Triggering receptor expressed on myeloid cells 2 deficiency exacerbates injury-induced inflammation in a mouse model of tauopathy

Atsuko Katsumoto1,2, Olga N. Kokiko-Cochran1,3, Shane M. Bemiller1,2, Guixiang Xu1,2, Richard M. Ransohoff1,4 and Bruce T. Lamb1,2*

1Department of Neurosciences, The Cleveland Clinic Lerner Research Institute, Cleveland, OH, United States, 2Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN, United States, 3Department of Neurosciences, College of Medicine, The Ohio State University, Columbus, OH, United States, 4Neuroinflammation Research Center, The Cleveland Clinic Lerner Research Institute, Cleveland, OH, United States

Traumatic brain injury (TBI) promotes several Alzheimer’s disease-like pathological features, including microtubule-associated protein tau (MAPT) accumulation within neurons. Macrophage activation in the injured hTau mouse model of tauopathy raises the question whether there is a relationship between MAPT pathology and alterations in macrophage phenotype following TBI. Triggering receptor expressed on myeloid cells 2 (TREM2) is a critical regulator of microglia and macrophage phenotype, but its mechanisms on TBI remain unclear. To address the association with TREM2 in TBI and MAPT pathology, we studied TREM2 deficiency in hTau injured mice (hTau; Trem2−/−) 3 (acute phase) and 120 (chronic phase) days after experimental TBI. At three days following injury, hTau;Trem2−/− mice exhibited reduced macrophage accumulation both in the cortex and hippocampus. However, to our surprise, hTau;Trem2−/− mice exposed to TBI augments macrophage accumulation in the corpus callosum and white matter near the site of tissue damage in a chronic phase, which results in exacerbated axonal injury, tau aggregation, and impaired neurogenesis. We further demonstrate that TREM2 deficiency in hTau injured mice promotes neuronal dystrophy in the white matter due to impaired phagocytosis of apoptotic cells. Remarkably, hTau;Trem2−/− exposed to TBI failed to restore blood-brain barrier integrity. These findings imply that TREM2 deficiency accelerates inflammation and neurodegeneration, accompanied by attenuated microglial phagocytosis and continuous blood-brain barrier (BBB) leakage, thus exacerbating tauopathy in hTau TBI mice.

KEYWORDS
TREM2, microglia, traumatic brain injury, tauopathy, neurodegeneration, blood-brain barrier, blood-brain barrier
Introduction

Traumatic brain injury (TBI) triggers neuroinflammation involving brain resident microglia and peripherally derived macrophages. Cytokines, chemokines, and molecular mediators are released within hours of a TBI (1, 2), which leads to microglia and astrocytes’ activation and the recruitment of peripheral immune cells. These responses primarily promote the clearance of tissue debris and the resolution of the inflammatory response. However, it can cause secondary injury; a prolonged dysregulated microglial/macrophage activity results in neuronal death and chronic neurodegeneration, including oligomeric and hyperphosphorylated tau proteins (3–7) and amyloid beta oligomers (8, 9). Therefore, it is crucial to elucidate the mechanisms underlying detrimental myeloid cell inflammation and regulate these responses to prevent progressive neuronal damage.

Triggering receptor expressed on myeloid cells 2 (TREM2) is a type I transmembrane receptor found in dendritic cells, osteoclasts, microglia, monocytes, and tissue macrophages (10, 11). Homozygous mutations in either TREM2 or its adapter protein DNAX-activating protein of 12 kDa (DAP12) result in Nasu-Hakola disease (NHD), a progressive neurodegenerative disorder with massive gliosis and demyelination in the subcortical white matter (12, 13). Heterozygous rare variants in TREM2 have been reported to increase the risk of Alzheimer’s disease (AD) (14, 15) and identified in other neurodegenerative disorders such as frontotemporal dementia and Parkinson’s disease as well (16, 17). Although exact mechanisms underlying NHD are unknown, it has been proposed that loss of TREM2 or DAP12 causes abnormalities in microglial survival, inflammatory response, and ability to clear neuronal debris (18, 19). TREM2 is also associated with hyperphosphorylated tau (20), another pathological hallmark for neurodegenerative diseases (21, 22). Silencing of brain TREM2 in the tau transgenic mice using a lentiviral-mediated strategy exacerbated tau pathology, possibly through neuroinflammation-induced hyperactivation of tau kinases (23). Using the same strategy, selective TREM2 overexpression on microglia was shown to ameliorate neuropathologies and spatial cognitive impairments in the tau transgenic mice, which suggests the protective role of TREM2 in tau pathology (24). However, the role of TREM2 in TBI is poorly understood. We recently showed that TBI increased TREM2 expression in C57BL/6 mice. After TBI, TREM2 deficient mice demonstrated reduced inflammatory cytokine production during the acute phase and reduced hippocampal atrophy during the chronic phase of recovery (25). TREM2 itself elicits tau phosphorylation (3, 5, 7). In addition, in a mouse model of tauopathy, the mice with TBI showed a persistent macrophage response and enhanced tau phosphorylation compared with controls (26, 27).

