures was used to classify group differences in rotarod and MWM performance. The between factors were genotype and injury, and the within-group factor was day of testing. Main effects and interaction effects were considered. Multiple analysis of variance (MANOVA) was used to analyze multiplex data. Non-normally distributed multiplex data was log-transformed before MANOVA. Statistical significance was determined at $p < 0.05$.

Results

TBI induces an age-dependent increase in the brain injury cavitation in R1.40 mice

Moderate FPI results in a cortical contusion that enlarges over time to become a visible brain injury cavity. FPI induced extensive damage to the cortex and underlying hippocampus of the injured hemisphere (Fig. 1A). At 3 DPI, no significant differences in lesion volume were detected between non-Tg and R1.40 TBI mice (Fig. 1B); however, the lesion cavity was smaller in R1.40 mice compared with non-Tg mice. At 120 DPI, an enlarged injury cavity was observed in both genotypes (Fig. 1A). At 120 DPI, there was a statistically significant increase in lesion volume in TBI R1.40 mice compared with 3 DPI ($t = 4.97$, $p < 0.01$, Fig. 1B), which was not seen in controls. From these data, we can conclude that over time,

FIG. 1. Lesion and hippocampal volume change over time. (A) Representative three-dimensional (3D) reconstructions show a cortical injury cavity at 3 days post-injury (DPI) in mice with traumatic brain injury (TBI), which is larger at 120 DPI ($n=6-7$ /group/time point). (B) Volumetric lesion analysis reveals a time-dependent increase in the size of the injury cavity in the R1.40 mice at 120 DPI compared with 3 DPI. (C) Representative 3D reconstructions show the ipsilateral hippocampus in brain and sham injured non-Tg and R1.40 mice at 3 and 120 DPI. (D) Volumetric hippocampal analysis reveals a time-dependent decrease in all groups, which reached statistical significance in R1.40 TBI and sham mice.

the size of the lesion cavity is stable in non-Tg mice but variable in R1.40 mice.

Volumetric analysis of the ipsilateral hippocampus revealed only modest changes after FPI in both genotypes at 3 DPI (Fig. 1C), which resulted in no statistically significant differences between groups at the acute post-injury time point (Fig. 1D). By 120 DPI, notable changes in hippocampal volume can be seen in TBI mice compared with sham mice (Fig. 1C); however, because of variability, no statistically significant differences were detected between TBI and sham mice (Fig. 1D) at this chronic time point. An age-dependent decrease in hippocampal volume can be seen in all groups, which reached statistical significance in R1.40 mice and was further enhanced by TBI (R1.40 sham, $t=2.614$, $p<0.05$; R1.40 TBI, $t=2.640$, $p<0.05$, Fig. 1C).

Brain injury induces dynamic regional microglial activation

A hallmark feature of human TBI is widespread neuroinflammation characterized by activation of brain-resident microglia, infiltration of monocytes, and abundant production of inflammatory mediators.^{28,29} At 3 DPI, we used Iba1 immunohistochemistry to

demonstrate altered macrophage morphology via FF analysis in TBI mice in the ipsilateral cortex ($F(1, 19)=49.58$, $p<0.001$), ipsilateral thalamus ($F(1, 19)=47.39$, $p<0.001$), and contralateral CA2 ($F(1, 19)=4.56$, $p<0.05$; Fig. 2A,B). By 120 DPI, macrophage morphological responses in TBI mice had waned in all brain regions analyzed; however, a persistent effect of injury was still detected in the ipsilateral cortex ($F(1, 20)=4.69$, $p<0.05$), ipsilateral thalamus ($F(1, 20)=61.00$, $p<0.001$), and contralateral CA2 ($F(1, 20)=4.67$, $p<0.05$) where a significant effect of genotype was also detected ($F(1, 20)=10.67$, $p=0.01$; Fig. 2C,D).

Taken together, these findings demonstrate that cortical macrophage activation dramatically decreases between 3 DPI and 120 DPI, although subcortical brain regions (i.e., thalamus) may be at risk for persistent alterations in macrophage activation. Because Iba1 recognizes both microglia as well as infiltrating monocytes, however, these studies could not discriminate between the effects of TBI on either microglia or monocytes in the R1.40 and non-Tg mice.

Altered acute inflammatory response in R1.40 mice exposed to TBI

Microglia of the healthy brain are CD45^{dim} and those found in the inflamed central nervous system (CNS) are often CD45^{int}, while infiltrating monocytes are CD45^{hi}.³⁰⁻³² At 3 DPI, CD45 staining near the injury cavities of TBI mice (Fig. 3A) showed an increase in immunoreactive cells in both R1.40 and non-Tg TBI animals compared with shams (ipsilateral cortex, $F(1, 21)=40.69$, $p<0.01$; ipsilateral thalamus, $F(1, 21)=37.86$, $p<0.001$; contralateral CA2, $F(1, 21)=18.03$, $p<0.01$) but with a surprising difference: in the ipsilateral thalamus, CD45 immunoreactivity in brain injured R1.40 mice was markedly and significantly less than that observed in non-Tg mice (genotype effect, $F(1, 21)=6.44$, $p<0.05$; interaction effect, $F(1, 21)=4.78$, $p<0.05$; Fig. 3B).

These findings indicated that the post-injury macrophage response was altered in R1.40 mice. Importantly, the only distinction between R1.40 and non-Tg mice at 2 months of age lies in overproduction of A β peptides in the CNS of transgenic animals. F4/80 immunoreactivity (Fig. 3C) confirmed the suppressed macrophage response by microglia and monocytes in R1.40 TBI mice compared with non-Tg TBI mice. A main effect of injury was detected in all brain regions (ipsilateral cortex, $F(1, 20)=9.62$, $p<0.01$; ipsilateral thalamus, $F(1, 20)=23.65$, $p<0.001$; contralateral thalamus, $F(1, 20)=61.44$, $p<0.001$; Fig. 3D). A main effect of genotype was detected in the ipsilateral thalamus ($F(1, 20)=9.96$, $p<0.01$) and contralateral CA2 region ($F(1, 20)=32.05$, $p<0.001$).

Finally, an interaction effect was detected in all brain regions (ipsilateral cortex, $F(1, 20)=7.36$, $p<0.05$; ipsilateral thalamus, $F(1, 20)=5.96$, $p<0.05$; contralateral thalamus, $F(1, 20)=19.43$, $p<0.001$; Fig. 3D). Together, the CD45 and F4/80 staining indicated that R1.40 mice mounted a subdued neuroinflammatory response to TBI at early time points.

Next we used CD68 and TREM2 immunohistochemistry to confirm the altered macrophage response in R1.40 TBI mice at 3 DPI. CD68 is a marker for macrophages, including microglia-derived macrophages. CD68 immunoreactivity around the lesion cavity was significantly higher in brain-injured mice compared with shams, $F(1, 44)=14.54$, $p<0.001$, Fig. 3E-F. A significant effect of genotype was also identified, $F(1, 44)=4.34$, $p<0.05$, indicating that CD68 expression was reduced in R1.40 mice compared with non-Tg mice; however, there was no significant interaction between injury and genotype.

FIG. 2. Traumatic brain injury (TBI) induces morphometric changes in Iba1+ macrophages. (A,C) Representative images of Iba1 immunostaining in sham and TBI mice in ipsilateral cortex (Ipsi-Ctx), ipsilateral thalamus (Ipsi-Thal), and contralateral CA2 (Contra-CA2) brain regions at 3 days post-injury (DPI) and 120 DPI, respectively. (B) Form factor (FF) analysis of Iba1 positive macrophages at 3 DPI revealed higher values in non-Tg and R1.40 TBI mice compared with sham mice in all brain regions analyzed ($n=6$ /group). (C) FF analysis at 120 DPI also revealed a primary effect of injury in all brain regions ($n=6$ /group). Error bars indicate standard error of the mean. Scale bars indicate 20 μ m. Main effect of injury, *, $p<0.05$, ***, $p<0.001$; main effect of genotype, #, $p<0.05$.

TREM2 is an immune receptor expressed on the cell membrane of myeloid cells, including microglia and tissue macrophages, and has been implicated in inflammation and phagocytosis.³³ TREM2 immunoreactivity predominantly surrounded the injury cavity at 3 DPI in non-Tg and R1.40 mice compared with shams who showed very little TREM2 expression throughout the brain (Fig. 3G). Quantification revealed that TREM2 immunoreactivity was significantly higher in TBI mice compared with shams, $F(1, 62)=25.45$, $p<0.001$. A significant genotype effect was also detected in TREM2 expression, $F(1, 62)=5.65$, $p<0.05$, as well as a significant interaction between injury and genotype, $F(1, 62)=8.80$, $p<0.01$, Fig. 3H. Together, these data lend support to the finding that the macrophage response to TBI is significantly reduced in R1.40 mice.

