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TRPM2 contributes to LPS/IFNγ-induced production of nitric oxide via the p38/JNK pathway in microglia

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ABSTRACT

Microglia are immune cells that maintain brain homeostasis at a resting state by surveying the environment and engulfing debris. However, in some pathological conditions, microglia can produce neurotoxic factors such as pro-inflammatory cytokines and nitric oxide (NO) that lead to neuronal degeneration. Inflammation-induced calcium (Ca^{2+}) signaling is thought to underlie this abnormal activation of microglia, but the mechanisms are still obscure. We previously showed that combined application of lipopolysaccharide and interferon γ (LPS/IFNγ) induced-production of NO in microglia from wild-type (WT) mice is significantly reduced in microglia from transient receptor potential melastatin 2 (TRPM2)-knockout (KO) mice. Here, we found that LPS/IFNγ produced a late-onset Ca^{2+} signaling in WT microglia, which was abolished by application of the NADPH oxidase inhibitor diphenylene iodonium (DPI) and ML-171. In addition, pharmacological blockade or gene deletion of TRPM2 channel in microglia did not show this Ca^{2+} signaling. Furthermore, pharmacological manipulation and Western blotting revealed that Ca^{2+} mobilization, the proline-rich tyrosine kinase 2 (Pyk2), p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun NH2-terminal kinase (JNK) contributed to TRPM2-mediated LPS/IFNγ-induced activation, while the extracellular signal-regulated protein kinase (ERK) did not. These results suggest that LPS/IFNγ activates TRPM2-mediated Ca^{2+} signaling, which in turn increases downstream p38 MAPK and JNK signaling and results in increased NO production in microglia.

1. Introduction

Microglia are immunocompetent cells in the central nerve system (CNS) that are important for preserving neural tissue [1,2]. Microglia are activated in pathological conditions such as Alzheimer's disease, Parkinson's disease, and neuropathic pain, and are important for both neuroprotection and neurotoxicity [3]. Activated microglia are characterized by their altered morphology, increased proliferation, and excessive production of inflammatory mediators, including cytokines/chemokines, super oxide, and nitric oxide (NO) [1,4]. Because these molecules contribute to neuronal damage during the courses of neurodegenerative diseases, the overactivation and dysregulation of microglia can have progressive neurotoxic consequences [5].

Several researchers have targeted microglia as a potential therapeutic avenue by focusing on the intracellular pathways that are essential for microglial functions [1–5]. One of the prime candidates is the pathway that regulates intracellular calcium concentration ($[Ca^{2+}]_i$). This pathway is important for enzyme and ion channel properties, cytoskeletal structures, and transcriptional regulation during microglial activation [6]. Activation of purinergic receptors, such as the P2X7 receptor, induces tumor necrosis factor (TNF) release in rat culture microglia in a Ca^{2+}-dependent manner [7]. Furthermore, lipopolysaccharide (LPS)-mediated activation of cultured murine microglia results in increases in $[Ca^{2+}]_i$ and production of pro-inflammatory factors, such as TNF and NO, which is significantly suppressed by the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) [8]. These data indicate that extracellular Ca^{2+} influx plays a pivotal role in the pathological activation of microglia. However, the molecular entities responsible for extracellular Ca^{2+} influx have not been fully elucidated. Therefore, investigations into the physiological roles of Ca^{2+}-permeable channels could contribute to the development of useful therapeutic interventions that target abnormal activation of microglia to treat neurodegenerative diseases.