We focused on a dual outcome of TREM2 deficiency in the TBI and tau mouse model in the current study. We sought to determine the relative contribution of TREM2 in TBI-induced inflammation followed by tau accumulation. First, we evaluated the inflammatory response to TBI in hTau;Trem2+/− mice. Next, we assessed the progression of tau pathology and neurodegeneration in regions of the inflamed white matter in hTau;Trem2−/− TBI mice. Finally, we examined the blood-brain barrier integrity in hTau;Trem2−/− TBI mice to determine if the exacerbated tau pathology in a specific area is related to BBB impairment.

Materials and methods

Mouse

To study the effects of TBI-induced inflammation in promoting AD-like phenotypes, the human MAPT transgenic strain was mated to a separate Mapt−/− knockout mouse (Jackson Laboratory #007251) that was predicted to be a functional null allele (hTau mice). This mouse was maintained on the C57BL/6 background. We also used a Trem2−/− mouse model (Trem2tm1(KOMP)Vlcg) as previously described (28). These mice were maintained on a C57BL/6 background. These mice were crossed with hTau mice to yield hTau;Trem2−/− mice. To study the effects of TBI in AD-like phenotypes, two-month-old hTau mice and hTau; Trem2−/− mice (mixed sex; n = 6 mice per group for immunohistochemistry, n = 10–12 mice per group for behavior tests) were used 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

We performed lateral fluid percussion injury to induce TBI as described before (25, 26, 29). Briefly, at two 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 μL) was administered subcutaneously before the 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. Twenty-four hours after surgical

---

Abbreviations: AD, Alzheimer’s disease; FJC, fluoroJade C; MAPT, microtubule-associated protein tau; TBI, traumatic brain injury; TREM2, triggering receptor expressed on myeloid cells 2.
peroxidases were quenched by incubating sections in 1% H2O2 in PBS at 4°C. Matched with Alexa Fluor 488 (1:500, Abcam), Caspase-3 (1:500, Cell signaling), GFAP (1:500, Bio-Rad), F4/80 (1:500; AbD Serotec), doublecortin antibodies were added overnight at 4°C: Iba1 (1:1,000; Wako Pure Chemical Industries), CD45 (1:500; AbD Serotec), CD68 (1:500; Bio-Rad), F4/80 (1:500; AbD Serotec), doublecortin (1:250; Cell Signaling), APP (1:200; DSHB), AT8 (1:500; Thermo Fisher Scientific), Ki-67 (1:200, DSHB), AT8 (1:500; Thermo Fisher Scientific), TUNEL (1:100; Roche), and AT180 (1:250; Cell Signaling). Mouse on Mouse Blocking and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAN... staining was performed as described (30). The slides were dehydrated again in graded ethanol for 2 min each. The slices were placed in xylene for another 5 min and coverslipped.

