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ApoE and TREM2 regulate LPS-induced inflammatory responses in a joint manner

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Abstract

Alzheimer's disease (AD) has been considered as the most common reason of dementia in the people. AD is associated with TREM2 genetic variants, an innate immune receptor located in the brain's microglia. Apo lipoprotein E (ApoE) and (TREM2) are strongly expressed in microglia that control responses of inflammatory respectively in the central nervous system (CNS). Here we demonstrate that LPS (lipopolysaccharide) down-regulates the appearance of small type Trem2 and ApoE in primary microglia. In Trem2-/- and apoE-/- rats, primary microglia, we found that both ApoE and Trem2 can respectively regulate LPS-induced inflammatory cytokine release. Importantly, primary microglia of *Trem2*/*ApoE* double knockout mice was hypersensitive to LPS stimulation when compared with single gene knockout mice. It suggested that ApoE and TREM2 regulate LPS-induced inflammatory responses in a joint manner. Our data indicate that ApoE-TREM2 dealings in microglia that plays serious roles in modulating neuron inflammation, and establishes a crucial connection among two proteins whose genes are closely associated with the risk of AD.

Keywords: ApoE, TREM2, LPS, microglia, inflammation.

1. Introduction

AD has been commonly form of dementia in people of old age with medication that is not allowed to change the disorder (De Strooper B. et al., 2012)(Karch C.M. et al., 2015). In late-onset AD (LOAD), APOE is the biggest risk factor (Liu C.C. et al., 2013) and encodes an Apo lipoprotein which binds and transports cholesterol and other lipids from glia to CNS (Bu G., 2009). TREM2 has been discovered AD risk gene recently (Mahley R.W. et al., 1996) which encodes an innate immune receptor (IR) expressed in microglia of the brain. Several TREM2 variants, such as R47H, R62H, D87N, Q33X, Y38C, and T66 M, were associated with enlarged risk of AD, Parkinson's disease (PD), front temporal dementia (FTD), lateral amyotrophic sclerosis (ALS), and essential tremor (Del-Aguila J.L. et al., 2012).

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TREM2 is an immunoglobulin (Ig) superfamily member which contains a codomain, a transmembrane domain along with a short cytoplasmic tail. DNAX-activating 12 kDa (DAP12) protein is required to mediate TREM2 signal transduction through a single activation motif (ITAM) based on the immune receptor tyrosine. Both TREM2 and DAP12 genetic mutations have been shown to be linked with disorders such as NHD (Nasu-Hakola) (Paloneva J. et al., 2002) and AD (Pottier C. et al., 2016). Furthermore, inside the CNS, both are preferentially expressed in microglia. Together they control microglia functions along with pro-inflammatory responses of inhibition (Zhong L. et al., 2015)(Zhong L. et al., 2017) and outward extension barriers of amyloid fibrils and axonal dystrophy (Sirkis D.W. et al., 2016)(Yuan P. et al., 2016). Another significant risk factor for developing LOAD is the APOE gene. APOE4allele, which raises the risk of AD by three-fold compared to APOE3, while APOE2 is protective (Corder E.H. et al., 1994).

Early studies indicated that the isoform ApoE π4 bound faster to Aβ and increased Aβ deposition are more readily associated with the isoforms of ApoE ÿ2 or π3 (Garai K. et al., 2014)(Bales K.R. et al., 2009). As a lipid conveyor protein abundantly expressed in brain cells, ApoE is packed with cholesterol as well as phospholipid to form complexes lipid-protein and binds to ApoE receptors on the surfaces of nerve cells, then released into the extracellular space, neurotransmissions and brain danger response (Yeh F.L. et al., 2016).