Transient receptor potential (TRP) channels are Ca^{2+}-permeable, nonselective cation channels. Twenty-eight different mammalian TRP genes that comprise six families (TRPC, TRPM, TRPV, TRPA,

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TRPP, and TRPML) have been identified [9]. TRPM2, a member of the TRPM family, has an adenosine 5′-diphosphoribose ribose (ADPR)-recognizing Nudix box motif in the intracellular C-terminal tail [10], and is activated by ADPR [10], reactive oxygen species (ROS) [11] and intracellular Ca^{2+} [12]. TRPM2 is highly expressed in a broad range of tissues, including brain tissue [13], and plays a role in hydrogen peroxide (H_2O_2)-induced Ca^{2+} influx and subsequent neuronal cell death [14]. In addition, TRPM2 is expressed at high levels in immune cells, including monocytes and macrophages, neutrophils, dendritic cells, T lymphocytes and microglia [10,13,15] Previous studies reported that TRPM2-mediated Ca^{2+} influx induces production of cytokines and chemokines in monocytes. This increase in cytokines and chemokines can aggravate inflammation [16,17]. We also recently showed that TRPM2 in microglia plays a role in NO production [18]. However the intracellular signaling mechanisms underlying these phenomena have not yet been clarified. Here, through comparisons of data from wild-type (WT) and TRPM2-KO mice, we provide evidence that LPS/IFNγ-mediated stimulation of microglia results in the induction of TRPM2-mediated Ca^{2+} signaling and increases in downstream p38 mitogen-activated protein kinase (MAPK) and c-Jun NH2-terminal kinase (JNK) signaling. These intracellular changes result in increased NO production in microglia.

2. Materials and methods

2.1. Reagents

Unless otherwise mentioned, all drugs and chemicals were obtained from Nacalai Tesque (Kyoto, Japan). LPS derived from *Escherichia coli* (0111:B4), ADPR, *N*-methyl-D-glucamine (NMDG), BAPTA, diphenylene iodonium (DPI), cremophore EL, sulphanilamide, phosphoric acid, naphthylethlenediamine dihydrochloride, sodium nitrite and phosphate inhibitor cocktail 2 were all obtained from Sigma–Aldrich Chemicals (St. Louis, MO). ML171 was purchased from Tocris Bioscience (R&D Systems; Minneapolis, MN). Rabbit antibodies against p38 MAPK, phospho-p38 MAPK, extracellular signal-regulated protein kinase (ERK), and phospho-ERK, were all purchased from Cell Signaling (Boston, MA). Peroxidase-conjugated goat anti-rabbit IgG was purchased from GE Healthcare (Chalfont St Giles, UK). Immobilon western chemiluminescent horseradish peroxidase (HRP) substrate was purchased from Millipore (Darmstadt, Germany).

2.2. Primary cultured microglia

All animals used in this study were treated in accordance with the guidelines of the Kyoto University Animal Experimentation Committee and the Japanese Pharmacological Society. Microglia cultures were prepared from newborn wildtype (WT) and TRPM2 knockout (TRPM2-KO) C57BL/6 mice (1–2 days). Dissociated cells were seeded on 75 cm^2 flasks in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% FBS (SAFC Biosciences), 5 mg/ml insulin (Biological Industries), and 1% antibiotic and antimycotic solution. The cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere. 2–3 weeks after seeding, the primary mixed glial cultures were shaken at 150 rpm for 90 min. The detached cells were then plated on either dishes or cover glasses for experiments at a density of approximately 1.5×10^5 cells/cm^2.

2.3. RT-PCR and quantitative RT-PCR

PCR amplification and fluorescence detection were performed using a Light Cycler 480 (Roche) with the following sets of primers (from 5′ to 3′ with position 1 being the first base of the start codon): for actin, 5′-ACTGGGACGACATGGAGAAG-3′ and 5′-GAGGCATACAGGGACAGCA-3′; and for mTRPM2, 5′-AAGGAACACAGACAATGCCTG-3′ and 5′-AGGATGGTCTTGTGGTTCGC-3′. The following conditions were used for the amplification process: 95 °C for 1 min; 35 cycles of 94 °C for 40 s, 58 °C for 40 s, and 72 °C for 60 s; 72 °C for 5 min for RT-PCR reactions or 95 °C for 5 min; and then 60 cycles of 95 °C for 10 s, 58 °C for 30 s, and 72 °C for 60 s for quantitative RT-PCR.

