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RESEARCH HIGHLIGHT

TREMble Before TREM2: The Mighty Microglial Receptor Conferring Neuroprotective Properties in TDP-43 Mediated Neurodegeneration

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Microglia were long hailed as solely inflammatory cells, and though they are responsible for the brain's immune response, they perform a variety of neuroprotective functions including the clearance of cellular and toxic debris [1]. One toxic molecule known to be cleared by microglia is the protein aggregate found in Alzheimer's Disease (AD), amyloid-ß. The triggering receptor expressed on myeloid cells 2 (TREM2) is a known receptor for ß-amyloid [2] and is exclusively expressed on microglia. Interestingly, microglial TREM2 variants have been described as a risk factor for AD in epidemiological studies [3].

Considering these findings, there have been recent investigations into potential TREM2 variants that may serve as risk factors for other proteinopathies, such as TAR DNA binding protein 43 (TDP-43) aggregation observed in patients with amyotrophic lateral sclerosis and frontotemporal dementia. To determine if TREM2 directly contributes to TDP-43 mediated neurodegeneration, Xie *et al*. [4] successfully applied a method of viral delivery through intracerebroventricular (icv) injection in P0 pups to overexpress human TDP-43 (hTDP-43). To evaluate whether TREM2 contributes to TDP-43-related motor deficits, motor function was evaluated in *Trem2*-knockout (KO) mice and wild-type (WT) controls following icv injection of hTPD-43. The results revealed that *Trem2*-KO mice not only displayed hindlimb clasping earlier than the WT, but also had delayed recovery and significant locomotor impairments in the rotarod test, indicating that TREM2 deficiency both accelerates the onset of and worsens hTDP-43-related motor dysfunction. Next, the authors demonstrated significant neuronal loss in the primary motor cortex, higher levels of both hTDP-43 and p-hTDP-43 in the soluble fraction, and more p-hTDP-43 in the insoluble fraction of *Trem2*-KO mice compared to the WT. Thus, the motor deficits in *Trem2*-KO mice correlated with higher levels of pathological hTPD-43.

The authors next set out to determine how TREM2-deficiency impacts microglial phenotypes in response to hTDP-43-related neurodegeneration. Mice expressing WT hTDP-43 possessed a subcluster of microglia with the distinctive $CD11c^{high}$ phenotype that was reduced in mice expressing *Trem2*-KO hTDP-43. Immunohistochemical analysis revealed higher $CD11c^{+}$ expression in microglia phagocytosing GFP-hTDP-43 than in non-phagocytic microglia, indicating that a TREM2-dependent $CD11c^{+}$ subpopulation may facilitate hTDP-43 phagocytosis (Fig. 1).

To test this possibility, they stereotactically injected GFP-hTDP-43 virus into the primary motor cortex of 2-month old mice. CD68, a marker for microglial lysosomal activity, was significantly reduced in *Trem2*-KO mice, and CD68-positive microglia had high CD11c expression and co-localized with GFP-hTDP-43. Consistent with this, WT mice contained a significantly higher number GFP-hTDP-43-containing microglia than *Trem2*-KO mice, which resulted in significantly higher hTDP-43 and p-TDP-43 accumulation relative to WT mice.

Furthermore, the reduced phagocytic phenotype in *Trem2*-KO mice suggests that microglia remain inactivated in response to hTDP-43 accumulation. Indeed, WT microglia exhibit a stereotypical reactive phenotype with

