TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer’s Disease Model

Highlights

- TREM2 deficiency augments Aβ accumulation and neuronal loss in a mouse model of AD
- TREM2 sustains the microglial response to Aβ plaques by promoting microglial survival
- TREM2 senses anionic lipids that have been found to interact with fibrillar Aβ
- TREM2 R47H mutation linked to Alzheimer’s disease impairs lipid recognition

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In Brief

TREM2 acts in microglia as a sensor for a wide array of lipids that are associated with β-amyloid accumulation and neuronal loss. The TREM2 mutation that has recently been identified as a risk factor for Alzheimer’s disease attenuates microglial detection of damage-associated lipids, providing a mechanistic basis for the genetic association.

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TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer’s Disease Model

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SUMMARY

Triggering receptor expressed on myeloid cells 2 (TREM2) is a microglial surface receptor that triggers intracellular protein tyrosine phosphorylation. Recent genome-wide association studies have shown that a rare R47H mutation of TREM2 correlates with a substantial increase in the risk of developing Alzheimer’s disease (AD). To address the basis for this genetic association, we studied TREM2 deficiency in the 5XFAD mouse model of AD. We found that TREM2 deficiency and haplosufficiency augment β-amyloid (Aβ) accumulation due to a dysfunctional response of microglia, which fail to cluster around Aβ plaques and become apoptotic. We further demonstrate that TREM2 senses a broad array of anionic and zwitterionic lipids known to associate with fibrillar Aβ in lipid membranes and to be exposed on the surface of damaged neurons. Remarkably, the R47H mutation impairs TREM2 detection of lipid ligands. Thus, TREM2 detects damage-associated lipid patterns associated with neurodegeneration, sustaining the microglial response to Aβ accumulation.

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder with histopathological hallmarks of β-amyloid (Aβ) plaques and neurofibrillary tangles in the brain (Huang and Mucke, 2012; Tanzi, 2013). Although disease etiology is incompletely understood, families with inherited early-onset AD have mutations in three proteins directly involved in the Aβ processing pathway, suggesting a key role for Aβ in disease pathogenesis. Early studies have shown that brain microglia accumulate around Aβ plaques and occasionally contain Aβ in both AD patients (D’Andrea et al., 2004; McGeer et al., 1987; Perlmuter et al., 1990) and transgenic mouse models of AD (Dickson, 1999; Frautschy et al., 1998; Stalder et al., 1999). Microglia contribute to Aβ clearance, at least in the early phases of neurodegeneration (El Khoury et al., 2007); however, the ability of microglia to clear Aβ may wane with age (Streit et al., 2004; Streit and Xue, 2009). At late stages of AD, microglia may paradoxically contribute to the disease by releasing pro-inflammatory cytokines in response to Aβ deposition (El Khoury et al., 2007; Hickman et al., 2008).

Recent genome-wide association studies (GWASs) have shown that a rare Arginine-47-Histidine (R47H) mutation of the triggering receptor expressed on myeloid cells 2 (TREM2) is associated with a substantial increase in the risk of developing AD (Guerreiro et al., 2013b; Jonsson et al., 2013). TREM2 is a cell-surface receptor of the Ig-superfamily that is expressed by microglia and osteoclasts in vivo (Kilaiainen et al., 2005; Paloneva et al., 2002; Schmid et al., 2002; Thrash et al., 2009) as well as monocyte-derived DCs, bone marrow-derived macrophages, and macrophage cell lines in vitro (Bouchon et al., 2001; Daws et al., 2001). Although TREM2 was detected in other cells of the CNS (Guerreiro et al., 2013b; Sessa et al., 2004), these observations have not been confirmed (Jiang et al., 2014). TREM2 binds anionic carbohydrates, anionic bacterial products, and various phospholipids (Cannon et al., 2012; Daws et al., 2003). It transmits intracellular signals through the associated transmembrane adaptor DAP12, which recruits the protein tyrosine kinase Syk, leading to phosphorylation of many downstream mediators, such as PLC-γ, PI-3K, and Vav2/3 (Ford and McVicar, 2009; Peng et al., 2010). Individuals homozygous for rare mutations that impair expression of either TREM2 or DAP12 develop lethal forms of progressive dementias such as Nasu-Hakola disease (NHD) and frontotemporal dementia (FTD) (Guerreiro et al., 2013a, 2013c; Kleinberger et al., 2014; Paloneva et al., 2002).