Accumulation of microglia and monocytes in the injured hemisphere at 3 DPI was also quantified using flow cytometry (Fig. 4A–E). A significant reduction in F4/80+/CD45^{low} cells (microglia) was identified in brain-injured mice compared with shams, $F(1, 16)=6.34$, $p<0.05$, Fig. 4D. The ratio of F4/80+/CD45^{high} cells (monocytes) was variable after TBI or sham injury, and no statistically significant differences were detected between groups, Fig. 4E. A significant interaction between genotype and injury was identified in the fraction of CD45+ cells (microglia, monocytes, and macrophages) expressing F4/80, $F(1, 1)=6.85$, $p<0.05$, Fig. 4C. This finding suggests that the effect of TBI on CD45+ cells expressing F4/80 varies in non-Tg and R1.40 mice. The ratio of F4/80+ cells is clearly reduced in R1.40 TBI mice ($M=0.74$, SEM=0.06) compared with non-Tg TBI mice ($M=1.20$, SEM=0.13).

Acutely after TBI, total myeloid cells numbers and relative proportions of microglia and monocytes in the CNS of the two genotypes were similar but their activation to macrophages was blunted in R1.40 animals.

TBI-induced neuroinflammation was surprisingly persistent in the ipsilateral thalamus of both non-Tg and R1.40 animals at the chronic 120 DPI time point as judged by quantitative CD45 (ipsilateral thalamus, $F(1, 19)=9.26$, $p<0.01$, Fig. 5A,B) and F4/80 immunohistochemistry (ipsilateral thalamus, $F(1, 18)=3.790$, $p<0.05$, Fig. 3C,D). F4/80 immunoreactivity was also enhanced in the contralateral CA2 region ($F(1, 18)=3.329$, $p<0.05$, Fig. 5C,D). There were no pairwise differences between R1.40 and non-Tg TBI mice at 120 DPI. Interestingly, however, when we compared 3 DPI and 120 DPI in R1.40 TBI mice, F4/80 staining was similar in all brain regions, suggesting the macrophage response was compromised at acute and chronic time points. CD68 and TREM2 expression was dramatically reduced in the cortex of all groups at 120 DPI (data not shown).

GFAP immunohistochemistry was performed to document astrogliosis at 3 and 120 DPI. Several GFAP+ cells were identified in the ipsilateral cortex and hippocampus of TBI mice at 3 DPI; however, no overt differences were apparent between genotypes. By 120 DPI, GFAP+ astrocytes displayed elongated processes with web-like morphology around the injury cavity in TBI mice only. GFAP was not detected in subcortical structures in any group at 3 or 120 DPI (data not shown). Together these data suggest that the presence of A β peptides in R1.40 mice potentially alters the macrophage response to TBI at both acute and chronic time points independent of astrogliosis.

FIG. 3. Distinct macrophage phenotype in brain injured R1.40 mice at 3 days post-injury (DPI). (A,C) Representative images of CD45 and F4/80 immunostaining in sham and mice with traumatic brain injury (TBI) in ipsilateral cortex (Ipsi-Ctx), ipsilateral thalamus (Ipsi-Thal), and contralateral CA2 (Contra-CA2) brain regions at 3 DPI. (B) Non-Tg and R1.40 TBI mice show similar levels of increased CD45 immunoreactivity compared with sham animals ($n=6$ /group) in all brain regions. R1.40 TBI mice showed a decrease in CD45 immunoreactivity compared with non-Tg TBI mice in the Ipsi-Thal. (D) F4/80 immunoreactivity was significantly elevated in non-Tg TBI mice compared with all other groups in each brain region analyzed ($n=6$ /group). (E,G) Representative images of CD68 and Trem2 immunostaining in sham and TBI mice at 3 DPI. (F) CD68 expression was significantly increased in TBI mice compared with shams with non-Tg TBI mice exhibiting the most CD68 expression ($n=3$ /group). (H) Trem2 expression was significantly elevated in non-Tg TBI mice compared with all other groups ($n=3$ /group). Error bars indicate standard error of the mean. Scale bars indicate 20 μ m. Main effect of injury: **, $p<0.01$; ***, $p<0.001$; Main effect of genotype: #, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$; interaction effect: +, $p<0.05$; +++, $p<0.001$.

FIG. 4. Proportion of microglia and monocytes are similar between non-Tg and R1.40 mice with traumatic brain injury (TBI). (A,B) Representative contour plots of microglia (CD45^{low}/F4/80 cells) and monocytes (CD45^{high}/F4/80 cells) in non-Tg and R1.40 mice with TBI at 3 days post-injury (DPI). (C) R1.40 TBI mice show a significant decrease in CD45⁺/F4/80⁺ compared with non-Tg TBI mice at 3 DPI ($n=3-7$ /group). (D,E) TBI induced a significant reduction in microglia (CD45^{low}/F4/80 cells) at 3 DPI; however, no significant differences in monocytes were identified at 3 DPI (CD45^{high}/F4/80 cells). Error bars indicate standard error of the mean; main effect of injury: *, $p<0.05$; interaction effect: +, $p<0.05$.

FIG. 5. Macrophage activation persists in subcortical structures at 120 days post-injury (DPI). (A,C) Representative images of CD45 and F4/80 immunostaining of both non-Tg and R1.40 sham and mice with traumatic brain injury (TBI) at 120 DPI. (B) CD45 immunoreactivity remained elevated in the ipsilateral thalamus (Ipsi-Thal) in both non-Tg and R1.40 TBI mice ($n=6/\text{group}$). (D) A main effect of injury was apparent in F4/80 immunoreactivity in the Ipsi-Thal and contralateral CA2 (Contra-CA2) region. Error bars indicate standard error of the mean. Scale bars indicate 20 μm . Main effect of injury: *, $p < 0.05$; **, $p < 0.01$. Ipsilateral cortex, Ipsi-Ctx.

Acute increase in post-injury APP expression declines at chronic time point

Abnormal accumulation of many AD-related proteins has been detected after human TBI, including APP and A β , which reflect axonal transport failure.^{9,34–37} To quantitate changes in post-injury APP levels, ipsilateral cortical extracts from separate groups of TBI and sham injured non-Tg and R1.40 mice underwent Western blot analysis using antibodies CT15 (for mouse and human APP) and 6E10 (for human APP only) at 3 DPI and 120 DPI (Fig. 6A,D), and normalized to GAPDH. At 3 DPI, a main effect of injury was identified in CT15 expression, $F(1, 18)=7.81$, $p < 0.05$, which was likely driven by enhanced CT15 expression in non-Tg TBI mice ($M=0.48$, $\text{SEM}=0.05$) compared with non-Tg sham mice ($M=0.23$, $\text{SEM}=0.07$, Fig. 6B). A main effect of genotype was also identified in CT15 expression at 3 DPI, $F(1, 18)=28.36$, $p < 0.001$; CT15 expression was enhanced in R1.40 mice compared with non-Tg mice regardless of injury group (Fig. 6B). As expected, 6E10 expression was only apparent in R1.40 mice resulting in a significant effect of genotype, $F(1, 19)=55.81$, $p < 0.001$, Fig. 6C.

By 120 DPI, APP expression was significantly reduced in non-Tg TBI mice ($M=0.21$, $\text{SEM}=0.03$) compared with non-Tg sham mice ($M=0.37$, $\text{SEM}=0.03$; main effect of injury $F(1, 20)=8.33$, $p < 0.01$, Fig. 6E), which was not observed between R1.40 TBI ($M=0.68$, $\text{SEM}=0.05$) and R1.40 sham mice ($M=0.75$, $\text{SEM}=0.05$). APP levels remained elevated in R1.40 mice compared with non-Tg mice at 120 DPI (CT15, main effect of genotype $F(1, 20)=79.83$, $p < 0.001$, Fig. 6E; 6E10, main effect of genotype $F(1, 20)=68.89$, $p < 0.001$, Fig. 6F), but a significant reduction in full-length human APP was also detected in R1.40 TBI mice compared with R1.40 sham mice (6E10, main effect of injury $F(1, 20)=5.90$, $p < 0.05$, Fig. 6F).

CT15 immunohistochemistry revealed accumulation of APP in the ipsilateral external capsule of TBI mice indicative of disrupted

axonal transport (Fig. 6I).³⁸ Somatic accumulation of APP was also apparent in the ipsilateral cortex of TBI mice as well as R1.40 sham mice at 3 DPI. By 120 DPI, somatic APP was evident throughout the ipsilateral cortex but not as prevalent in the white matter of TBI mice (Fig. 6I). Further, immunohistochemistry suggested that TBI did not advance the appearance of A β plaques because no aggregates of APP could be detected. This was confirmed with ELISA, which demonstrated no significant differences in accumulation of A β 1–40 between R1.40 TBI and sham mice at 3 DPI (Fig. 6G) or 120 DPI (Fig. 6H).