Microglia continuously surveys the brain to preserve order and stability, providing trophic support for neurons, phagocytizing cellular debris, and responding to foreign invaders (Krasemann S. et al., 2017). Recent papers have shown that all three major human ApoE isoforms bind to human TREM2 (Yeh F.L. et al., 2016)(Atagi Y. et al., 2015). Clinical confirmation of such an association would effectively relate the two main factors of genetic risk for AD pathogenesis to biological functions of the microglia. We used Trem2 / ApoE double knockout mice in this study to provide the first proof of ApoEcooperates with TREM2 to regulate initiation and propagation. We verified that LPS stimulation inhibited both ApoEand Trem2 expressionin primary microglia. In addition, LPSstimulated Wide-type primary microglia, ApoEknockout, Trem2 knockout, and Trem2 / ApoE double knockout mice and elevated the expression of TNF-α and IL-1βto varying degrees.

2. Material and Methods

2.1. Reagents and Antibodies

Life Technologies had been synthesizing the quantitative RT-PCR primers. Roche purchased SYBR Green for RT-PCR quantitative. Sigma obtained the colony-stimulating factor (GM-CSF) and LPS for granulocyte macrophage. The antibodies that has been used in this work are as following: anti-β-Actin, ApoE anti-mouse (Cell Signaling Technology), TREM2 anti-mouse (R&D), IgG antibody anti-rabbit goat and IgG antibody anti-goat combined with radish peroxidase pet.

2.2. Isolation and Culture of Mouse Primary Microglia

The microglia primary cultures have been prepared as follows. Both mouse studies were performed in a protocol approved by the Animal Ethics Committee. In short, on postnatal day 1 to 3, mixed wild-type glial cells, ApoE-KO, Trem2-KO, ApoE / Trem2-DKOmice, were put on flasks as well as grown in DMEM (Gibco) accompanied with 10 per cent heat-inactivated bovine fetal serum (FBS) (Gibco). Level of 25 ng / mL GM-CSF and 10 per cent FBS has been revised three days later. After 10-12 days in cultivation, primary microglia was harvested by trembling (200 rpm, 20min) and afterwards once every 3 days (up to four harvests).

2.3. Genotyping

Genomic DNA was collected in Thailand from tissue samples of mice and subsequently analyzed using ApoEor Trem2genotyping. DNA was extracted using the kit of DNA extraction (TIAN-GEN), as instructed by the manufacturer. In addition, DNA has been diluted to 10ng / μL for nuclease-free water genotyping. Initial activation of AmpliTaq DNAPolymerase at 94 ° C for 3min, accompanied by 40 cycles at 94 ° C for 30 seconds, annealing at 55 ° C for 30 seconds at 72 ° C for 45 seconds, the Trem2 -/- genotype PCR amplification protocol was the following: The detection of ApoE -/- was as follows: after pre-denaturation for 10 min at 98 ° C, the mixture was amplified for 35 cycles at 98 ° C with 10 seconds of denaturation followed by annealing at 68 ° C for 30 seconds at 72 ° C. Myoclonic supported use of the primers. In a 1.5 per cent agarose, ample were electrophoresed after PCR.

2.4. Western Blotting

Primary microglia has been lysed at the appropriate times in the lysis buffer containing 1 percent “NP-40, 50 mM Tris-HCl, pH 8.0, 150 mM” of sodium chloride accompanied with protease and cocktails of phosphatase inhibitors. The kit of BCA protein assay has been used to test protein concentrations as instructed by the manufacturer. HRP-conjugated antibodies to evaluate the equivalent quantity of proteins. Proteins were visualized by means of ECL Western blotting reagents. We quantified bands that were immune reactive using Image.