**Immunohistochemistry**

Mice were deeply anesthetized with a combination of ketamine and xylazine and perfused with PBS. Brains were fixed overnight in 4% PFA in PBS, cryoprotected in 30% sucrose, and frozen in OCT. 30 μm coronal sections were prepared on a cryostat and stored in PBS at 4°C. Medial and lateral sections were used for each animal. For 3,3'-diaminobenzidine (DAB) staining, endogenous peroxidases were quenched by incubating sections in 1% H2O2 in PBS for 30 min. Sections were blocked in 5% NGS/0.3% Triton X-100 in 1× PBS for 1 h. The following primary antibodies were added overnight at 4°C: Iba1 (1:1,000; Wako Pure Chemical Industries), CD45 (1:500; AbD Serotec), CD68 (1:500; Bio-Rad), F4/80 (1:500; AbD Serotec), doublecortin (1:250; Cell Signaling), APP (1:1000; Invitrogen), and AT180 (1:500; Thermo Fisher Scientific). Mouse on Mouse Blocking and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAIN Elite ABC kit (Vector Laboratories) and VECTASTAN... immunoreactivity above the threshold was automatically calculated, and the same threshold cut-point was applied to images across the group. Because the differences between groups across the signal spectrum are not constant, information on the pixel intensity histograms for all experiments is shown in Supplementary Figure 1. Microglia phagocytosis was analyzed by assessing the co-localization of Iba-1 and Lamp-1 puncta. Using the ImageJ software (19), Pearson’s correlation coefficient was calculated. For microglial morphology analysis, a total of 15–20 microglia were selected randomly from the white matter tract near the lesion from each group. Individual cells were thresholded, skeletonized, and analyzed for ramifications (process length/cell and endpoints/cell) using the ImageJ AnalyzeSkeleton plugin, which outputs a total number of branches along with branch junctions. Fractal analysis (FracLac for ImageJ) was used to quantify cell complexity (fractal dimension), cell shape (span ratio), and cell size (density). All data acquisition and analysis were made in a blinded manner.

**Statistical analysis**

All data are presented as mean ± standard error of the mean (SEM). All statistical analysis was completed with GraphPad Prism 5 software (San Diego, CA). A mixed model factorial analysis of variance (ANOVA) followed by the Bonferroni post hoc test was used to evaluate group differences. Comparisons between two groups were analyzed using Student’s t-test (two-tailed; unpaired) at a 95% confidence interval. Statistical significance was determined at 

\( p < 0.05 \), \( p < 0.01 \) **, \( p < 0.005 \) ***, and \( p < 0.001 \) ****.
Results

TREM2 deficiency in hTau TBI mice attenuates macrophage activation in the acute phase (3 days post-injury)

To address the effect of TREM2 deficiency on TBI-induced neuroinflammation in the mouse model of tauopathy, hTau and hTau;Trem2−/− mice were subjected to moderate lateral FPI, and the inflammatory reaction was analyzed three days post-injury (dpi) by immunohistochemistry (Figure 1). In hTau mice, macrophage and microglial marker F4/80 expression was massive in the injured site (Figures 1B, D). In addition, magnifying observation revealed the increased F4/80 expression in the cortex, the corpus callosum, and the hippocampus compared to sham-operated mice (Figure 1C). Similarly, hTau;Trem2−/− mice showed increased F4/80 expression in the injured brain. However, this myeloid cell activation was significantly reduced in the cortex and hippocampus of hTau; Trem2−/− TBI mice compared to hTau-TBI mice (Figures 1B, D).

TREM2 loss of function in hTau TBI mice leads to sustained inflammation in the chronic phase (120 days post-injury)

The previous report showed that TREM2 has different roles in AD-related myeloid cell functions early and late in disease progression (31). Therefore, we next assessed whether TREM2 deficiency might differentially affect neuroinflammation in hTau mice at a later time point. At 120 dpi, which represents a chronic time point that is also consistent with our previous work (7, 25, 26, 32, 33), accumulation of cells positive for macrophage markers, F4/80, CD45, Iba-1, and CD68, were robust in the corpus callosum and white matter near the site of tissue damage in hTau;Trem2−/−-injured mice (Figure 2A; Supplementary Figure 2A). These immunoreactive areas were significantly increased in TREM2 deficient TBI mice (Figure 2B). This was accompanied by astrocytosis shown by GFAP immunostaining (Supplementary Figure 2B). Morphological analysis revealed that Iba-1+ cell ramification was decreased in hTau; Trem2−/− TBI mice compared to hTau;Trem2−/− sham mice, and there was a significant increase in the density both in hTau TBI mice and hTau;Trem2−/− TBI mice. (Supplementary Figure 3). Overall, we observed a consistent immunoreactive pattern for microglia/macrophages in hTau;Trem2−/− TBI mice.