Altered macrophage response coincides with expression of inflammatory cytokines

Multiplex cytokine array was used to examine inflammatory mediators after TBI or sham injury in non-Tg and R1.40 mice at 3 and 120 DPI (Table 2). Of the 25 inflammatory mediators examined, significant differences in expression of four chemokines, six cytokines, and one growth factor were identified at 3 DPI. Overall, non-Tg TBI mice exhibited enhanced expression of chemokines, proinflammatory cytokines, and the growth factor compared with non-Tg sham mice at 3 DPI. Conversely, chemokine expression was similar between R1.40 TBI and sham mice while cytokine and the growth factor expression were reduced in R1.40 TBI mice compared to R1.40 sham mice at 3 DPI. R1.40 TBI mice consistently displayed reduced expression of inflammatory molecules compared with non-Tg mice, which was identified by multiple interaction effects between genotype and injury (Table 2).

By 120 DPI, significant differences in expression of 4 chemokines, 10 cytokines, and 2 growth factors were identified (Table 2). Although a main effect of injury was detected in expression of interferon-gamma (IFN- γ), interleukin-6 (IL-6), and fibroblast growth factor-2 (FGF-2), a dominant effect of genotype was identified in expression of all molecules. Overall, expression of inflammatory molecules was markedly higher in R1.40 mice

compared with non-Tg mice regardless of injury group (Table 2). Collectively, these results illustrate the complexity of the post-injury inflammatory response and highlight genotype specific as well as time dependent changes in cytokine expression; however, the lack of robust differences between TBI groups suggests that cytokines are not primarily responsible for the altered macrophage response in R1.40 TBI mice.

Altered macrophage response does not affect post-injury tau pathology

To quantitate changes in post-injury tau pathology, ipsilateral cortical extracts were subjected to Western blot analysis using

antibodies AT8, AT180, and Tau5 at 3 and 120 DPI. At 3 DPI, a main effect of injury was identified in AT8 expression $F(1, 20) = 8.0, p < 0.05$. On closer examination, the ratio of AT8/Tau5 expression was relatively similar between non-Tg TBI ($M = 0.02$, $SEM = 0.00$) and non-Tg sham mice ($M = 0.01$, $SEM = 0.00$); however, AT8/Tau5 expression was higher R1.40 TBI mice ($M = 0.03$, $SEM = 0.00$) compared with R1.40 sham mice ($M = 0.01$, $SEM = 0.00$). No other significant differences were identified in tau phosphorylation between groups at 3 or 120 DPI.

Immunohistochemistry was also performed using antibodies AT8 and AT180 at 3 and 120 DPI. Some region-specific differences in tau phosphorylation were detected in mice with TBI; however, variability in the extent of this phosphorylation prevented the detection of any significant differences between groups (data not shown). Together these data suggest that the altered post-injury macrophage response seen in R1.40 mice is not mediated by abnormal tau pathology.

TBI causes genotype-specific behavioral deficits

To examine the chronic effects of TBI-induced neuroinflammation in promoting AD-like behavioral phenotypes, routine motor and cognitive tests were performed as mice aged to 120 DPI. The rotarod test was used to examine motor function. All mice performed similarly during baseline training, but TBI mice fell off the rotating rod much faster than sham animals during the first week after injury. Notably, TBI mice continued to exhibit deficits in rotarod performance several months after TBI. A significant interaction effect between genotype and injury was detected, $F(1, 48) = 18.5, p < 0.001$, indicating that performance changed across time in all groups (Fig. 7A).

Spatial working memory was evaluated via an examination of spatial alternation in the Y maze. R1.40 animals exposed to TBI exhibited an age-dependent decline in working memory that

FIG. 6. Traumatic brain injury (TBI) causes time dependent changes in APP expression. (**A,D**) Western blot of cortical extracts were probed with antibodies CT15, 6E10, and GAPDH at 3 and 120 days post-injury (DPI) respectively. (**B**) There was a significant increase in APP in non-Tg TBI mice compared with non-Tg shams at 3 DPI. R1.40 TBI mice displayed higher levels of APP than non-Tg TBI mice, but no differences in APP expression were identified between R1.40 TBI and R1.40 sham mice at 3 DPI. (**C**) No difference in human APP expression was found between R1.40 TBI and R1.40 sham mice at 3 DPI. As expected, human APP was undetectable in non-Tg mice. (**E**) There was a significant reduction in APP in non-Tg TBI mice compared with non-Tg shams at 120 DPI. APP levels were reduced in non-Tg mice compared with R1.40 mice regardless of injury group. (**F**) R1.40 TBI mice displayed a significant reduction in human APP expression compared with R1.40 sham mice at 120 DPI (3 DPI, $n = 5-6$ /group; 120 DPI, $n = 6$ /group). Human APP was undetectable in non-Tg mice. (**G,H**) Levels of Aβ 1-40 in ipsilateral cortex homogenates were higher in R1.40 mice compared with non-Tg mice at 3 and 120 DPI, respectively; however these differences were not statistically significant ($n = 5-6$ /group). (**I**) Representative images of CT15 immunostaining in sham and TBI mice at 3 DPI (top two rows) and 120 DPI (bottom row). APP accumulation was apparent in the ipsilateral external capsule and cortex at 3 DPI in mice with TBI. By 120 DPI, only R1.40 mice showed enhanced APP immunostaining in the ipsi-cortex ($n = 3$ /group/time point). Error bars indicate standard error of the mean. Scale bars indicate 20 μm. Main effect of injury: *, $p < 0.05$; main effect of genotype: #, $p < 0.05$; ###, $p < 0.001$.

TABLE 2. MULTIPLEX CYTOKINE ARRAY REVEALS GENOTYPE DEPENDENT EXPRESSION PROFILE

Post-injury time point	Category	Variable	Non-Tg Sham ($\mu\text{g}/\text{mg}$)	Non-Tg TBI ($\mu\text{g}/\text{mg}$)	R1.40 TBI ($\mu\text{g}/\text{mg}$)	R1.40 Sham ($\mu\text{g}/\text{mg}$)	Main effect of injury	Main effect of genotype	Interaction effect
3 DPI	Chemokine	IP-10	340 $\pm$ 50	480 $\pm$ 50	340 $\pm$ 80	250 $\pm$ 30		*	
		MCP-1	230 $\pm$ 20	380 $\pm$ 30	208 $\pm$ 40	290 $\pm$ 30	*		*
		MIG	350 $\pm$ 40	420 $\pm$ 30	400 $\pm$ 60	570 $\pm$ 60			*
		MIP-1 α	520 $\pm$ 70	780 $\pm$ 60	600 $\pm$ 80	570 $\pm$ 60	*		
	Cytokine	IFN- γ	150 $\pm$ 10	220 $\pm$ 10	190 $\pm$ 30	280 $\pm$ 30		*	**
		IL-1 α	300 $\pm$ 40	410 $\pm$ 40	280 $\pm$ 40	350 $\pm$ 50			*
		IL-1 β	540 $\pm$ 60	540 $\pm$ 60	280 $\pm$ 40	320 $\pm$ 40		***	
		IL-5	180 $\pm$ 20	220 $\pm$ 10	180 $\pm$ 30	300 $\pm$ 30			*
		IL-10	770 $\pm$ 90	600 $\pm$ 90	460 $\pm$ 50	670 $\pm$ 80	*		
		IL-13	640 $\pm$ 80	790 $\pm$ 90	530 $\pm$ 70	780 $\pm$ 60			*
		VEGF	120 $\pm$ 10	130 $\pm$ 10	80 $\pm$ 10	100 $\pm$ 10		**	
120 DPI	Chemokine	IP-10	300 $\pm$ 10	380 $\pm$ 20	500 $\pm$ 60	460 $\pm$ 20		**	
		MCP-1	400 $\pm$ 10	460 $\pm$ 30	600 $\pm$ 80	650 $\pm$ 50		**	
		MIG	810 $\pm$ 40	990 $\pm$ 60	1270 $\pm$ 100	1190 $\pm$ 60		***	
		MIP-1 α	950 $\pm$ 50	1160 $\pm$ 70	1640 $\pm$ 120	1550 $\pm$ 160		***	
	Cytokine	GM-CSF	110 $\pm$ 10	150 $\pm$ 10	200 $\pm$ 40	200 $\pm$ 10		**	
		IFN- γ	460 $\pm$ 10	570 $\pm$ 10	630 $\pm$ 60	560 $\pm$ 40	*	*	
		IL-1 α	460 $\pm$ 20	540 $\pm$ 50	850 $\pm$ 90	770 $\pm$ 40		***	
		IL-1 β	510 $\pm$ 30	620 $\pm$ 20	680 $\pm$ 40	790 $\pm$ 110		**	
		IL-2	250 $\pm$ 0	270 $\pm$ 10	310 $\pm$ 20	290 $\pm$ 20		**	
		IL-5	460 $\pm$ 20	610 $\pm$ 30	690 $\pm$ 70	690 $\pm$ 30		*	
		IL-6	550 $\pm$ 20	720 $\pm$ 30	750 $\pm$ 40	670 $\pm$ 30	**	*	
		IL-12	70 $\pm$ 0	90 $\pm$ 10	140 $\pm$ 20	120 $\pm$ 10		***	
		IL-13	1070 $\pm$ 50	1270 $\pm$ 90	1350 $\pm$ 80	1440 $\pm$ 100		*	
		IL-17	30 $\pm$ 0	30 $\pm$ 0	50 $\pm$ 0	50 $\pm$ 0		***	
	Growth factor	FGF-2	11330 $\pm$ 370	11730 $\pm$ 500	10810 $\pm$ 310	9350 $\pm$ 310	*	**	
		VEGF	110 $\pm$ 10	100 $\pm$ 0	130 $\pm$ 10	120 $\pm$ 0		**	