The association between the R47H mutation of TREM2 and the increased risk for late-onset AD suggests that microglia may require TREM2 to respond to Aβ deposition and to limit neuronal degeneration. Consistent with this hypothesis, we recently showed that APPPS1-21 transgenic mice, an AD model with rapid deposition of Aβ, have a marked decrease in the number and size of Aβ-associated microglia when they lack one copy of the Trem2 gene, although this defect did not increase Aβ accumulation (Ulrich et al., 2014). The mechanisms underlying this altered microglial response and its impact on Aβ deposition have not been delineated. To address these questions, we studied TREM2 deficiency in the 5XFAD mouse model of AD, in which
Aβ deposition develops less rapidly than in APPPS1-21 mice (Oakley et al., 2006). We find that both TREM2 deficiency and haploinsufficiency augment Aβ accumulation due to a dysfunctional response of microglia, which become apoptotic rather than undergoing activation and proliferation. We further show that TREM2 sustains microglial survival by synergizing with colony stimulating factor-1 receptor (CSF-1R) signaling. Finally, we demonstrate that TREM2 binds to a broad array of anionic lipids, which were found in association with fibrillar Aβ and are also exposed during neuronal and glial cell death. Remarkably, the R47H mutation impairs TREM2 binding to anionic lipids. We conclude that TREM2 is a receptor that detects damage-associated lipids, thereby enabling microglia to sense Aβ accumulation and cell damage, as well as supporting microglial survival and Aβ reactive microgliosis.

RESULTS

TREM2 Modulates Aβ Accumulation
We examined the deposition of Aβ aggregates in Trem2−/− mice bred to 5XFAD transgenic mice (APPSwF1Lon, PSEN1*M146L*L286V), an accelerated mouse model of AD (Oakley et al., 2006). Staining of matched coronal brain sections from Trem2−/−5XFAD mice and control 5XFAD mice at 8.5 months of age with a monoclonal antibody (mAb) against Aβ revealed significantly increased Aβ accumulation in the hippocampal but not cortical regions of Trem2−/−5XFAD mice (Figures 1A, 1B, and S1A). Trem2+/−5XFAD mice had an intermediate phenotype, although it was not statistically significant (p = 0.104). We also determined levels of Aβ40 and Aβ42 in the hippocampus and cortex of these mice by ELISA. While levels of soluble Aβ40 and Aβ42 were similar (Figures 1C and S1B), we detected a significant increase in insoluble, guanidine-extracted Aβ40 and Aβ42 in the hippocampal regions of Trem2−/−5XFAD mice compared to 5XFAD mice (Figures 1D and 1E). Moreover, there was a significant effect of TREM2 gene copy number on insoluble Aβ protein levels in the hippocampus, whereas levels of insoluble Aβ40 and Aβ42 in the cortex were equivalent across all three genotypes (Figures S1C and S1D). We also found that the loss of layer V neurons, a feature of 5XFAD mice (Eimer and Vassar, 2013; Oakley et al., 2006), was more prominent in Trem2−/− 5XFAD mice (Figures 1F and 1G). Trem2+/−5XFAD mice presented an intermediate phenotype. Collectively, these data suggest that TREM2 modulates Aβ accumulation, limiting neuronal...
loss. The lack of a significant difference in Aβ accumulation in the cortices of Trem2–/–5XFAD mice and 5XFAD mice may be the result of the fast kinetics of Aβ deposition in 5XFAD mice, such that the potential cortical differences are no longer detectable at 8.5 month of age.