TREM2 deficiency in hTau mice promotes TBI-induced axonal damage and impairs neurogenesis in the chronic phase

Axonal injury is an important pathological feature of TBI, and beta-amyloid precursor protein (APP) immunohistochemistry was performed to assess impaired axonal transport. Consistent with
previous studies (7, 34), APP immunoreactivity was enhanced in the body of the corpus callosum and the external capsule in injured hTau mice (Figure 3A). Notably, hTau;Trem2−/− mice showed prominent APP staining in the corpus callosum and the external capsule linked with it compared to the injured hTau mice (Figure 3B). Higher magnification showed APP accumulation in axonal bulbs or swellings in this area. Quantifying axonal pathology as the ratio of APP immunoreactivity in areas including corpus callosum documented that axonal pathology was significantly worsened in hTau;Trem2−/− mice compared to hTau mice (Figure 3C). These observations indicate that TREM2 deficiency increases axonal injury, as monitored with APP immunoreactivity, after TBI in hTau mice.

Neurogenesis is also affected by TREM2 deficiency in hTau TBI mice. Immunohistochemistry and quantitative morphometry for neuroblast marker the neuronal migration protein, doublecortin (DCX), in hTau and hTau;Trem2−/− groups of mice showed a significant decrease in DCX in injured hTau; Trem2−/− mice, suggesting impaired neurogenesis (Figures 3D, E and H). Furthermore, the hTau;Trem2−/− mouse showed immature cells with short neurites, while the hTau mouse demonstrated more developed cells with branches (Figures 3F, G).

Both injury and TREM2 genotype affect aggregated Tau levels in the chronic phase

To determine whether hTau;Trem2−/− TBI mice display accumulation of aggregated tau, Gallyas silver staining was performed (Figure 4). In response to TBI, increased staining at the injury site in the white matter of both hTau and hTau; Trem2−/− mice (Figure 4A) were observed. Increased tau aggregation was also detected in hTau;Trem2−/− mice with sham. Strikingly, more prominent Gallyas positive neurons in white matter and dystrophic neurites were identified in hTau; Trem2−/− TBI mice (Figures 4A, B). Compared to small, granular staining in hTau TBI and hTau;Trem2−/− sham mice, hTau; Trem2−/− TBI mice showed larger and speckled staining. Because tau aggregates in the hTau mice appear after nine months of age (35), our data demonstrate that pathologic tau...
aggregation is affected in the hTau;Trem2−/− mice, and the injury further accelerates it.

**Injury accelerates neurodegeneration in hTau;Trem2−/− mice in the chronic phase**

Next, we assessed whether the white matter’s inflammation and tau aggregation led to neurodegeneration by immunohistochemistry for the degenerating neuron-specific antigen fluororoJade-C (FJC) in hTau and hTau;Trem2−/− groups of mice (Figure 5A). Surprisingly, there were significant levels of degenerating neurons within the white matter near the site of tissue damage in the hTau;Trem2−/− TBI group compared to the hTau TBI group (Figure 5A). No FJC-positive cells were observed in sham groups. Quantifying the FJC+ area demonstrated a significant increase in the hTau;Trem2−/− mice compared to hTau TBI mice (Figure 5B).
In addition, immunolabelling of dystrophic neurites using anti-Lysosome-associated membrane glycoprotein 1 (Lamp-1) or anti-Ubiquitin antibodies showed robust numbers of the swollen dystrophic neurites in the white matter near the site of tissue damage (Figures 5C, D and quantified in 5E, F).

Macrophage phagocytic function is impaired in hTau;Trem2−/− injured mice in the chronic phase

TREM2-positive myeloid cells are involved in tissue debris clearance (36–38) and amyloid clearance (19, 39). Therefore, to examine whether TREM2 deficiency could lead to compromised phagocytic capacity, Iba-1 positive cells co-localization with Lamp-1-positive dystrophic neurites in the white matter tract near lesion were analyzed (Figure 6A). Although Iba-1 positive cells were prominent (Figure 6B), Lamp1-positive phagocytic macrophages were significantly less in hTau;Trem2−/− injured mice (Figure 6C).