Multiplex cytokine array was used to examine post-injury inflammatory mediators in ipsilateral cortical extracts. Notable inflammatory mediators that were statistically significant are listed above (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Values are presented as mean $\pm$ standard error of the mean $\mu\text{g}/\text{mg}$ of protein in a 25 μL sample of brain homogenate. TBI, traumatic brain injury; DPI, days post-injury.

FIG. 7. Traumatic brain injury (TBI) induces time dependent changes in motor and cognitive function. (A) Mean latency to fall from the rotating rod was shorter in TBI mice at all post-injury time points compared to shams regardless of genotype. No between genotype differences in rotarod performance were detected in sham and TBI mice ($n = 12-14/\text{group}$). (B) No significant differences between groups in average spontaneous alternation in the Y-maze. R1.40 TBI mice showed a significant reduction in percent spontaneous alternation between arms from 7 days post-injury (DPI) to 90 DPI. (C) Average total arm entries were similar between TBI groups; however, sham mice demonstrated a significant reduction in arm entries over time ($n = 12-14/\text{group}$). (D) A significant interaction effect between injury and genotype was detected in latency to reach the hidden platform at 120 DPI ($n = 12-14/\text{group}$). (E) Average acquisition index shows that latency to reach the platform significantly decreases within a testing day in R1.40 compared with non-Tg mice. (F) Average savings index shows that latency to reach the platform significantly increases between testing days in R1.40 compared with Non-Tg mice. Error bars indicate standard error of the mean. Main effect of injury: *, $p < 0.05$; ***, $p < 0.001$; Interaction effect: ++, $p < 0.01$; +++, $p < 0.001$.

reached statistical significance by 90 DPI ($t=2.138$, $p<0.05$), whereas B6 animals exposed to TBI did not exhibit any impairments at any age examined (Fig. 7B) or when compared with sham counterparts. Importantly, there were no significant differences in arm entries between brain-injured groups at any testing time point suggesting that motor deficits did not interfere with cognitive assessment (Fig. 7C). A significant decrease in arm entries was detected in sham animals; however, because no motor deficits were identified in rotarod testing, this decrease is attributed to habituation to the testing arena over time.

Spatial reference memory was assessed in the hidden platform version of the MWM beginning at 120 DPI. TBI resulted in longer latencies to reach the hidden goal platform in both non-Tg and R1.40 mice; however, repeated measures ANOVA revealed no main effect of injury or genotype. A significant interaction effect was detected, $F(1, 48)=9.51$, $p<0.01$, indicating that latency to reach the platform changed across time in all groups (Fig. 7D). No group differences were observed in average swim speed or latency to reach a visible platform (data not shown), excluding the possibility that the data were confounded by motor or visual deficits. Although sham mice spent more time than TBI mice in the goal quadrant during probe testing, no significant differences were detected between groups.

The water maze is an integrative task that necessitates coordinate deployment of encoding, consolidation, storage, and retrieval, all of which are distinct memory processes. To better assess these behavioral components, we used two different learning indices to quantify within- and between-day learning.²⁷ The acquisition index, which measures within-day learning, in non-Tg TBI mice was less than one suggesting that the time to reach the goal platform was similar between trial 1 and trial 4 within any given memory testing day. Surprisingly, the acquisition index in R1.40 TBI mice averaged around 13 and was significantly higher than brain-injured non-Tg mice ($t=3.373$, $p<0.01$, Fig. 7E). This finding demonstrates that R1.40 TBI mice show tremendous improvement in performance between trial 1 and trial 4 within any given memory testing day.

Next, we used a savings index, which quantifies how much learned information is transferred or “saved” across days and provides an indirect measure of memory consolidation. The savings index in non-Tg TBI mice averaged around two, indicating that there was only slight improvement from one day to the next in the latency to reach the goal platform. Interestingly, the savings index in R1.40 TBI mice averaged around negative six, suggesting that performance significantly worsened from one day to the next ($t=2.147$, $p<0.05$, Fig. 7F). Together, these data demonstrate that non-Tg and R1.40 TBI mice maintain unique profiles of within and between day learning in the water maze task even though average latency to reach the platform was similar. R1.40 mice display a deficit in transferring information from one day to the next but are able to overcome this deficit by quickly improving their performance within each day, providing further evidence of a time-dependent impairment in cognitive performance.

Discussion

Numerous studies have documented that previous incidence of TBI may lead to AD-related pathologies, neuroinflammation, neurodegeneration, and dementia.^{1,6,39} Because neuroinflammation is a rapid response to brain trauma as well as an early feature of AD, we hypothesized that any alteration in the post-injury neuroinflammatory response could have significant effects on long-

term AD-like features. In an attempt to better understand the relationship between TBI and AD, many experimental paradigms have been used; however, results from these studies remain inconclusive.

While some experimental rodent studies demonstrate a worsening of AD-like pathology, several have found no effect or even an improvement of AD-like pathology after TBI.^{40–46} Importantly, many of the experimental studies based their results on the presence or advancement of A β plaques alone with less attention given to other mechanisms that may be contributing to altered post-injury pathology. Our data lend support to recent studies demonstrating that accumulating A β significantly alters the post-injury macrophage response to a moderate TBI and correlates with chronic cavity enlargement and time-dependent behavioral impairment.⁴⁷

Brain injury disrupts many biological pathways that trigger progressive neurodegeneration, including brain atrophy, neuronal loss, and axonal degeneration that last months to years after the initial insult as demonstrated by both clinical and experimental studies.^{6–9,22,23,48–50} Mechanisms contributing to these pathological events are not well established; however, they are often associated with neuroinflammation. Post-injury neuroinflammation is cited as a double-edged sword capable of mediating short-term beneficial effects but subsequently facilitating secondary brain damage.⁵¹ Although the neuroinflammatory response to TBI encompasses many cell types, we have focused our efforts on characterizing the activated macrophage response with specific emphasis on brain resident microglia and infiltrating monocytes in non-Tg and R1.40 mice.

In our model, we found that a single moderate FPI caused obvious cortical damage at 3 DPI, which extended into the hippocampus at 120 DPI in both non-Tg and R1.40 mice. Although cavity enlargement was apparent in both genotypes, only the R1.40 mice showed a statistically significant, time-dependent increase in lesion size. Notably, FPI resulted in cortical tissue loss similar to other studies examining moderate to severe TBI^{52,53}; however, the extensive hippocampal damage in these studies could be from the location of injury.

Visible edema was present after FPI regardless of genotype, which could have resulted in dura rupture and further enhanced the post-injury brain damage. Mortality rates did not exceed 20%, and no signs of infection were present at any post-injury time point. As expected, macrophage activation was evident around the injury cavity at 3 DPI and substantially declined by 120 DPI in both injured genotypes. Persistent macrophage activation was notable in the ipsilateral thalamus of both non-Tg and R1.40 TBI mice at 120 DPI. These data support previous experimental findings from the subacute and chronic phase of TBI in which macrophage activation was also apparent in the thalamus.²²

Surprisingly, we detected a substantial reduction in the macrophage response to TBI in R1.40 mice compared with controls via immunohistochemical and flow cytometry studies at 3 DPI. The severity of TBI was similar between R1.40 and non-Tg mice, and all post-injury assessment parameters were exactly the same. Importantly, anatomical coordinates, regardless of injury size, were used for quantification of CD45, F4/80, CD68, and TREM2 immunoreactivity. Although there was a trend for brain injured as well as sham R1.40 mice to display fewer activated monocytes compared with non-Tg controls in flow cytometry studies, this difference was not statistically significant.