**TREM2 Is Required for Reactive Microgliosis**

How does lack of TREM2 impact Aβ accumulation? Although TREM2 expression has been reported in CNS cells other than microglia (Guerrero et al., 2013b; Sessa et al., 2004), this finding is controversial (Jiang et al., 2014). Indeed, a recently published RNA sequencing (RNA-seq) data set demonstrated that Trem2 is specifically expressed in microglia, but not other cells in the CNS under steady-state conditions (Butovsky et al., 2014). We also found that Trem2 expression is further upregulated in microglia isolated from 5XFAD mice during Aβ deposition (Figures S2A and S2B). Thus, we focused our studies on microglia. One of the many effects of Aβ deposition is the induction of reactive microgliosis, which involves the expansion of microglia and conversion to an activated state (Ransohoff and Cardona, 2010). Microgliosis predominantly involves the proliferation of brain-resident microglia, with some contribution from blood-borne monocytes and microglia migrating from adjacent non-damaged brain areas (El Khoury et al., 2007; Grathwohl et al., 2009; Malm et al., 2005; Mildner et al., 2011; Simard et al., 2006; Staldner et al., 2005). To evaluate the impact of TREM2 deficiency on Aβ-induced microglial responses in 5XFAD mice, we examined transcriptional profiles of microglia purified from 5XFAD and Trem2–/–5XFAD mice as well as transgene negative wild-type (WT) and Trem2–/– littermates (Figure S2A). To evaluate changes in global transcription profiles, we first performed principle component analysis (PCA) of the top 15% most variable transcripts. We noticed that WT and Trem2–/– replicates clustered closely, suggesting a limited impact of Trem2 deficiency in the steady state, which was confirmed by a volcano plot comparing the two groups (Figures 2A and 2B). In contrast, 5XFAD microglial replicates were dramatically different from WT replicates (Figure 2A), and a volcano plot revealed that 5XFAD microglia expressed many more transcripts including those associated with microglial activation (MHC-II, CD11c), production of inflammatory cytokines (interleukin-1β [IL-1β], tumor necrosis factor-α [TNF-α], IL-12, and SPP1), and neurotrophic factors (insulin growth factor 1 [IGF-1] and VEGFA) (Figure 2C). Trem2–/–5XFAD microglia had an intermediate behavior in the principle component analysis compared to 5XFAD and WT microglia. To further interrogate how Trem2 deficiency affected the microglial response to Aβ deposition, we selected the transcripts upregulated 2-fold between 5XFAD and WT microglia (Figure 2C) and compared the expression of these transcripts among the entire data set. We found that Trem2–/–5XFAD microglia failed to upregulate these transcripts and behaved more similarly to WT microglia, as shown by hierarchical clustering and expression-by-expression plots (Figures 2D and 2E). Flow cytometric analysis of isolated microglia confirmed phenotypic changes in 5XFAD microglia consistent with increased activation, including a marked increase in cell size and strong upregulation of MHC-II, CD11c, and CD11b (Figures S2C–S2G). We also confirmed increased expression of inflammatory cytokine transcripts by qPCR in whole-brain lysates of 5XFAD mice (Figures S2I–S2L). However, in Trem2–/–5XFAD mice, these changes were markedly attenuated (Figures 2D, 2E, and S2C–S2L). In fact, Trem2–/–5XFAD microglia were phenotypically more similar to WT microglia in steady state than 5XFAD microglia. Overall, these results implied that TREM2 is required for reactive microgliosis.

**Microglia Fail to Colocalize with Aβ Plaques in Trem2–/– Mice**

Initial staining of microglia in coronal brain sections with Iba-1 revealed very similar distribution of microglia in Trem2–/–, Trem2+/–, and WT adult mice (Figures S3A–S3C). However, co-staining of coronal brain sections from Trem2–/–5XFAD and 5XFAD mice with Iba-1 and X-34, to visualize microglia and Aβ plaques, respectively, showed remarkable differences. We found that Trem2–/–5XFAD mice had reduced Iba-1 reactivity both in the hippocampi and cortices compared to 5XFAD mice (Figures 3A–3D). This was particularly evident in the areas surrounding Aβ plaques (Figures 3E and 3F; Movies S1, S2, and S3), suggesting a preferential reduction of microgliosis near amyloid depositions. Trem2–/–5XFAD mice also had a partial reduction of amyloid-associated Iba-1 reactivity.

Examination of a second model of AD, APPPS1-21 mice that have been bred to Cx3cr1GFP/+ mice in order to visualize endogenous microglia, confirmed that complete TREM2 deficiency results in a marked reduction of GFP+ microglial clusters around Aβ plaques (Figures S3D–S3F). This corroborates our previous observation that TREM2 haploinsufficiency correlates with fewer amyloid-associated microglia in APPPS1-21xCx3cr1GFP/+ mice (Ulrich et al., 2014). Moreover, since CX3CR1 marks brain-resident microglia (Ransohoff and Cardona, 2010), these results also suggest that TREM2 deficiency primarily affects the response of brain-resident microglia to Aβ.