Abnormal blood-brain barrier leakage in hTau;Trem2−/− mice in chronic phase

Given our finding that TREM2 deficiency leads to chronic inflammation and neurodegeneration in a specific area, we hypothesized that this area would be sites of inflammatory cells and molecules entry. Apart from the normal staining in periventricular structures, IgG staining demonstrated a clear and significant increase of BBB leakage in the white matter near the site of tissue damage in hTau;Trem2−/− mice, but not in other groups (Figure 7).
Discussion

In this study, we investigated hTau mice with a loss-of-function mutation in TREM2 both in the acute and chronic phases after TBI. TREM2 deficiency in hTau mice reduced cortical and hippocampal myeloid cell immunoreactivity near the injury site during the acute phase of TBI. During the chronic phase of recovery, reactive myeloid cells accumulate in the white matter tract of the corpus callosum, specifically deep in the injured region. This area in hTau;Trem2-/- TBI mice had severe neuronal damage and tau aggregation, accompanied by degenerating neurons and dystrophic neurites. Furthermore, microglia/macrophage in hTau;Trem2-/- mice failed to phagocytose degrading neurons in the white matter (Table 1).

Our findings demonstrate that TREM2 deficiency accelerated neurodegeneration caused by TBI in hTau mice. Silencing of TREM2 in wild-type mice facilitates the hyperphosphorylation of endogenous tau, kinase activation, and neuroinflammation (23), although no neurodegenerative changes nor spatial learning deficits were observed. Similarly, TREM2 deficiency promotes tau pathology and aggregation via enhanced kinase activation in the mouse model of tauopathy (23, 40, 41). It has also been demonstrated that elevated levels of microglial-derived IL-1β induce tau pathology and neurodegeneration (42, 43). Furthermore, the chronic phase of hTau TBI mice showed persistent macrophage response that results in compromised spatial recognition (26). Thus, TBI-induced inflammation may be sufficient to promote tau pathology in hTau;Trem2-/- mice.

In contrast, opposing roles of TREM2 on tauopathy models have been reported. TREM2 deficiency or R47H variant of TREM2 in PS19 mice mitigates inflammatory gene expression and protects against brain atrophy at nine months (44, 45). This microglial opposing response might have resulted from the different genotypes or the stage of the disease examined. PS19 mice, which express a human tau transgene containing a P301S mutation, induce severe tangle deposition, gliosis, and neurodegeneration by nine months. Whereas hTau mice we used in our study, which express only human tau isoforms, develop mild age-associated tau pathology as indicated by accumulation of hyperphosphorylated tau begins from six months, and aggregated tau and paired helical filaments detectable at nine months. In 9 months PS19 model, microglia react strongly, and TREM2 may work detrimental. On the other hand, TREM2 may be beneficial in six months hTau model. Considering TREM2 also has different roles in AD-related myeloid cell functions early and late in disease progression (31, 46), TREM2 is generally protective for microglial activation and tau pathology in the early stage, then changes their phenotype to accelerate neurodegeneration and inflammation in a later stage.

Regional variations in TREM2 expression may contribute to the varying microglial function in the white matter. TREM2 deficiency hindered the accumulation of microglia with abnormal morphology with age, especially in the corpus callosum, which is rich in myelin that may trigger solid TREM2 signaling (18). In the cuprizone model of demyelination, TREM2 deficiency hampered the microglial response for myelin debris clearance, which led to axonal dystrophy and persistent demyelination. Other groups also showed
that inhibiting microglial TREM2 expression impaired phagocytosis of apoptotic neurons (33) or the internalization of amyloid (31). In our hTau TBI model of neuroinflammation, mice were at six months of age in the chronic phase, where the microglial number does not differ between genotypes. Our data demonstrated that reactive microglia/macrophages were localized in the white matter where tau aggregation was enhanced in hTau;Trem2-/- TBI mice. Although co-labeling of phosphorylated tau with microglia/macrophage-specific Iba-1 was not performed, impaired phagocytosis of dystrophic neurons suggests a deficiency in clearance or phagocytosis of abnormal tau in this region (Figure 6). Tau pathology was much milder in hTau;Trem2-/- sham mice, which indicates TBI could further affect tau clearance. A previous study showed that TREM2 deficiency in hTau mice increased activation of the stress signaling JNK, GSKβ, ERK, and that downstream tau hyperphosphorylation and aggregation (40), supporting the idea that TREM2 deficiency competes with efficient tau clearance in the white matter.