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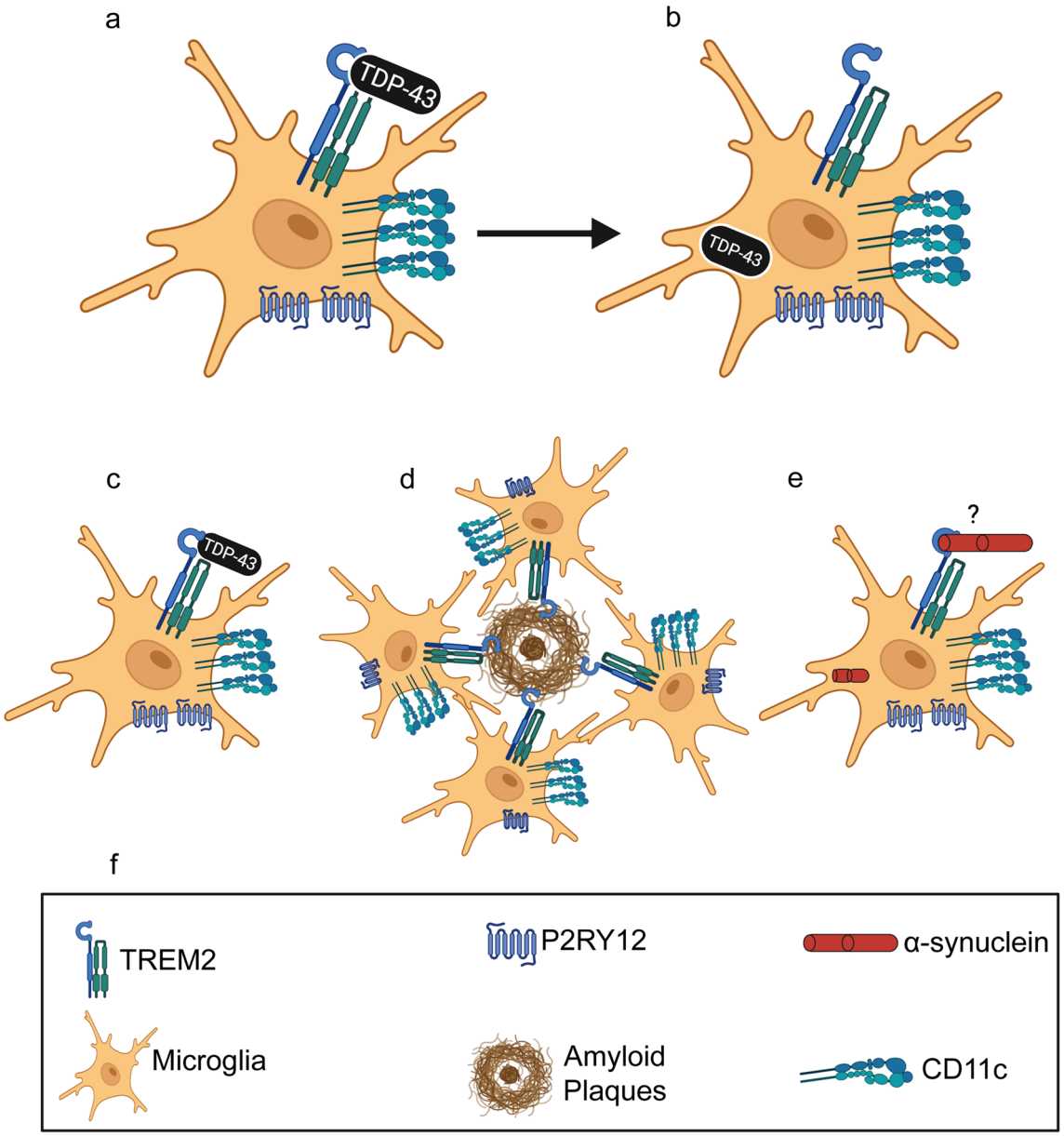

Fig. 1 Schematic illustrating the roles of TREM2-mediated phagocytosis in various proteinopathies **A** The central finding of this paper shows that TREM2 on CD11c^{high} microglial cells directly interacts with TDP-43 resulting in **B** phagocytosis of TDP-43 and maintained expression of the homeostatic gene P2RY12. **C, D** Comparison of microglia in hTDP-43-mediated neurodegeneration to the plaque-associated CD11c^{high} disease-associated microglia (DAM) phenotype present in Alzheimer's Disease. Unlike hTPD-43-mediated neurodegeneration, DAM microglia have downregulated homeostatic genes, such as P2RY12. **E** Theoretical representation of TREM2 interactions with α-synuclein. It is known that microglia phagocytose neuron-released α-synuclein, but does this occur *via* a TREM2-mediated mechanism? What is the state of microglia CD11c expression in other proteinopathies, and would a subcluster of CD11c^{high} microglia play a protective role in other proteinopathies similar to AD and ALS? Future studies should seek to clarify this. **F** figure legend. This figure was created using BioRender.

an enlarged soma and shortened processes upon hTDP-43 expression, all of which is attenuated in microglia lacking TREM2 expression. In addition, the homeostatic markers P2RY12 and TMEM119 were markedly downregulated in WT microglia but not TREM2-deficient microglia in response to hTDP-43 accumulation. Together, these results indicate that TREM2 expression on microglia is critical for directing their phagocytic clearance of TDP-43 and regulating their transition from a homeostatic state into activation.

Next, the authors set out to determine how hTDP-43 interacts with TREM2 to activate microglial phagocytic activity. To assess if there is a direct physical interaction between

hTDP-43 and TREM2, the authors applied co-immunoprecipitation in transfected HEK293T cells with Myc-tagged human TREM2 or control plasmids. The authors successfully immunoblotted for hTDP-43 following hTREM2 immunoprecipitation using an hTREM2 antibody. To further define the interaction between TREM2 and hTDP-43, the authors used liquid chromatography with tandem MS (LC-MS/MS) and obtained annotated MS/MS spectra for both hTREM2 and hTDP-43 from anti-TREM2 immunoprecipitates. They identified the C-terminal fragment (residues 216–414) of hTDP-43 as the domain interacting with hTREM2 by co-transfecting HEK cells with Myc- hTREM2 and GFP-hTPD-43. Bead-immobilized GFP antibodies were used to capture GFP or GFP-tagged proteins. Finally, the authors applied SILAC (stable isotope labeling with amino-acids in cell culture)-based quantitative proteomics to study the hTREM2 interactome. hTREM2 was labeled with heavy amino-acids, and the analysis of mass spectra revealed 157 potential hTREM2-interacting proteins, including hTDP-43.