To further quantify the number of microglia around Aβ plaques, we recorded the coordinates (x, y, and z) of all visible microglial cell bodies and the location of Aβ plaques in each z stack confocal image and calculated the number of microglia within 30 μm radius of the plaques (defined as plaque-associated microglia) and non-plaque-associated microglia. While no statistically significant difference was observed among non-plaque-associated microglia (Figure S4A), we noted a high degree of microglial clustering around amyloid plaques in 5XFAD mice (average 4.28 microglia per plaque), which gradually decreased in Trem2–/–5XFAD mice (average 3.42 microglia per plaque) and Trem2−/−5XFAD mice (average 2.36 microglia per plaque) (Figures 4A and 4B). To confirm the “negligence” of microglial responses to Aβ in the absence of TREM2, we compared the actual frequency of microglia per plaque to that obtained by Monte Carlo simulations where the same numbers of microglia and plaques observed in z stack images were positioned by chance in each genotype (Figure S4B). The probability that observed microglial frequencies per plaque fell outside of simulated random frequencies was inversely proportional to Trem2 gene copy number (Figure 4C). Moreover, while 27.9% of microglial distribution in 5XFAD mice with respect to Aβ plaques was increased expression of inflammatory cytokine transcripts by qPCR in whole-brain lysates of 5XFAD mice (Figures S2I–S2L). However, in Trem2–/–5XFAD mice, these changes were markedly attenuated (Figures 2D, 2E, and S2C–S2L). In fact, Trem2–/–5XFAD microglia were phenotypically more similar to WT microglia in steady state than 5XFAD microglia. Overall, these results implied that TREM2 is required for reactive microgliosis.

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not explained statistically by chance, the frequency of non-random microglial distribution was reduced to 9.5% in Trem2<sup>−/−</sup> 5XFAD mice (Figure 4D).

Another feature of reactive microgliosis is morphological transformation. In 5XFAD mice, plaque-associated microglia showed morphological changes associated with microglial activation, including a partial retraction and a slight hypertrophy of the microglial cell processes as well as an increase in size (Figures 4E–4G). These changes in microglial morphology were significantly attenuated in Trem2<sup>−/−</sup> 5XFAD and Trem2<sup>+/−</sup> 5XFAD mice (Figures 4E–4G) and were paralleled by an increased distance between microglia and the center of their associated plaques (Figure 4H). Collectively, these data indicate that TREM2 is essential for the microglial response to Aβ plaques.

**Figure 2. TREM2 Deficiency Impairs Aβ-Induced Transcriptional Program in Microglia**

Transcriptional analysis of microglia isolated from hippocampi and cortices of 8.5-month-old Trem2<sup>−/−</sup> 5XFAD, 5XFAD, Trem2<sup>−/−</sup>, and WT mice. (A) Top 15% most variable transcripts were subjected to principle component analysis (PCA). Plot shows two-dimensional (PC2 versus PC3) comparison of transcriptional changes in all classes analyzed. WT and Trem2<sup>−/−</sup> bone marrow-derived macrophages were used as references. (B) Volcano plot comparing microglial transcripts in Trem2<sup>−/−</sup> and WT mice. TREM2 transcript is indicated. (C)Volcano plot comparing microglial transcripts in 5XFAD and WT mice. Numbers in plots (B) and (C) indicate probes that are significantly upregulated or downregulated (±2-fold, p < 0.05, Student’s t test). Representative transcripts are indicated. (D and E) Visualization of Aβ-induced changes in microglial transcripts from (C). (D) A heatmap displays hierarchical clustering of all samples analyzed. (E) A scatterplot compares these transcriptional changes in Trem2<sup>−/−</sup> 5XFAD and 5XFAD microglia. Representative transcripts are shown. See also Figure S2.

**TREM2 Deficiency Affects Microglial Survival in 5XFAD Mice**

Why is TREM2 required for Aβ reactive microgliosis? We first hypothesized that TREM2 may be necessary for Aβ uptake and microglial activation. We initially investigated the impact of TREM2 deficiency on microglial activation in vitro. For this analysis, we used primary microglia isolated from adult mice and expanded in the presence of optimal amounts of CSF-1 and TGF-β (Figure S5A), as they closely resemble microglia in vivo (Butovsky et al., 2014). TREM2 deficiency did not affect microglial expansion, migration, or TNF-α secretion in response to Aβ (Figures S5B–S5D). In contrast, Trem2<sup>−/−</sup> microglia produced significantly more TNF-α than WT microglia in response to lipopolysaccharide (LPS), consistent with previous demonstrations that TREM2 attenuates cytokine responses to certain TLR ligands.
Moreover, TREM2 deficiency had very little impact on microglial uptake of Aβ aggregates (Figure S5E; Movie S4) or their subsequent proteolytic processing, as demonstrated by similar degradation of the intracellular concentration of Aβ after initial loading (Figure S5F). Thus, TREM2 deficiency does not engender a direct defect in phagocytosis of Aβ.