2.5. Quantitative RT-PCR

One microgram of RNA was reversed to cDNA on the first-strand. Quantitative PCR has been executed by using the “Fast Start Universal SYBR Green Master (Roche)”. The primary sequences TREM2, ApoE, IL-1β, TNF-α, and β-actin were as follows: Trem2-Forward: 5'Forward: 5’- • TCATAGGG

CAAGACCT-3';Trem2-Reverse:5'-GCTGCTCTCTTTGTC-3';ApoE-Forward:5'-CACACAAGAACTGCGCAC-3',ApoE-Reverse:5'-CGTAGATCCT

CCATGTCGC-3';IL-1β-Forward:5'-CCTGCAGCAGGAGTGAGTGAT-3';IL-1β-Reverse: 5'-TGTGCTGCTGAGTGCTGCT-3';TNF-α-Forward:5'-AGCCCACTGCT-3' GTCGTAGCAACCAC-3;TNF-α-Reverse:5'-AGGTACAACCCGGCTGC A-3';β-actin-Forward:5'-AGTGACGTGACTGACCGTA-3'; β-actin-Reverse:5'-GCCAGCAGCAGTCTCTC-3''.

2.6. Statistical Analyses

The analysis has been calculated by means of GraphPad Prism, along with all data has been obtainable as mean ±SEM. It analyzed three separate experiments using unpaired t-test, one-way ANOVA. These values of probabilities has been obtained: *, p<0.05; * *, p<0.01; * *, p<0.001.

3. Results

3.1. LPS inhibits ApoE and TREM2 expression in primary microglia

This has found that the appearance of ApoE and Trem2 has been linked with LPS-induced inflammation (Baitsch D. et al., 2011)(Zheng H. et al., 2016). We confirmed that LPS stimulation meaningfully suppressed ApoE and Trem2 appearance in the primary microglia of Wide-type mice. The ApoEandTrem2 mRNA levels has been meaningfully down-regulated in primary microglia stimulated in LPS (Fig. 1A-B). Consistently, we pretreated microglia with specified concentrations of LPS to further detect how LPS modulates the appearance of the APOE and TREM2 protein. The protein levels of ApoEandTrem2 were similarly down-regulated by LPS (Fig. 1C-E). Therefore we infer that the expression of ApoE and TREM2 in primary microglia was inhibited by LPS.

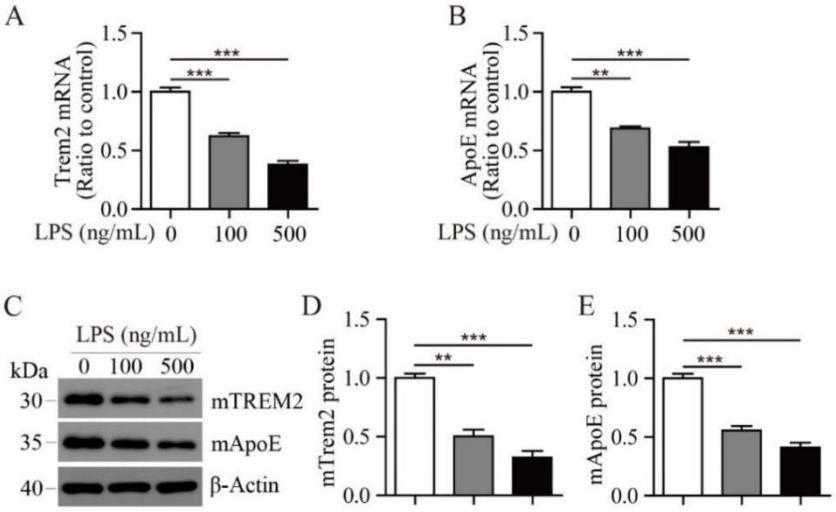

Figure 1: **(A-B) Wide-sized primary microglia are treated with specified concentrations of LPS (0, 100 and 500 ng / mL). In addition, RNA has been extracted as well as quantitative RT-PCR has been used as a bar graph for evaluating the relative mRNA levels of Trem2(A) or ApoE(B). The vehicle treatment mRNA level in microglia was used as control.β-actin was used as internal control (C-E) Cytoplasmic extracts from small primary microglia and analyzed to formouse-TREM2 and mouse-ApoE (C) by Western blotting antibodies. Bar graphs display the quantification of Western blots as TREM2 (D) or ApoE (E) ratios. The ratio of protein levels in non-LPS treatment cells was used as control.β-actin. * *, p<0.01; * *,p<.001.**