A related trend was observed in the multiplex data, in which various chemokines, including interferon gamma-induced protein 10 (IP-1; CXCL10); monocyte chemoattractant protein-1 (MCP-1, CCL2), monokine induced by gamma interferon (MIG, CXCL9),

and macrophage inflammatory protein-1 (MIP-1 α , CCL4) were similar between R1.40 TBI and sham mice but enhanced in non-Tg TBI mice compared with non-Tg sham mice. Notably, the multiplex data was acquired from cortical lysates, and thus macrophage specific expression of inflammatory molecules could not be identified.

This is the first set of experiments to demonstrate increased TREM2 expression after moderate TBI at 3 DPI. Apparent loss of function mutations in *TREM2*, a gene exclusively expressed by microglia and monocytes (as well as dendritic cells), was demonstrated to be genetically associated with AD in late 2012.^{11,12} In addition to AD, heterozygous mutations in *TREM2* have also been identified as a risk factor for frontotemporal dementia, Parkinson disease, and amyotrophic lateral sclerosis.^{54–57} Given the strong genetic association between *TREM2* and neurodegeneration, the central role of neuroinflammation in TBI, including activation resident microglia and infiltration of peripheral monocytes and the selective expression of TREM2 within these cells, studies further defining the role of TREM2 in TBI are urgently needed.

Recent experimental stroke studies have linked TREM2 expression to the subacute phase of recovery and detected a surprising attenuation of the inflammatory response in TREM2 deficient mice.^{58,59} Interestingly, the reduced phagocytic response was associated with worsened neurological recovery.⁵⁹ These results clearly demonstrate that TREM2 is capable of modulating the neuroinflammatory and functional response to brain injury and warrants further investigation in the context of TBI.

These results indicated that the presence of A β peptides and oligomers influence the post-injury macrophage response to TBI. Microglia interact with A β through a complex cell surface receptor system that promotes clearance and phagocytosis.⁶⁰ Recent reports indicate a time-dependent effect of A β on microglial physiology. In particular, AD model mice that overproduced A β developed striking impairment of microglial function, which could be rescued by antibody-mediated clearance of A β .⁶¹ Further, amyloidogenic peptides attenuate neuroinflammation, including amelioration of paralysis, in a mouse model of multiple sclerosis.⁶²

Recent studies have also revealed that increases in A β after spinal cord injury spare white matter from damage and promote behavioral recovery.⁶³ These observations have consequences for understanding the role of microglia and macrophages in AD: for example, extracellular oligomeric A β modulates microglial phagocytosis of fibrillar A β and disrupts clearance of A β deposits.⁶⁴ Conversely, it is also pertinent, as in the present study, to examine how the presence of A β modifies the microglial and macrophage reaction to pathologies such as TBI and thereby clarify how modulating macrophage function can affect outcomes. Our present findings support the concept that macrophage dysfunction occurs in the presence of A β in R1.40 mice and influences outcomes after a secondary neuroinflammatory challenge such as TBI.

Macrophage activation substantially declined by 120 DPI; however, notable differences in the macrophage response were still detectable between groups. R1.40 TBI mice did not display a reduced macrophage response similar to non-Tg TBI mice. Instead, the macrophage response in R1.40 TBI mice was relatively similar to that observed at 3 DPI and even increased in the ipsilateral thalamus.

Although there were some subtle differences in expression of inflammatory cytokines between non-Tg and R1.40 TBI mice, multiplex experiments highlighted a genotype specific and time-dependent trend in cytokine expression. Overall, cytokine expression was higher in R1.40 mice than non-Tg mice, and cytokine

expression increased at 120 DPI compared with 3 DPI. This finding is consistent with previous studies showing that cytokine expression is enhanced in R1.40 mice at 6 months of age.⁶⁵ TBI did not induce a unique cytokine expression profile in R1.40 mice that corresponded with the altered macrophage response. Future studies examining cell specific changes (i.e., microglia and monocytes) in post-injury cytokine expression would potentially provide a better tool for identifying mediators of the altered macrophage response.

Consistent with previous experimental studies and indicative of diffuse axonal injury, we detected an increase in full-length APP at 3 DPI in non-Tg TBI mice compared with non-Tg shams.^{66,67} A similar difference was not detected between R1.40 TBI and sham mice at 3 DPI; however, both groups had higher levels of APP compared with non-Tg mice. By 120 DPI, full-length APP levels significantly declined in non-Tg TBI mice compared with their respective shams. Although APP levels remained similar between R1.40 TBI and sham mice, a significant decrease in full-length human APP was detected after TBI at 120 DPI. The distinction in expression of human-APP could reflect genotype specific differences in post-injury axonal integrity and further suggests that the altered macrophage response in R1.40 mice influences AD-related pathological features.

The presence of mutant human APP is the driving force behind AD-like pathology in R1.40 mice,^{20,21} and a reduction in expression of human APP at 120 DPI in mice with TBI could account for the lack of A β aggregation. Accumulating evidence suggests that APP has neuroprotective qualities, and sAPP α has specifically been shown to improve outcome after TBI.⁶⁸ More recent experimental and clinical studies suggest that the dynamics of APP accumulation and A β are very complex after brain trauma, and the role of low- and high-molecular weight A β oligomers resulting from amyloidogenic processing of APP remains unclear.^{69–71} Future studies are needed to better characterize post-injury processing of APP and development of A β in non-Tg as well as R1.40 mice at chronic time points because the relationship between post-injury A β and persistent inflammation remains unclear.

Unique trends in behavioral outcome measures were observed. Mice with TBI displayed chronic deficits in balance and coordination in the rotarod task. This unexpected finding could be attributed to the moderate injury severity that resulted in substantial tissue loss in the somatosensory cortex as well as subcortical macrophage activation. Further, the slightly rostral craniectomy position may have facilitated a prominent motor deficit.⁷² TBI did not induce deficits in spatial working memory at 7 DPI, but R1.40 brain-injured mice displayed increased spontaneous alternation compared with all other groups. This trend was not statistically significant but could reflect injury-induced anxiety or hyperactivity occurring as a result of the location or severity of brain injury. TBI in R1.40 mice resulted in a time-dependent decrease in spontaneous alternation between 7 and 90 DPI, which was not observed in any other group. This finding suggests that spatial working memory declines over time in R1.40 mice after TBI; however, one cannot exclude the possibility that this statistically significant result is dependent on the increase in spontaneous alternation observed at 7 DPI. This observation highlights the need for analysis of anxiety-like behavior in future studies.

TBI induced deficits in spatial reference memory as assessed by the MWM, but no statistically significant differences in latency to reach the submerged goal platform were found between R1.40 and non-Tg TBI mice. This finding is contrary to some other studies demonstrating that TBI worsens cognitive performance in AD mice compared with controls.^{45,73–76} R1.40 and

non-Tg mice were 6 months old at 120 DPI, and this is still 6–8 months before A β plaques develop in R1.40 mice. Examining longer post-injury time points might provide a better opportunity to characterize global cognitive decline. Future studies might also benefit from including the visible platform training before memory testing.

The use of two learning indices provided valuable insight to the potential differences between TBI groups in within- and between-day performance. The results indicate that R1.40 TBI mice are deficient in transferring information from one day to the next compared with non-Tg TBI mice. On the other hand, R1.40 TBI mice are superior to non-Tg TBI mice in transferring information from one trial to the next within any one day of testing. Together with the Y-maze data, these results suggest that TBI induces a modest time-dependent cognitive impairment in R1.40 mice that is not evident in non-Tg mice.

Volumetric analysis revealed a time-dependent decrease in the ipsilateral hippocampus that was enhanced by TBI in R1.40 mice. It is possible that hippocampal tissue loss combined with persistent macrophage activation in the thalamus, which is critical in several aspects of cognition including memory, executive function, and attention,⁷⁷ contributed to unique trends in behavioral impairment.

Conclusion

This study demonstrates that TBI induces a distinct neuroinflammatory response in the genomic-based R1.40 mouse model of AD. These experiments suggest that microglia and infiltrating monocytes in the presence of accumulating A β respond differently to a secondary immune challenge such as TBI and the macrophage response is compromised in R1.40 mice. At more chronic time points after TBI, however, R1.40 mice exhibit enhanced neurodegeneration, persistent macrophage activation, and also unique impairments in components of memory consolidation and retrieval in spatial reference memory. These studies suggest that animals prone to the development of AD exhibit a unique inflammatory response to TBI that has different short- and long-term consequences with only modest worsening of cognitive function several months post-injury.

Similar findings were recently reported in a double gene-targeted knock-in mouse model of AD, but delayed post-injury neuroinflammation correlated with significant cognitive impairment.⁴⁷ The difference in functional outcome measures are likely because of several variables, including the mouse model used, the experimental injury model, as well as post-injury time points examined. Collectively these results emphasize that accumulating A β alters the immune response to brain injury at both acute and chronic time points.