Previous studies have suggested that the CSF-1-CSF-1R pathway promotes reactive microgliosis (Chitu and Stanley, 2006) and Aβ clearance (Mitrasinovic et al., 2003); consistent with this, CSF1-deficient osteopetrotic (op/op) mice are characterized by increased deposition of Aβ, scarcity of microgliosis and neuronal loss (Kaku et al., 2003). We had previously demonstrated that TREM2 signaling via its associated adaptor DAP12

Figure 3. TREM2 Deficiency Leads to Reduced Microgliosis in 5XFAD Mice
Microgliosis in 8.5-month-old Trem2−/−5XFAD, Trem2+/−5XFAD, and 5XFAD mice.
(A and B) Matching coronal sections were stained with Iba-1 (red) for microglia and X-34 (green) for amyloid plaques. Representative z stack images with maximum projection are shown.
(C and D) Quantification of total Iba-1 reactivity per high-power field (HPF) in hippocampi and cortices.
(E and F) Quantification of microgliosis associated with plaques of similar sizes in hippocampi and cortices.
Original magnification 20× (A, B, upper panels), 40× (A, B, lower panels); scale bar, 10 μm (A, B, upper panels), 50 μm (A, B, lower panels). *p < 0.05, **p < 0.01, ****p < 0.0001, one-way ANOVA. Data represent analyses of a total of eight of ten 5XFAD, eight of 12 Trem2+/−5XFAD mice, and eight of 16 Trem2−/−5XFAD mice. Bars represent mean ± SEM. See also Figure S3 and Movies S1, S2, and S3.

(Hamerman et al., 2006; Turnbull et al., 2006).
synergizes with CSF-1R signaling to promote survival of macrophages (Otero et al., 2009, 2012). Specifically, TREM2/DAP12 were required to induce activation of the Syk tyrosine kinase pathway downstream of CSF-1R (Otero et al., 2009; Zou et al., 2008). Thus, we hypothesized that TREM2 may synergize with CSF-1-CSF-1R signaling to sustain reactive microgliosis during Aβ deposition. We initially tested this hypothesis in vitro by measuring the survival of adult primary microglial cultures from WT and Trem2–/– mice in the presence of graded concentrations of CSF-1 (10%, 1%, and 0.1% L-cell conditioned medium [LCM]). While TREM2 deficiency did not affect viability at high concentrations of CSF-1 (10% and 1%), Trem2–/– microglia were markedly less viable than WT microglia in 0.1% CSF-1 (Figures 5A–5C). We next purified microglia from Trem2–/–5XFAD and 5XFAD mice and cultured them in medium containing low levels of CSF-1 (0.1% LCM) for 5 days. Trem2–/–5XFAD microglia were significantly less viable than 5XFAD microglia (Figure 5D). Since CSF-1R captures CSF-1 and targets it for degradation (Stanley and Chitu, 2014), we hypothesized that CSF-1R deficiency might affect viability at low concentrations of CSF-1. Indeed, CSF-1R blockade reduced viability of 5XFAD microglia, confirming that the pro-survival effect of TREM2 cannot replace that of CSF-1R, but only synergize with it (Figure 5D).

TREM2 Is a Sensor for Anionic and Zwitterionic Lipids that Accumulate in the CNS during Aβ Deposition

To evaluate the impact of TREM2 deficiency on microglial apoptosis in vivo, we analyzed coronal sections of Trem2–/– 5XFAD and 5XFAD mice by TUNEL staining. Markedly more TUNEL+ microglia were evident in Trem2–/– mice than the very few observed in control 5XFAD mice (Figures 5E and 5F), corroborating a role for TREM2 in maintaining microglial survival during reactive microgliosis. Consistent with this, significantly fewer microglia were recovered from the cortices and hippocampi of Trem2–/–5XFAD mice than from 5XFAD mice (Figure 5G). We postulate that reactive microgliosis is associated with increased CSF-1 uptake by CSF-1R and degradation restricting CSF-1 range of action, such that microglia in close proximity must compete for CSF-1. Because of their inability to survive CSF-1 limitation, TREM2-deficient microglia are incapable of sustaining reactive microgliosis and undergo apoptosis rather than becoming activated and expanding.