3.2. ApoE and TREM2 inhibits LPS-induced cytokines production

Previous studies showed that mRNA levels of pro-inflammatory cytokines in the presence of LPS increased significantly from primary microglia knockout Trem2 or ApoE gen (Zheng H. et al., 2016)(Liu Y. et al., 2015). To check the role of APOE and TREM2 in mediating the responses of inflammatory to pathogenic stimuli, we have used gene-deficient mice to knock out primary microglia (Fig. 2A) and ex expression of Trem2 or ApoE. These data indicate that both ApoE and TREM2 are important to suppress the development of pro-

inflammatory cytokines when exposing microglial cells to LPS stimules.

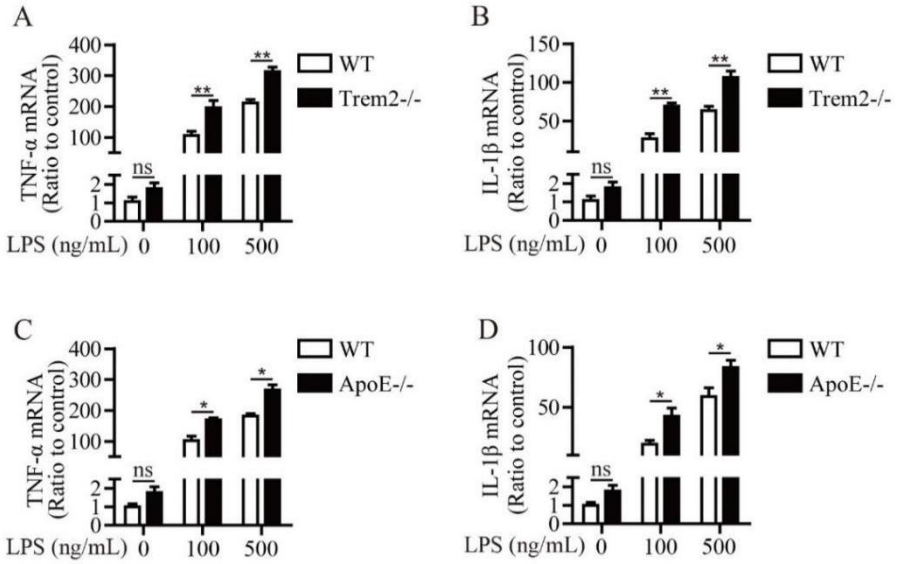

Figure 2: **(A-B) Wide-type primary microglia and Trem2 -/- mice with specified LPS concentrations (0, 100 and 500 ng / mL) were treated for 4 hours. RNA was extracted using quantitative RT-PCR (n=3, bidirectional ANOVA) as well as the relative mRNA levels of TNF-α(A) and IL-1β(B) were calculated as bar graph. (D-E) The relative mRNA levels shown as bar graphs of TNF-α (C) and IL-1β (D) has been analyzed by quantitative RT-PCR in wide-type and ApoE/- microglial cells. As an internal regulation β-actin was used. The vehicle treatment level mRNA in Wide-type microglia was used as the trigger.**

3.3. Enhanced LPS-induced inflammatory responses in Trem2- and ApoE-deficient microglia

Since we observed an anti-inflammatory role in microglia of ApoE and TREM2, we further investigated whether ApoE and TREM2 in some way suppressed the production of inflammatory cytokines. Trem2-/-, ApoE-/-, Trem2 / ApoE-double knockout mice was isolated from primary microglia and subjected to additional LPS therapy. Ironically, deficiencies of the ApoE / Trem2double gene significantly increased the development of primary microglia-stimulated inflammatory cytokines TNF-α and IL-1βin LPS compared to other classes (Fig. 3A-B). Interestingly, the levels of these inflammatory cytokines has been substantially higher in the primary microglia of dual gene knockout mice than the single gene knockout microglia which is consistent with our hypothesis.