2.4. Measurement of $[Ca^{2+}]_i$

TRPM2-mediated increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were estimated by measurements of the Ca^{2+}-sensitive fluorescent dye fura 2 acetoxymethyl ester (AM) (Dojindo) with a fluorescence imaging system (AQUACOSMOS/ORCA-AG, Hamamatsu Photonics). Cells grown on 10 mm diameter coverslips (Matsunami) were incubated in Krebs–Ringer buffer (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.4) containing 5 μM fura-2 AM and 0.005% cremophore EL for 40 min at RT. The cells were then rinsed with buffer and the coverslips were transferred to a recording chamber on the stage of an inverted fluorescence microscope (TE300, Nikon). Fura-2 fluorescence signals were obtained with excitation wavelengths of 340 nm and 380 nm, and emission wavelengths of 500 nm, and were recorded at 10 s intervals. Drug solutions were applied at room temperature by perfusion into the recording chamber.

2.5. Measurement of NO release

Measurement of NO release was performed as previously described [18]. NO production was analyzed by measuring the accumulated levels of its stable metabolite, nitrite, in the microglial culture supernatants using a Griess reagent system (1% sulphanilamide, 2.5% phosphoric acid, and 0.1% naphthylethlenediamine dihydrochloride) for 10 min at room temperature. The absorbance of diazonium compound was measured at 540 nm with a microplate reader (Model 680, Bio-Rad Laboratories). Absolute levels of nitrite were determined by referring to a standard curve that was obtained using defined concentrations of sodium nitrite.

2.6. Western blotting

Immunoblotting analyses were performed on whole-cell lysates, as previously described [19]. Cells grown in 35 mm dishes were harvested and lysed in a buffer (20 mM Tris–HCl, 100 mM NaCl, 1% TritonX-100, 40 mM NaF, 1 mM EDTA, and 1 mM EGTA) supplemented with 1% protease inhibitor cocktail set III (Calbiochem) and 1% phosphatase inhibitor cocktail 2. Aliquots of lysate were diluted with an equal volume of sample buffer (124 mM Tris–HCl, 10% glycerol, 4% SDS, 100 mM dithiothreitol, 0.02% bromophenol blue, pH 6.8) and loaded onto a 10% SDS–polyacrylamide gel. Proteins were blotted onto Immobilon-P PVDF transfer membranes (Millipore). The membranes were exposed to a blocking step and then incubated overnight at 4 °C with a rabbit antibody against phospho-p38 MAPK, p38 MAPK, phospho-ERK, or ERK (1:1000). The following day, the membranes were briefly washed and then incubated with peroxidase-conjugated goat anti-rabbit IgG (1:10,000) for 1 h at RT. Specific bands were then detected with immobilon western chemiluminescent HRP substrate.

2.7. Statistical analyses

All data are expressed as mean ± SEM. Statistical analyses were performed with Student's *t*-tests or with one-way analysis of

variance (ANOVA), followed by Tukey multiple comparison tests. Prism 5 software (Graph Pad) was used for the analyses. Probability values less than 5% ($P < 0.05$) were considered significant.

3. Results

3.1. LPS and IFNγ-induced $[Ca^{2+}]_i$ elevation is abolished in TRPM2-KO microglia

We first examined whether murine cultured microglia expressed functional TRPM2. RT-PCR revealed that TRPM2 mRNA was detectable in microglia isolated from WT but not TRPM2-KO mice (Fig. 1A). Next, to investigate whether TRPM2 is important for LPS (100 ng/ml) and IFNγ (10 ng/ml) (LPS/IFNγ)-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) elevation in microglia, we performed Ca^{2+} imaging experiments. Application of LPS/IFNγ elicited delayed and gradual $[Ca^{2+}]_i$ increases in a large proportion of WT microglia (Fig. 1B, C and E). However, these Ca^{2+} responses were nearly abolished in TRPM2-KO microglia (Fig. 1D and E). Pharmacological blockade of TRPM2 by a TRPM2 antagonist miconazole (10 μM) also suppressed the Ca^{2+} response (Fig. 1F). When the extracellular solution was changed to a 0 mM extracellular Ca^{2+} ($[Ca^{2+}]_o$) solution, the Ca^{2+} response was eliminated (Fig. 1G). TRPM2 is opened by ROS such as H_2O_2 [11], which are mainly produced by NADPH oxidase in microglia. Interestingly, LPS/IFNγ-induced increase in $[Ca^{2+}]_i$ was completely suppressed by the NADPH oxidase inhibitor DPI (10 μM; Fig. 1H) and ML-171 (10 μM; Fig. 1I), suggesting that LPS/IFNγ-evoked $[Ca^{2+}]_i$ increases in microglia are dependent on TRPM2-mediated signaling.