Recently microglial transcriptomic states, termed disease-associated microglia (DAM) (47) and white matter-associated microglia (WAM) (48), have been identified. DAM is proposed to detect damage in the CNS partly through the TREM2 signaling pathway, and acquiring the DAM transcriptome requires a sequential two-stage process (47, 49). Because the transition from stage 1 (intermediate state) to stage 2 DAM (a full DAM signature) requires a TREM2 signal, microglia from TREM2 deficient mice that we used in this study might remain in an intermediate state. TBI appears to induce DAM2 signatures, which may serve neuroprotective functions and prevent tau pathology (50), whereas a loss of TREM2 function prevents microglia from switching to a full DAM2 state (47). These impaired TREM2 functions possibly prevent microglia from boosting phagocytosis, chemotaxis, and lipid metabolism and drive tau accumulation and neurodegeneration in hTau;Trem2-/- TBI mice. WAM, which shares parts of the DAM gene signature and depends on TREM2 signaling, might also be incompletely developed in TREM2-deficient mice.

BBB disruption has often been observed in patients with TBI (51). Microglia migrate to vessels in a CCR5-dependent manner to maintain BBB integrity after systemic inflammation (52). However, when inflammation persists, activated microglia impede the maintenance of tight junctions by releasing inflammatory modulators and phagocytosing astrocytic end-feet and endothelial cells, leading to impairment of BBB integrity and function (52–55). We observed abnormal BBB leakage in the white matter deep to the injured cortex of hTau;Trem2-/- mice, while little or no leakage was present in hTau TBI mice. It is plausible that TREM2-positive cells contribute to BBB restoration through unknown mechanisms. Since low TREM2-expressing microglia results in incomplete BBB (56), there is a possibility that hTau;Trem2-/- mice could have BBB vulnerability congenitally. This possibility has also been supported by other work showing impaired angiogenesis in TREM2-deficient mice in the ischemic stroke model (57). In addition, inflammatory

| TABLE 1 | TREM2 functions in different genetic backgrounds. |
|---------|-----------------------------------------------|
| B6 TBI | Trem2 +/- TBI (25) | hTau TBI (24) | hTau;Trem2 +/- TBI [our study] |
| Acute phase | Microglial/macrophage activation | + | Less | More | Less | Robust in white matter |
| Chronic phase | Phagocytic function | N/A | N/A | Preserved | Impaired |
| | BBB restoration | N/A | N/A | Proper | Impaired |
| | Brain atrophy | + | Less | Not significant | Accelerated |
| | Tauopathy | N/A | N/A | + | Not significant |
| | Cognition | Impaired | Rescued | Compromised use of spatial search strategies | Not significant |
mediators such as IL-6 caused by TBI may prevent BBB restoration in hTau;Trem2−/− TBI mice.

These results suggest that TREM2 deficiency amplifies chronic inflammation and facilitates neurodegeneration in the injured brain in hTau mice. Specifically, abnormal BBB leakage in the white matter highlights the crucial role of TREM2-positive cells in preventing the infiltration of peripheral macrophages. Further elucidation of TREM2 signaling is needed to clarify mechanisms underlying our observations and may provide deeper insights into the role of TREM2 in regulating inflammation following TBI. Future studies aim to seek the mechanisms between MAPT pathologies and TREM2 deficiency.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic.

Author contributions

AK performed immunohistochemistry and wrote the manuscript. O.K-C. designed, performed surgeries and TBIs, and co-edited the manuscript. SB performed silver staining. GX helped with mouse strain maintenance. RR provided funding and expertise in data analysis and interpretation and co-edited the manuscript, and BL provided funding and expertise in data analysis and co-edited the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Department of Defense grant (W81XWH-14-0265).

Acknowledgments

We thank the Rodent Behavioral Core at the Lerner Research Institute within the Cleveland Clinic for support. We thank Dr. Imad Najm for the use of the fluid percussion device. We have permission to cite the conference paper of the World Congress of Neurology 2017 from Elsevier and Copyright Clearance Center (License number 5343890984223).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.978423/full#supplementary-material

References

1. Lloyd E, Somera-Molina K, Van Eldik LJ, Watterson DM, Wainwright MS, et al. Suppression of acute proinflammatory cytokine and chemokine upregulation by post-injury administration of a novel small molecule improves long-term neurologic outcome in a mouse model of traumatic brain injury. J Neuroinflamm (2008) 5:28. doi: 10.1186/1742-2094-5-28

2. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. Nat Rev Immunol (2014) 14(7):463–77. doi: 10.1038/nri3705