TREM2 Is a Sensor for Anionic and Zwitterionic Lipids that Accumulate in the CNS during Aβ Deposition

We next sought to identify the ligand(s) that trigger TREM2 signaling during Aβ deposition. Since TREM2 binds anionic carbohydrates, anionic bacterial products, and phospholipids (Cannon et al., 2012; Daws et al., 2003), we focused on lipids that have been shown to accumulate during Aβ deposition and

Figure 4. TREM2 Deficiency Diminishes the Capacity of Microglia to Cluster around Aβ Plaques

Frequencies of plaque-associated microglia in 8.5-month-old Trem2–/–5XFAD, Trem2+/–5XFAD, and 5XFAD mice were determined. (A) Heatmap shows frequencies of microglia in relation to Aβ plaques shown as white squares. (B) Summary of frequencies of plaque-associated microglia in all analyzed genotypes. (C and D) Microglial clustering around plaques in 5XFAD, Trem2+/–5XFAD, and Trem2–/–5XFAD mice were compared to Monte Carlo simulations that assume total randomness between plaques and microglia. Probabilities that any given microglia-plaque cluster are non-random are shown in (C). Pie charts show frequencies of microglia-plaque clusters that cannot be statistically explained as random (p < 0.05) (D). (E) Morphology of plaque-associated microglia highlighting the shape of cell bodies (red) and primary processes (cyan). (F–H) Plaque-associated microglia are analyzed for their surface area (cell body only), average length of primary processes, and distance from the center of adjacent Aβ plaque.

Original magnification: 20 x; scale bar, 15 μm. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, one-way ANOVA. Data represent analyses of a total seven mice per group (A–D) and a total of five mice per group (E–G). Bars represent mean ± SEM. See also Figure S4.
might stimulate microglia. These included negatively charged phospholipids, which have been shown to associate with Aβ in lipid membranes (Ahyayauch et al., 2012; Nagarathinam et al., 2013); membrane phospholipids, such as phosphatidylserine, which are exposed by damaged neurons and glial cells; and anionic and zwitterionic non-phosphate lipids, such as sulfatides and sphingomyelin, which are released by damaged myelin. We transfected human TREM2 in reporter cells that express GFP under the control of NFAT, such that Ca²⁺ mobilization turns on GFP expression when TREM2 is engaged. Incubation of TREM2 reporter cells with many of these lipids activated reporter activity, although to differing extents, with phosphatidylcholine (PC) and sphingomyelin (SM) performing best in these assays (Figures 6A and 6B). Similar results were obtained with a mouse TREM2 reporter (data not shown). Addition of a blocking TREM2 antibody abolished reporter activation by all ligands, demonstrating specificity (Figure 6B). Interestingly, other potential candidates, such as cardiolipin, which is released by damaged mitochondria, did not significantly activate the TREM2 reporter despite its phospholipid structure. This suggests that the ability to engage TREM2 may only partially depend on the presence of negatively charged moieties like phosphoric acid (Figures 6A and 6B). Furthermore, TREM2 reporter activation was not detected with plate-bound synthetic or extracted Aβ (data not shown). In agreement with the ability of phosphatidylserine (PS) to activate TREM2 reporter cells, apoptotic cells, which expose PS on the cell surface, also activated TREM2 reporter cells (Figure 6C). However, microglia isolated from Trem2−/−5XFAD and 5XFAD mice engulfed apoptotic cells equally well (Figures 6D and 6E). Thus, TREM2 is not directly involved in phagocytosis of apoptotic cells. We conclude that TREM2 is a sensor for several anionic and zwitterionic lipids that are exposed during Aβ deposition as well as during neuronal and glial cell death.

**R47H Mutation Impairs TREM2 Recognition of Lipid Ligands**

What is the impact of the R47H mutation on TREM2 ligand recognition? We generated TREM2 R47H reporter cells and compared their response to identified ligands to that of TREM2 reporter cells. The R47H mutation considerably reduced reporter activation in response to many ligands, including phosphatidic acid (PA), phosphatidyglycerol (PG), PS, phosphatidylinositol (PI), and sulfatides (Figures 7A–7G). The R47H mutation had less impact on SM recognition and very little influence on PC-mediated activation. Importantly, the R47H mutation did not detectably affect cell-surface expression or signaling of TREM2, as assessed by stimulating the R47H reporter cells with a plate-bound anti-TREM2 antibody (Figure 7H). Thus, these data suggest that the R47H reduces the overall capacity of TREM2 to bind anionic ligands.