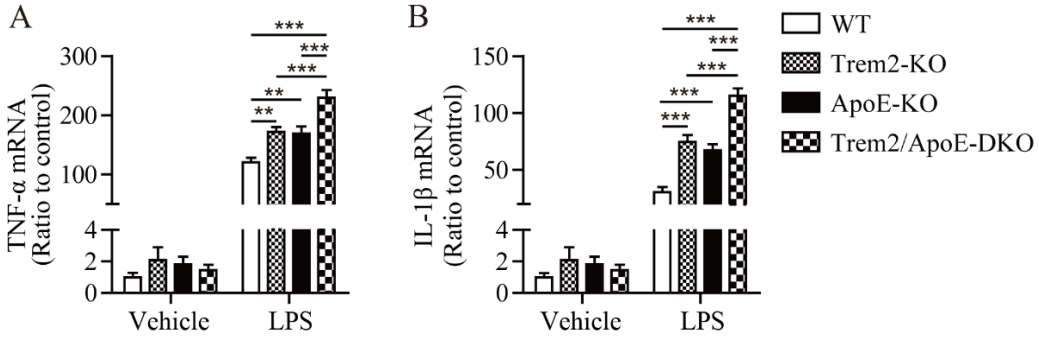

Figure 3: **(A-B) microglia cells from WT, Trem2-/-, ApoE-/-, Trem2 / ApoE-double knockout mice has been enthused with 100 ng / mL LPSor vehicle power, and cells were lysed after 4 h. The mRNA levels of TNF-α (A) and IL-1β (B) shown as bar graphs has been calculated by quantitative RT-PCR. In addition, β-actin has been used as internal regulation. Vehicle treatment mRNA levels in WT microglia have been used as controls.**

4. Discussion

This study showed that, when primary microglia are exposed to LPS, TREM2 and ApoE both suppress pro-inflammatory cytokine production. Importantly, the negative regulation of inflammatory response in primary microglia Trem2 / ApoE double knockout mice induced by LPS was more severe. Our data indicated that ApoE and TREM2 control inflammatory responses triggered by LPS in a joint manner and showed that the involvement of ApoE-TREM2 in microglia played critical roles in neuron inflammatory modulation.

TREM2 and APOE contribute genetically to Alzheimer's disease. The studies of genetic depletion TREM2 or its signaling adapter-DAP12 in the key mouse microglia have shown a substantial increase in pro-inflammatory cytokine levels. Evidence has shown that TREM2 has been cellular receptor for ApoE which helps to understand TREM2 's function in neurodegenerative disorders(Bailey C.C. et al., 2015). Nevertheless, ApoE has not been presented only as a free protein (Strittmatter W.J. et al., 1993). These diverse types will possibly result in variations in binding and signaling via TREM2 (Yeh F.L. et al., 2016). Krasemann and others Show that APOE and TREM2 regulate the transition between homeostatic phenotypes and microglia-associated damage (Jendresen C. et al., 2017)(Krasemann S. et al., 2017)(Pimenova A.A. et al., 2017). TREM2-ApoElikely was used for the regulation of apoptotic neuronal phagocytosis and Aβ plague (Yeh F.L. et al., 2016)(Atagi Y. et al., 2015)]. HowmightaTREM2 / ApoE interaction contributes to other pathogenesis of the AD? Some findings have identified critical roles in regulating the then euro inflammatory environment for TREM2 and ApoE, and the to some extent of the ApoE / TREM2 interaction to activate downstream signaling cascades most likely plays an important role for MAPK signaling pathway. By modifying the pathway might TREM2-ApoE, the modulation of the microglial neurodegenerative phenotype acts as a means of restoring homeostatic microglia and treating neurodegenerative disorders.

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