3. Goldstein LE, Fisher AM, Tagge CA, Zhang XL, Vellisek I, Sullivan JA, et al. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. Sci Transl Med (2012) 4(134):134ra60. doi: 10.1126/scitranslmed.3003716

4. Hawkins BE, Krishnamurthy S, Castillo-Carranza DL, Sengupta U, Prough DS, Jackson GR, et al. Rapid accumulation of endogenous tau oligomers in a rat model of traumatic brain injury: possible link between traumatic brain injury and sporadic tauopathies. J Biol Chem (2013) 288(23):17042–50. doi: 10.1074/jbc.M113.472746

5. Gerson J, Castillo-Carranza DL, Sengupta U, Bodani R, Prough DS, DeWitt DS, et al. Tau oligomers derived from traumatic brain injury cause cognitive impairment and accelerate onset of pathology in htau mice. J Neurotrauma (2016) 33(22):2034–43. doi: 10.1089/neu.2015.4262

6. Kondo A, Shahpasand K, Mannix R, Qiu J, Moncaster J, Chen CH, et al. Antibody against early driver of neurodegeneration cis p-tau blocks brain injury and tauopathy. Nature (2015) 523(7561):431–6. doi: 10.1038/nature14658
21. Lill CM, Rengmark A, Pihlstrom L, Fogh I, Shatunov A, Sleiman PM, et al. TREM2 disruption in a mouse model of Alzheimer’s disease. J Neurotrauma (2019) 138(4):613. doi: 10.1089/neuro.2018.1075-y

22. Jay TR, Miller CM, Cheng PJ, Graham LC, Bel谋求r, S. Broihier ML, et al. Fluid percussion injury and experimental brain injury in the rat. J Neurotrauma (2015) 12(1):288. doi:10.1089/neuro.2014.24322

23. Nishiyama J, Lyeth BG, Portshlock IT, Findling RL, Harm RJ, Marronaro A, et al. Fluid percussion injury and experimental brain injury in the rat. J Neurotrauma (1987) 4(7):111–19. doi:10.1089/neuro.1987.67.1.1101

24. Bhakar K, Konerth M, Kokiko-Cochran ON, Cardire A, Ransohoff RM, Lamb BT. Regulation of tau pathology by the microglial fractalkine receptor. Neuron (2010) 68(1):19–31. doi:10.1016/j.neuron.2010.08.023

25. Jay TR, Hirsch AM, Broihier ML, Miller CM, Nelson LE, Ransohoff RM, et al. Disease progression evidence-dependent effects of TREM2 deficiency in a mouse model of Alzheimer’s disease. J Neurosci. (2016). doi:10.1523/JNEUROSCI.2110-16.2016

26. Kokiko-Cochran O, Ransohoff L, Veenstra M, Lee S, Saber M, Sikora M, et al. Altered neuroinflammation and behavior after traumatic brain injury in a mouse model of Alzheimer’s disease. J Neurotrauma (2016) 33(7):625–40. doi:10.1089/neu.2015.3970

27. Katsumoto A, Miranda AS, Butovsky O, Teixeira AL, Ransohoff RM, Lamb BT, et al. Lpiquinomod attenuates inflammation by modulating macrophage functions in traumatic brain injury mouse model. J Neuroinflamm (2018) 15 (1):26. doi:10.1186/s12974-018-1075-y

28. Ojo IO, Mouan B, Almagal M, Leary P, Lynch C, Abdullah L, et al. Chronic repetitive mild traumatic brain injury results in reduced cerebral blood flow, axonal injury, gliosis, and increased T-tau and tau oligomers. J Neuropathol Exp Neurol (2016) 75(7):696–711. doi:10.1097/nen.000000000000001

29. Hsieh CL, Koike M, Spusta SC, Niemi EC, Yenari M, Nakamura MC, et al. A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. J Neurochem (2009) 109(4):144-156. doi:10.1111/j.1471-4149.2009.06042.x

30. Xiang X, Werner G, Bohrmann B, Liese A, Mazaheri F, Capell A, et al. TREM2 deficiency reduces the efficacy of immunotherapeutic amyloid clearance. EMBO Mol Med (2016) 8(9):992–1004. doi:10.15252/emmm.201606370

31. Cowper S, McCray TJ, Allan K, Formica SV, Xu G, Wilson G, et al. TREM2 deficiency exacerbates tau pathology through dysregulated kinase signaling in a mouse model of tauopathy. Mol Neurodegener (2017) 12:174. doi:10.1186/s12974-017-0216-6