**DISCUSSION**

This study showed that TREM2 modulates Aβ accumulation in the 5XFAD mouse model of AD, thereby reducing neuronal damage. The importance of TREM2 in Aβ clearance is underscored by the fact that even the loss of one copy of Trem2 gene is sufficient to increase Aβ accumulation. TREM2 acts in microglia by supporting Aβ-reactive microgliosis, a process of expansion and activation that leads to microglial clustering around Aβ plaques and subsequent Aβ removal (Ransohoff and Cardona, 2010). In the absence of TREM2, this microgliosis is impaired. In fact, microglia from Trem2−/−5XFAD mice are unable to survive, as evidenced by the accumulation of apoptotic microglia around Aβ plaques. Cells involved in TREM2-dependent microgliosis had phenotypic features of brain resident microglia, such as expression of CX3CR1. However, it is possible that monocytes from peripheral blood contribute to microgliosis and that TREM2 supports their survival as well.

Previous studies have shown that CSF-1-CSF-1R signaling is essential for microgliosis in response to Aβ (Chitu and Stanley, 2006; Kaku et al., 2003; Mitrasinovic et al., 2003). Since CSF-1 is rapidly consumed during this process (Stanley and Chitu, 2014), there is probably a limited supply of CSF-1 surrounding the Aβ plaques. Our results demonstrate that TREM2 provides a signal that is necessary for survival of microglia at low CSF-1 concentrations. We postulate that TREM2 acts as a costimulatory molecule that sustains survival of microglia, which are activated and proliferate in the presence of Aβ. Previous studies of cultured myeloid cells indicate that TREM2 may synergize with CSF-1-CSF-1R signaling to activate the protein tyrosine kinase Syk, which, in turn, activates multiple downstream mediators, such as ERK, PI-3K, and Akt (Zou et al., 2008). In addition, TREM2 may provide survival signals through activation of anti-apoptotic mediators such as β-catenin (Otero et al., 2009) and Mcl-1 (Peng et al., 2010). It is also possible that TREM2 is necessary to support increased microglial metabolism during activation.

Why is TREM2 activated during Aβ accumulation? Previous studies have indicated that TREM2 binds phospholipids, such as PS, and acts as a scavenger receptor for apoptotic cells that might be generated during neuronal damage (Hsieh et al., 2009; Takahashi et al., 2005, 2007). In our study, we demonstrate that TREM2 is a sensor for a broad array of acidic and zwitterionic lipids, which may or may not contain a phosphoric acid moiety. Membranes containing these lipids strongly interact with Aβ, facilitating the formation of fibrillar Aβ.
Moreover, some TREM2 lipidic ligands accumulate on the cell surface of neurons and glial cells damaged by Aβ accumulation, such as PS (Eckert et al., 2005; McLaurin and Chakrabartty, 1996), or are released by damaged myelin, such as SM and sulfatides. In contrast, TREM2 did not directly bind Aβ. Consistent with its ability to bind anionic lipids, the TREM2 extracellular domain is rich in arginine residues that may form salt bridges with polyanions. Remarkably, we found that the R47H mutation associated with AD affected the binding of multiple lipid ligands, although to differing extents. Most likely, the R47H mutation is sufficient to considerably reduce the binding affinity of TREM2 extracellular domain for most anionic ligands. Structural studies will be essential to validate this model.

Our findings demonstrated that TREM2 functions as a microglial sensor that is alerted by damage-induced molecules that share a common lipidic backbone and an anionic group. In contrast with previous reports (Hsieh et al., 2009; Takahashi et al., 2005, 2007), we found that the engagement of TREM2 does not directly mediate phagocytosis of apoptotic cells. However, TREM2 signaling may indirectly support phagocytosis by promoting survival of activated microglia. It has been shown that individuals homozygous for rare mutations that impair expression of either TREM2 or DAP12 develop lethal forms of progressive, early-onset dementia such as Nasu-Hakola disease (NHD) and frontotemporal dementia (Guerreiro et al., 2013a, 2013c; Kleinberger et al., 2014; Paloneva et al., 2002). Although the pathology of these forms of dementia differs from that of AD and often involves demyelination, our study suggests that TREM2 may be required for microglia to sense glycolipids such as SM and sulfatides that are exposed on damaged myelin sheaths; thus, TREM2 binding to these glycolipids may trigger the microglial response to damaged myelin, which is necessary to clear myelin residues and produce trophic factors that induce repair and remyelination. While the R47H mutation associated with AD did not entirely abolish ligand binding, mutations associated with Nasu-Hakola disease result in a complete lack of TREM2 expression (Kleinberger et al., 2014), which may explain the distinct pathology and more dramatic clinical course of this disease.