A further definition of the inflammatory response, including the role of infiltrating monocytes and microglia and different states of activation of these cells, is needed to gain insight into the potential factors that drive long-term functional outcome measures after TBI. Our results compliment previous studies demonstrating that manipulating the innate immune response can significantly alter AD pathogenesis,^{78,79} and some balance is required between pro- and anti-inflammatory mediators at both acute and chronic time points. Further, the results of these studies implicate A β in the neuroinflammatory response and emphasize the importance of post-injury neuroinflammation in mediating long-term AD-like phenotypes. Acknowledging the altered macrophage response in the presence of A β will be critical in optimizing experimental models to better characterize the relationship between TBI and AD.

Acknowledgments

We thank Anne Coteleur for assistance with flow cytometry analysis, Mark Whiting for assistance with behavioral analysis, and Grahame Kidd for assistance with volumetric analysis and 3D reconstruction. We also thank the Rodent Behavioral Core and the Flow Cytometry Core for technical advice and support.

This work was supported by the Department of Defense (W81XWH-12-1-0629, EDMS 5629, Bruce Lamb) and National Eye Institute (EY022358, Samuel Crish).

Author contributions: O.K.-C. designed, performed, and analyzed all experiments and wrote the manuscript. L.R. analyzed behavioral experiments and performed and analyzed immunohistochemical and biochemical experiments; M.V. performed and analyzed immunohistochemical experiments; S.L. performed and analyzed flow cytometry experiments; M.S. performed volumetric analysis of brain tissue; M.S. and R.T. performed and analyzed immunohistochemical experiments; G.X. performed and analyzed ELISA experiments; S.B. performed and analyzed biochemical experiments; G.W. performed and analyzed multiplex assay experiments; S.C. provided expertise in analysis of multiplex data; K.B. assisted with biochemical experiments and provided expertise in analysis; Y.-S.L. assisted with brain injuries; R.M.R. provided expertise and co-edited the manuscript; B.T.L. provided funding and expertise and co-edited the manuscript.

Author Disclosure Statement

No competing financial interests exist.

References

1. Nemetz, P.N., Leibson, C., Naessens, J.M., Beard, M., Kokmen, E., Annegers, J.F., and Kurland, L.T. (1999). Traumatic brain injury and time to onset of Alzheimer's disease: a population-based study. *Am. J. Epidemiol.* 149, 32–40.
2. Schofield, P.W., Tang, M., Marder, K., Bell, K., Dooneief, G., Chun, M., Sano, M., Stern, Y., and Mayeux, R. (1997). Alzheimer's disease after remote head injury: an incidence study. *J. Neurol. Neurosurg. Psychiatry* 62, 119–124.
3. Fleminger, S., Oliver, D.L., Lovestone, S., Rabe-Hesketh, S., and Giora, A. (2003). Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. *J. Neurol. Neurosurg. Psychiatry* 74, 857–862.
4. Plassman, B.L., Havlik, R.J., Steffens, D.C., Helms, M.J., Newman, T.N., Drosdick, D., Phillips, C., Gau, B.A., Welsh-Bohmer, K.A., Burke, J.R., Guralnik, J.M., and Breitner, J.C. (2000). Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology* 55, 1158–1166.
5. Mortimer, J.A., van Duijn, C.M., Chandra, V., Fratiglioni, L., Graves, A.B., Heyman, A., Jorm, A.F., Kokmen, E., Kondo, K., Rocca, W.A., Shalat, S.L., Soininen, H., and Hofman, A. (1991). Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *Int. J. Epidemiol.* 20, Suppl 2, S28–S35.
6. Johnson, V.E., Stewart, J.E., Begbie, F.D., Trojanowski, J.Q., Smith, D.H., and Stewart, W. (2013). Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain* 136, 28–42.
7. Johnson, V.E., Stewart, W., and Smith, D.H. (2012). Widespread tau and amyloid-Beta pathology many years after a single traumatic brain injury in humans. *Brain Pathol.* 22, 142–149.
8. Maxwell, W.L., MacKinnon, M.A., Stewart, J.E., and Graham, D.I. (2010). Stereology of cerebral cortex after traumatic brain injury matched to the Glasgow outcome score. *Brain* 133, 139–160.
9. Gentleman, S.M., Nash, M.J., Sweeting, C.J., Graham, D.I., and Roberts, G.W. (1993). Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. *Neurosci. Lett.* 160, 139–144.
10. Johnson, V.E., Stewart, W., and Smith, D.H. (2010). Traumatic brain injury and amyloid-beta pathology: a link to Alzheimer's disease? *Nat. Rev. Neurosci.* 11, 361–370.

11. Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., Cruchaga, C., Sassi, C., Kauwe, J.S., Younkin, S., Hazrati, L., Collinge, J., Pocock, J., Lashley, T., Williams, J., Lambert, J.C., Amouyel, P., Goate, A., Rademakers, R., Morgan, K., Powell, J., St George-Hyslop, P., Singleton, A., Hardy, J., and Alzheimer Genetic Analysis, G. (2013). TREM2 variants in Alzheimer's disease. *N. Engl. J. Med.* 368, 117–127.
12. Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P.V., Snaedal, J., Bjornsson, S., Huttenlocher, J., Levey, A.I., Lah, J.J., Rujescu, D., Hampel, H., Giegling, I., Andreassen, O.A., Engedal, K., Ulstein, I., Djurovic, S., Ibrahim-Verbaas, C., Hofman, A., Ikram, M.A., van Duijn, C.M., Thorsteinsdottir, U., Kong, A., and Stefansson, K. (2013). Variant of TREM2 associated with the risk of Alzheimer's disease. *N. Engl. J. Med.* 368, 107–116.
13. Griciuc, A., Serrano-Pozo, A., Parrado, A.R., Lesinski, A.N., Asselin, C.N., Mullin, K., Hooli, B., Choi, S.H., Hyman, B.T., and Tanzi, R.E. (2013). Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron* 78, 631–643.
14. Luber-Narod, J., and Rogers, J. (1988). Immune system associated antigens expressed by cells of the human central nervous system. *Neurosci. Lett.* 94, 17–22.
15. Rogers, J., Luber-Narod, J., Styren, S.D., and Civin, W.H. (1988). Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. *Neurobiol. Aging* 9, 339–349.
16. McGeer, P.L., Itagaki, S., and McGeer, E.G. (1988). Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol.* 76, 550–557.
17. Thal, D.R., Schultz, C., Dehghani, F., Yamaguchi, H., Braak, H., and Braak, E. (2000). Amyloid beta-protein (A β)-containing astrocytes are located preferentially near N-terminal-truncated A β deposits in the human entorhinal cortex. *Acta Neuropathol.* 100, 608–617.
18. Vlad, S.C., Miller, D.R., Kowall, N.W., and Felson, D.T. (2008). Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology* 70, 1672–1677.
19. McGeer, P.L., Schulzer, M., and McGeer, E.G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 47, 425–432.
20. Lamb, B.T., Sisodia, S.S., Lawler, A.M., Slunt, H.H., Kitt, C.A., Kearns, W.G., Pearson, P.L., Price, D.L., and Gearhart, J.D. (1993). Introduction and expression of the 400 kilobase amyloid precursor protein gene in transgenic mice [corrected]. *Nat. Genet.* 5, 22–30.
21. Lamb, B.T., Call, L.M., Slunt, H.H., Bardel, K.A., Lawler, A.M., Eckman, C.B., Younkin, S.G., Holtz, G., Wagner, S.L., Price, D.L., Sisodia, S.S., and Gearhart, J.D. (1997). Altered metabolism of familial Alzheimer's disease-linked amyloid precursor protein variants in yeast artificial chromosome transgenic mice. *Hum. Mol. Genet.* 6, 1535–1541.
22. Carbonell, W.S., and Grady, M.S. (1999). Regional and temporal characterization of neuronal, glial, and axonal response after traumatic brain injury in the mouse. *Acta Neuropathol.* 98, 396–406.
23. Carbonell, W.S., Maris, D.O., McCall, T., and Grady, M.S. (1998). Adaptation of the fluid percussion injury model to the mouse. *J. Neurotrauma* 15, 217–229.
24. Cardona, A.E., Huang, D., Sasse, M.E., and Ransohoff, R.M. (2006). Isolation of murine microglial cells for RNA analysis or flow cytometry. *Nat. Protoc.* 1, 1947–1951.
25. Fiala, J.C. (2005). Reconstruct: a free editor for serial section microscopy. *J. Microsc.* 218, 52–61.
26. Bhaskar, K., Konerth, M., Kokiko-Cochran, O.N., Cardona, A., Ransohoff, R.M., and Lamb, B.T. (2010). Regulation of tau pathology by the microglial fractalkine receptor. *Neuron* 68, 19–31.
27. Whiting, M.D., and Kokiko-Cochran, O.N. (in press). Assessment of cognitive function in the water maze task: maximizing data collection and analysis in animal models of brain injury, in: *Injury Models of the Central Nervous System: Traumatic Brain Injury*. C.E. Dixon and R. Hayes (eds). Springer: New York, N.Y.
28. Holmin, S., Soderlund, J., Biberfeld, P., and Mathiesen, T. (1998). Intracerebral inflammation after human brain contusion. *Neurosurgery* 42, 291–299.
29. Engel, S., Schluesener, H., Mittelbronn, M., Seid, K., Adjodah, D., Wehner, H.D., and Meyermann, R. (2000). Dynamics of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage-related proteins MRP8 and MRP14. *Acta Neuropathol.* 100, 313–322.
30. Sedgwick, J.D., Schwender, S., Imrich, H., Dorries, R., Butcher, G.W., and ter Meulen, V. (1991). Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. *Proc. Natl. Acad. Sci. U. S. A.* 88, 7438–7442.
31. Carson, M.J., Reilly, C.R., Sutcliffe, J.G., and Lo, D. (1998). Mature microglia resemble immature antigen-presenting cells. *Glia* 22, 72–85.
32. Campanella, M., Sciorati, C., Tarozzo, G., and Beltramo, M. (2002). Flow cytometric analysis of inflammatory cells in ischemic rat brain. *Stroke* 33, 586–592.
33. Takahashi, K., Rochford, C.D., and Neumann, H. (2005). Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J. Exp. Med.* 201, 647–657.
34. Smith, D.H., Uryu, K., Saatman, K.E., Trojanowski, J.Q., and McIntosh, T.K. (2003). Protein accumulation in traumatic brain injury. *Neuromolecular Med.* 4, 59–72.
35. Roberts, G.W., Gentleman, S.M., Lynch, A., Murray, L., Landon, M., and Graham, D.I. (1994). Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 57, 419–425.
36. Roberts, G.W., Gentleman, S.M., Lynch, A., and Graham, D.I. (1991). beta A4 amyloid protein deposition in brain after head trauma. *Lancet* 338, 1422–1423.
37. Uryu, K., Chen, X.H., Martinez, D., Browne, K.D., Johnson, V.E., Graham, D.I., Lee, V.M., Trojanowski, J.Q., and Smith, D.H. (2007). Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. *Exp. Neurol.* 208, 185–192.
38. Jiang, Y., and Brody, D.L. (2012). Administration of COG1410 reduces axonal amyloid precursor protein immunoreactivity and microglial activation after controlled cortical impact in mice. *J. Neurotrauma* 29, 2332–2341.
39. Ikonomic, M.D., Uryu, K., Abrahamson, E.E., Ciallella, J.R., Trojanowski, J.Q., Lee, V.M., Clark, R.S., Marion, D.W., Wisniewski, S.R., and DeKosky, S.T. (2004). Alzheimer's pathology in human temporal cortex surgically excised after severe brain injury. *Exp. Neurol.* 190, 192–203.
40. Murai, H., Pierce, J.E., Raghupathi, R., Smith, D.H., Saatman, K.E., Trojanowski, J.Q., Lee, V.M., Loring, J.F., Eckman, C., Younkin, S., and McIntosh, T.K. (1998). Twofold overexpression of human beta-amyloid precursor proteins in transgenic mice does not affect the neuromotor, cognitive, or neurodegenerative sequelae following experimental brain injury. *J. Comp. Neurol.* 392, 428–438.
41. Nakagawa, Y., Nakamura, M., McIntosh, T.K., Rodriguez, A., Berlin, J.A., Smith, D.H., Saatman, K.E., Raghupathi, R., Clemens, J., Saido, T.C., Schmidt, M.L., Lee, V.M., and Trojanowski, J.Q. (1999). Traumatic brain injury in young, amyloid-beta peptide overexpressing transgenic mice induces marked ipsilateral hippocampal atrophy and diminished A β deposition during aging. *J. Comp. Neurol.* 411, 390–398.
42. Nakagawa, Y., Reed, L., Nakamura, M., McIntosh, T.K., Smith, D.H., Saatman, K.E., Raghupathi, R., Clemens, J., Saido, T.C., Lee, V.M., and Trojanowski, J.Q. (2000). Brain trauma in aged transgenic mice induces regression of established A β deposits. *Exp. Neurol.* 163, 244–252.
43. Pierce, J.E., Trojanowski, J.Q., Graham, D.I., Smith, D.H., and McIntosh, T.K. (1996). Immunohistochemical characterization of alterations in the distribution of amyloid precursor proteins and beta-amyloid peptide after experimental brain injury in the rat. *J. Neurosci.* 16, 1083–1090.
44. Abrahamson, E.E., Ikonomic, M.D., Ciallella, J.R., Hope, C.E., Paljug, W.R., Isanski, B.A., Flood, D.G., Clark, R.S., and DeKosky, S.T. (2006). Caspase inhibition therapy abolishes brain trauma-induced increases in A β peptide: implications for clinical outcome. *Exp. Neurol.* 197, 437–450.
45. Abrahamson, E.E., Ikonomic, M.D., Dixon, C.E., and DeKosky, S.T. (2009). Simvastatin therapy prevents brain trauma-induced increases in beta-amyloid peptide levels. *Ann. Neurol.* 66, 407–414.
46. Loane, D.J., Pocivavsek, A., Moussa, C.E., Thompson, R., Matsuoka, Y., Faden, A.I., Rebeck, G.W., and Burns, M.P. (2009). Amyloid