32. Lee SH, Meiland WT, Xie L, Gandham VD, Ngo H, Barck KH, et al. TREM2 restrains the enhancement of tau accumulation and neurodegeneration by betaamyloid pathology. Neuron (2021) 109(3):1283–1301.e6. doi:10.1016/j.neuron.2021.02.010

33. Maphis N, Xu G, Kokiko-Cochran ON, Jiang S, Cardore A, Ransohoff RM, et al. Reactive microglia drive tau pathology and contribute to the spreading of pathological tau in the brain. Brain (2019) 142(1):178–755. doi:10.1093/brain/avz508

34. Maphis N, Xu G, Kokiko-Cochran ON, Cardore A, Ransohoff RM, Lamb B, et al. Loss of tau rescues inflammation-mediated neurodegeneration. Front Neurosci (2019) 13:916. doi:10.3389/fnins.2019.00916

35. Leys C, Ulrich J, Dink M, Bostock F, Radoslav J, Remolina Serrano I, et al. TREM2 deficiency attenuates neuroinflammation and protects against neurodegeneration in a mouse model of tauopathy. Proc Natl Acad Sci USA (2017) 114(43):11524–9. doi:10.1073/pnas.1710311114

36. Gratzer M, Leyns C, E, Sauerbeck A-D, St-Pierre M-K, Xiang M, Kim N, et al. Impact of TREM2 R47H variant on tau pathology-induced gliosis and neurodegeneration. J Clin Invest (2020) 130(9):4954–68. doi:10.1172/JCI138179

37. Mathys H, Adakian C, Gao F, Young J. Z, Manet E, Hemberg M, et al. Temporal tracking of microglia activation in neurodegeneration at single-cell resolution. Cell Rep (2017) 21(2):266–80. doi:10.1016/j.celrep.2017.09.039

38. Fujii M, Nishiyama J, Lyeth BG, Portshlock IT, Findling RL, Harm RJ, Marronaro A, et al. Fluid percussion injury and experimental brain injury in the rat. J Neurotrauma (2015) 12(1):288. doi:10.1089/neuro.2014.24322
50. Todd BP, Chimenti MS, Luo Z, Ferguson PJ, Bassuk AG, Newell EA, et al. Traumatic brain injury results in unique microglial and astrocyte transcriptomes enriched for type I interferon response. *J Neuroinflamm* (2021) 18(1):151. doi: 10.1186/s12974-021-02197-w

51. Shlosberg D, Benfli M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat Rev Neurol* (2010) 6(7):393–403. doi: 10.1038/nrneurol.2010.74

52. Haruwaka K, Ikegami A, Tachibana Y, Ohno N, Konishi H, Hashimoto A, et al. Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. *Nat Commun* (2019) 10(1):5816. doi: 10.1038/s41467-019-13812-z

53. da Fonseca AC, Matias D, Garcia C, Amaral R, Geraldo LH, Freitas C, et al. The impact of microglial activation on blood brain barrier permeability in brain diseases. *Front Cell Neurosci* (2014) 8:362. doi: 10.3389/fncel.2014.00362

54. Sumi N, Nishio-Kawata T, Takata F, Matsumoto J, Watanabe T, Shuto H, et al. Lipopolysaccharide-activated microglia induce dysfunction of the blood-brain barrier in rat microvascular endothelial cells co-cultured with microglia. *Cell Mol Neurobiol* (2010) 30(2):247–53. doi: 10.1007/s10571-009-9446-7

55. Jolivel V, Bicker F, Bename F, Ploen R, Keller S, Gollan R, et al. Perivascular microglia promote blood vessel disintegration in the ischemic penumbra. *Acta Neuropathol* (2015) 129(2):279–95. doi: 10.1007/s00401-014-1372-1

56. Schmid CD, Sautkulis LN, Danielson PE, Cooper J, Hasel KW, Hilbush BS, et al. Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult murine microglia. *J Neurochem* (2002) 83(6):1309–20. doi: 10.1046/j.1471-4159.2002.01243.x

57. Kawabori M, Kacimi R, Kauppinen T, Calosing C, Kim JY, Hsieh CL, et al. Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. *J Neurosci* (2015) 35(8):3384–96. doi: 10.1523/JNEUROSCI.2620-14.2015