**EXPERIMENTAL PROCEDURES**

**Mice**

Trem2−/− mice were generated as previously described. 5XFAD mice were purchased from the Jackson Laboratory (MMRRC) and crossed to Trem2−/− mice to generate Trem2+/− 5XFAD and Trem2−/− 5XFAD mice. All mice were bred and housed in the same animal facility.

**Preparation of Brain Samples**

For histological analysis 5XFAD mice, APPPS1-21 and transgene negative controls were anesthetized with ketamine and perfused with ice-cold PBS. Right-brain hemispheres were fixed in 4% PFA overnight and placed in 30% sucrose before freezing and cutting on a freezing sliding microtome. Serial 40-μm coronal sections of the brain were collected from the rostral anterior commissure to caudal hippocampus as landmarks. For biochemical and mRNA expression analysis, cortices and hippocampi of the left-brain hemispheres were carefully dissected out and flash frozen in liquid nitrogen.

**Immunohistochemistry and Microscopy**

For detailed procedures, see Extended Experimental Procedures.

**Gene Expression Analysis**

For frozen brain tissues, RNA was extracted using a RNeasy mini kit according to manufacture protocol (QIAGEN). Microglia were fluorescence-activated cell-sorted (FACS) directly into RLT-plus lysis buffer, and RNA extraction was performed using a RNeasy micro kit according to manufacture protocol (QIAGEN). Primers for qPCR analysis are provided in Table S1. For detailed procedure on microarray analysis, see Extended Experimental Procedures.
**ELISA**

All levels were assessed using sandwich ELISAs as described (Kim et al., 2009). For detailed procedure, see Extended Experimental Procedures.

**Ex Vivo Microglia Cultures**

Primary adult microglia culture was generated as previously described (Butovsky et al., 2014). Briefly, purified adult microglia were cultured in the presence of 15% LCM media (Otero et al., 2009) and 10 ng/ml human TGF-β (PeproTech) for 7 days before experiments. For details on in vitro assays performed, see Extended Experimental Procedures.

**Reporter Assay**

2B4 GFP-NFAT reporter T cells were stably transfected with murine or human TREM2 cDNAs. Cells were cultured with apoptotic thymocytes in round-bot-
tom 96-well plates or plated onto high-absorbance flat-bottom plate coated with various lipids at indicated concentration. Reporter cells were assessed af-
ter overnight incubation. Reporter activity (%) is defined as %GFP+ cells sub-
tracted from background (vehicle controls).

**Statistics**

Data in figures are presented as mean ± SEM. All statistical analysis was per-
formed using Prism (GraphPad). Statistical analysis to compare the mean
values for multiple groups was performed using a one-way or two-way
ANOVA with correction for multiple comparisons. Comparison of two groups
was performed using a two-tailed unpaired t test (Mann-Whitney). Values were accepted as significant if p ≤ 0.05.

**ACCESSION NUMBERS**

All data have been deposited at GEO (GSE65067).

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Extended Experimental Procedures, five
figures, one table, and four movies and can be found with this article online
at http://dx.doi.org/10.1016/j.cell.2015.01.049.

**AUTHOR CONTRIBUTIONS**

Y.W., J.D.U., M.L.R., S.S., and B.H.Z. performed two-photon and confocal im-
aging analyses; Y.W., B.H.Z., and G.M.K. performed computational analyses;
K.M., K.L.Y., and J.R.C. analyzed Aβ; Y.W. performed ex vivo cell-
culture experiments; M. Cella and Y.W. generated TREM2 reporter cells and
performed reporter assays; and S.G. generated and maintained Trem2−/−and Trem2+/−/5XFAD mice. D.M.H. supervised research on APPPS1-21 mice and
provided critical comments. M. Colonna supervised the entire project;
M. Colonna and Y.W. wrote the manuscript.

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