- precursor protein secretases as therapeutic targets for traumatic brain injury. *Nat. Med.* 15, 377–379.
47. Webster, S.J., Van Eldik, L.J., Watterson, D.M., and Bachstetter, A.D. (2015). Closed head injury in an age-related Alzheimer mouse model leads to an altered neuroinflammatory response and persistent cognitive impairment. *J. Neurosci.* 35, 6554–6569.
 48. Smith, D.H., Chen, X.H., Pierce, J.E., Wolf, J.A., Trojanowski, J.Q., Graham, D.I., and McIntosh, T.K. (1997). Progressive atrophy and neuron death for one year following brain trauma in the rat. *J. Neurotrauma* 14, 715–727.
 49. Pierce, J.E., Smith, D.H., Trojanowski, J.Q., and McIntosh, T.K. (1998). Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience* 87, 359–369.
 50. Bramlett, H.M., Dietrich, W.D., Green, E.J., and Busto, R. (1997). Chronic histopathological consequences of fluid-percussion brain injury in rats: effects of post-traumatic hypothermia. *Acta Neuropathol.* 93, 190–199.
 51. Morganti-Kossmann, M.C., Rancan, M., Stahel, P.F., and Kossmann, T. (2002). Inflammatory response in acute traumatic brain injury: a double-edged sword. *Curr. Opin. Crit. Care* 8, 101–105.
 52. Ferreira, A.P., Rodrigues, F.S., Della-Pace, I.D., Mota, B.C., Oliveira, S.M., de Campos Velho Gewehr, C., Bobinski, F., de Oliveira, C.V., Brum, J.S., Oliveira, M.S., Furian, A.F., de Barros, C.S., dos Santos, A.R., Ferreira, J., Figuera, M.R., and Royes, L.F. (2014). HOE-140, an antagonist of B2 receptor, protects against memory deficits and brain damage induced by moderate lateral fluid percussion injury in mice. *Psychopharmacology* 231, 1935–1948.
 53. Bolkvadze, T., and Pitkanen, A. (2012). Development of post-traumatic epilepsy after controlled cortical impact and lateral fluid-percussion-induced brain injury in the mouse. *J. Neurotrauma* 29, 789–812.
 54. Guerreiro, R.J., Lohmann, E., Bras, J.M., Gibbs, J.R., Rohrer, J.D., Gurunlian, N., Dursun, B., Bilgic, B., Hanagasi, H., Gurvit, H., Emre, M., Singleton, A., and Hardy, J. (2013). Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. *JAMA Neurol.* 70, 78–84.
 55. Rayaprolu, S., Mullen, B., Baker, M., Lynch, T., Finger, E., Seeley, W.W., Hatanpaa, K.J., Lomen-Hoerth, C., Kertesz, A., Bigio, E.H., Lippa, C., Josephs, K.A., Knopman, D.S., White, C.L., 3rd, Caselli, R., Mackenzie, I.R., Miller, B.L., Boczarska-Jedynak, M., Opala, G., Krygowska-Wajs, A., Barcikowska, M., Younkin, S.G., Petersen, R.C., Ertekin-Taner, N., Uitti, R.J., Meschia, J.F., Boylan, K.B., Boeve, B.F., Graff-Radford, N.R., Wszolek, Z.K., Dickson, D.W., Rademakers, R., and Ross, O.A. (2013). TREM2 in neurodegeneration: evidence for association of the p.R47H variant with frontotemporal dementia and Parkinson's disease. *Mol. Neurodegener.* 8, 19.
 56. Lill, C.M., Rengmark, A., Pihlstrom, L., Fogh, I., Shatunov, A., Sleiman, P.M., Wang, L.S., Liu, T., Lassen, C.F., Meissner, E., Alexopoulos, P., Calvo, A., Chio, A., Dizdar, N., Faltraco, F., Forsgren, L., Kirchheiner, J., Kurz, A., Larsen, J.P., Liebsch, M., Linder, J., Morrison, K.E., Nissbrandt, H., Otto, M., Pahnke, J., Partch, A., Restagno, G., Rujescu, D., Schnack, C., Shaw, C.E., Shaw, P.J., Tuman, H., Tynes, O.B., Valladares, O., Silani, V., van den Berg, L.H., van Rheenen, W., Veldink, J.H., Lindenberger, U., Steinhagen-Thiessen, E., Slagen Consortium, Teipel, S., Perneckzy, R., Hakonarson, H., Hampel, H., von Arnim, C.A., Olsen, J.H., Van Deerlin, V.M., Al-Chalabi, A., Toft, M., Ritz, B., and Bertram, L. (2015). The role of TREM2 R47H as a risk factor for Alzheimer's disease, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Parkinson's disease. *Alzheimers Dement.* Epub ahead of print.
 57. Cady, J., Koval, E.D., Benitez, B.A., Zaidman, C., Jockel-Balsarotti, J., Allred, P., Baloh, R.H., Ravits, J., Simpson, E., Appel, S.H., Pestronk, A., Goate, A.M., Miller, T.M., Cruchaga, C., and Harms, M.B. (2014). TREM2 variant p.R47H as a risk factor for sporadic amyotrophic lateral sclerosis. *JAMA Neurol.* 71, 449–453.
 58. Sieber, M.W., Jaenisch, N., Brehm, M., Guenther, M., Linnartz-Gerlach, B., Neumann, H., Witte, O.W., and Frahm, C. (2013). Attenuated inflammatory response in triggering receptor expressed on myeloid cells 2 (TREM2) knock-out mice following stroke. *PLoS One* 8, e52982.
 59. Kawabori, M., Kacimi, R., Kauppinen, T., Calosing, C., Kim, J.Y., Hsieh, C.L., Nakamura, M.C., and Yenari, M.A. (2015). Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. *J. Neurosci.* 35, 3384–3396.
 60. Lee, C.Y., and Landreth, G.E. (2010). The role of microglia in amyloid clearance from the AD brain. *J. Neural Transm.* 117, 949–960.
 61. Krabbe, G., Halle, A., Matyash, V., Rinnenthal, J.L., Eom, G.D., Bernhardt, U., Miller, K.R., Prokop, S., Kettenmann, H., and Heppner, F.L. (2013). Functional impairment of microglia coincides with Beta-amyloid deposition in mice with Alzheimer-like pathology. *PLoS One* 8, e60921.
 62. Grant, J.L., Ghosn, E.E., Axtell, R.C., Herges, K., Kuipers, H.F., Woodling, N.S., Andreasson, K., Herzberg, L.A., Herzberg, L.A., and Steinman, L. (2012). Reversal of paralysis and reduced inflammation from peripheral administration of beta-amyloid in TH1 and TH17 versions of experimental autoimmune encephalomyelitis. *Sci. Transl. Med.* 4, 145ra105.
 63. Pajoohesh-Ganji, A., Burns, M.P., Pal-Ghosh, S., Tadvalkar, G., Hokenbury, N.G., Stepp, M.A., and Faden, A.I. (2014). Inhibition of amyloid precursor protein secretases reduces recovery after spinal cord injury. *Brain Res.* 1560, 73–82.
 64. Pan, X.D., Zhu, Y.G., Lin, N., Zhang, J., Ye, Q.Y., Huang, H.P., and Chen, X.C. (2011). Microglial phagocytosis induced by fibrillar beta-amyloid is attenuated by oligomeric beta-amyloid: implications for Alzheimer's disease. *Mol. Neurodegener.* 6, 45.
 65. Bhaskar, K., Maphis, N., Xu, G., Varvel, N.H., Kokiko-Cochran, O.N., Weick, J.P., Staugaitis, S.M., Cardona, A., Ransohoff, R.M., Herrup, K., and Lamb, B.T. (2014). Microglial derived tumor necrosis factor- α drives Alzheimer's disease-related neuronal cell cycle events. *Neurobiol. Dis.* 62, 273–285.
 66. Itoh, T., Satou, T., Nishida, S., Tsubaki, M., Hashimoto, S., and Ito, H. (2009). Expression of amyloid precursor protein after rat traumatic brain injury. *Neurol. Res.* 31, 103–109.
 67. Ciallella, J.R., Ikonovic, M.D., Paljug, W.R., Wilbur, Y.I., Dixon, C.E., Kochanek, P.M., Marion, D.W., and DeKosky, S.T. (2002). Changes in expression of amyloid precursor protein and interleukin-1 β after experimental traumatic brain injury in rats. *J. Neurotrauma* 19, 1555–1567.
 68. Corrigan, F., Thornton, E., Roisman, L.C., Leonard, A.V., Vink, R., Blumbergs, P.C., van den Heuvel, C., and Cappai, R. (2014). The neuroprotective activity of the amyloid precursor protein against traumatic brain injury is mediated via the heparin binding site in residues 96–110. *J. Neurochem.* 128, 196–204.
 69. Washington, P.M., Morffy, N., Parsadanian, M., Zapple, D.N., and Burns, M.P. (2014). Experimental traumatic brain injury induces rapid aggregation and oligomerization of amyloid- β in an Alzheimer's disease mouse model. *J. Neurotrauma* 31, 125–134.
 70. Brody, D.L., Magnoni, S., Schwetye, K.E., Spinner, M.L., Esparza, T.J., Stocchetti, N., Zipfel, G.J., and Holtzman, D.M. (2008). Amyloid- β dynamics correlate with neurological status in the injured human brain. *Science* 321, 1221–1224.
 71. Schwetye, K.E., Cirrito, J.R., Esparza, T.J., Mac Donald, C.L., Holtzman, D.M., and Brody, D.L. (2010). Traumatic brain injury reduces soluble extracellular amyloid- β in mice: a methodologically novel combined microdialysis-controlled cortical impact study. *Neurobiol. Dis.* 40, 555–564.
 72. Floyd, C.L., Golden, K.M., Black, R.T., Hamm, R.J., and Lyeth, B.G. (2002). Craniectomy position affects morris water maze performance and hippocampal cell loss after parasagittal fluid percussion. *J. Neurotrauma* 19, 303–316.
 73. Brody, D.L., and Holtzman, D.M. (2006). Morris water maze search strategy analysis in PDAPP mice before and after experimental traumatic brain injury. *Exp. Neurol.* 197, 330–340.
 74. Uryu, K., Laurer, H., McIntosh, T., Pratico, D., Martinez, D., Leight, S., Lee, V.M., and Trojanowski, J.Q. (2002). Repetitive mild brain trauma accelerates Abeta deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis. *J. Neurosci.* 22, 446–454.
 75. Tajiri, N., Kellogg, S.L., Shimizu, T., Arendash, G.W., and Borlongan, C.V. (2013). Traumatic brain injury precipitates cognitive impairment and extracellular Abeta aggregation in Alzheimer's disease transgenic mice. *PLoS One* 8, e78851.
 76. Smith, D.H., Nakamura, M., McIntosh, T.K., Wang, J., Rodriguez, A., Chen, X.H., Raghupathi, R., Saatman, K.E., Clemens, J., Schmidt, M.L., Lee, V.M., and Trojanowski, J.Q. (1998). Brain trauma induces massive hippocampal neuron death linked to a surge in beta-amyloid levels in mice overexpressing mutant amyloid precursor protein. *Am. J. Pathol.* 153, 1005–1010.

77. Aggleton, J.P., and Brown, M.W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *The Behavioral and brain sciences* 22, 425-444; discussion 444-489.
78. El Khoury, J., Toft, M., Hickman, S.E., Means, T.K., Terada, K., Geula, C., and Luster, A.D. (2007). Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. *Nat Med* 13, 432-438.
79. Town, T., Laouar, Y., Pittenger, C., Mori, T., Szekely, C.A., Tan, J., Duman, R.S., and Flavell, R.A. (2008). Blocking TGF-beta-Smad2/3 innate immune signaling mitigates Alzheimer-like pathology. *Nat Med* 14, 681-687.

Address correspondence to:
Bruce T. Lamb, PhD
Department of Neurosciences
Cleveland Clinic
NC30, 9500 Euclid Avenue
Cleveland, OH 44195
E-mail: lambb@ccf.org