To confirm *in vivo* the interaction between TREM2 and TDP-43, an inducible mouse model of ALS-rNLS8, was generated. Immunoblotting against mTREM2 and hTDP-43 after immunoprecipitation from rNLS28 brain lysates revealed co-immunoprecipitation of endogenous mouse TREM2 with hTDP-43. LC- MS/MS on anti-TREM2 immunoprecipitates from rNLS8 mouse cortex revealed the presence of both mouse TREM2 and hTDP-43 in the immunoprecipitates. To determine if the interaction between TREM2 and TDP-43 occurs in human tissue as well, the authors successfully co-immunoprecipitated the hTDP-43-TREM2 complex from lysates of human ALS spinal cord.

The study by Xie *et al* is an elegant one, expanding our appreciation for roles of TREM2 in neurodegeneration beyond AD to TDP-43 pathology, as they show roles for TREM2 in (1) physically interacting with TDP-43 *in vitro*, *in vivo*, and in humans, (2) generating a CD11c^{high} microglial phagocytic subpopulation to clear accumulated proteins with (3) consequences to reduce neuronal damage and improve motor functions. CD11c, also known as integrin-alpha X, has been classically used to identify myeloid dendritic cells [5]. However, a previous study demonstrated in the context of AD that TREM2 is necessary for the transition of microglia to what was classified as a disease-associated microglial (DAM) phenotype [6]. These amyloid plaque-associated cells are also CD11c^{high} and were found to phagocytose amyloid-ß (Aß). Interestingly, this microglial phenotype was further found in the spinal cord of ALS mice, and hence, these two studies combined reveal a microglial subset characterized by TREM2 regulation of high CD11c expression and phagocytic activity, with these microglia ultimately playing a protective role throughout disease progression. In contrast to the CD11c^{high} DAM microglia, where homeostatic genes such as P2RY12 are downregulated, Xie *et al* found that microglia in hTDP-43 pathology maintain their expression.

Beyond TREM2 interactions with Aß and TDP-43, might these two papers suggest a possible interaction between TREM2 and aggregating proteins in other proteinopathies? Indeed, the central region of α-synuclein was first purified from the amyloid plaques of AD patients [7]. Of α-synuclein's three regions, the central non-amyloid component region is critical for amyloid fibril formation [8]. Furthermore, α-synuclein and tau are known to directly interact with one another [9], the results of which include promoting each protein's fibrillation [9]. In light of this, perhaps CD11c^{high} microglia are present in other proteinopathies where TREM2 binds aggregating monomers and promotes their phagocytosis? To that end, it has been shown in Parkinson's disease models that TREM2 deficiency exacerbates α-synuclein-induced neurodegeneration and neuroinflammation [10], and that microglia clear neuron-derived α-synuclein [11]. Might this be *via* TREM2 interactions? Future studies should clarify this.

Beyond the role of TREM2 in other proteinopathies, understanding how downstream activation of CD11c occurs following TDP-43 binding to TREM2 remains undetermined. One study [12] using mouse bone-marrow-derived dendritic cells demonstrated through chromatin immunoprecipitation that a transcriptional complex consisting of JunD and Fra2, transcription factors belonging to the AP-1 family, bind directly to the CD11c promoter to regulate its expression. TREM2 forms a receptor adaptor complex with DNAX-activating protein of 12 kDa. There are multiple downstream signaling pathways that can be activated following binding of extracellular ligands, but all begin with a Syk tyrosine kinase-generated signal. With regard to the receptor-mediated phagocytosis pathway, Syk activates Vav guanine nucleotide exchange factors which then signal to the downstream GTPases Rac1/Cdc42 and the Arp2/3 protein complex [13]. Though the mechanism regulating JunD recruitment to the CD11c promoter in dendritic cells has not been determined, it has been shown in T cell lymphocytes that JNK activation is strengthened through an alliance between Rac GTPase and Syk tyrosine kinase-produced signals. JNK is known to phosphorylate newly-synthesized JunD and promote its transcriptional activity [14]. In light of this, perhaps JunD activity at the CD11c promoter downstream of TREM2–TDP43 interactions is responsible for CD11c expression. This idea is supported by a study showing increased transcriptional expression of microglial JunD sampled from single-nuclei RNA sequencing of *post-mortem* AD and control brains with a range of Aβ and phospho-tau pathology [15]. Specifically, transcriptomic network characterization of microglial cells in brain regions with Aβ and phospho-tau pathology revealed that JunD is one upregulated

transcription factor that promotes microglial autophagy, phagocytosis, and proteostasis. Given CD11c's expression in disease-associated microglia and their presence in AD pathology, it is possible that JunD could regulate CD11c expression in TDP-43 mediated neurodegeneration, which will have to be addressed by future studies.

Taken together, this is the first study to show that TDP-43 can serve as a ligand for TREM2 in human spinal cord and mouse cortex. TREM2 regulates the microglial phagocytosis of TDP-43, while CD11c is highly expressed in these phagocytic microglia. Thus, TREM2 activation confers neuroprotective properties on microglia in ALS pathology, and future studies can seek to determine if $CD11c^{+}$ phagocytosing microglia can play an antigen-presenting role in ALS pathology specifically and neurodegeneration broadly.

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