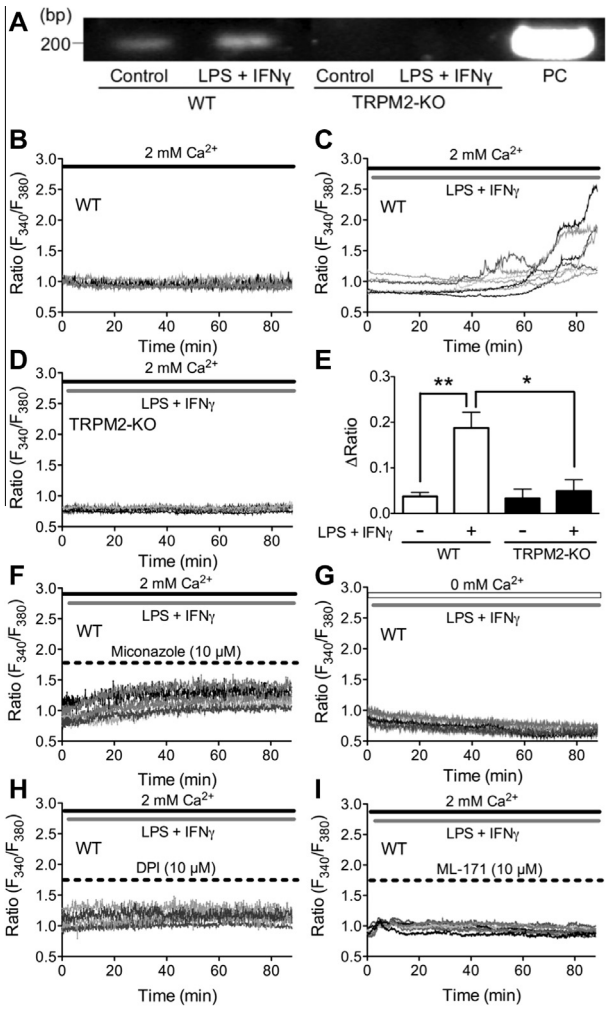

Fig. 1. TRPM2 is functionally expressed in cultured microglia. (A) RT-PCR analysis of cultured mice microglia showing expression levels of TRPM2 mRNA with or without LPS (100 ng/ml) and IFNγ (10 ng/ml). Shown is positive control (PC) mRNA of plasmid-DNA containing mTRPM2 sequences. (B–I) Ca^{2+} imaging experiments of cultured microglia from WT (B, C, F-I) and TRPM2-KO (D) mice. Microglia cultured on glass coverslips were loaded with fura-2 AM and were subjected to 2 mM Ca^{2+} solutions (B–D, F, H, I) or to a Ca^{2+} free solution (G). In panels C, D, F–I, cells were treated with LPS (100 ng/ml) and IFNγ (10 ng/ml). Representative traces of fluorescence ratios obtained from 70–93 cells on one coverslip are shown. Indicated drug applications or manipulations of extracellular Ca^{2+} occurred during the periods indicated by the horizontal bars. Panel E is the quantitative analysis of $[Ca^{2+}]_i$ elevation from 3–5 coverslips of WT microglia (open columns) and TRPM2-KO microglia (filled columns). The ΔFura-2 ratio was estimated by determining the mean maximal increases of $[Ca^{2+}]_i$ observed during the recording periods. $^{**}P < 0.01$, $^{***}P < 0.001$.

3.2. Ca^{2+} chelators and Pyk2 inhibitors suppress LPS/IFNγ-induced NO production via TRPM2

Activated microglia are known to release cytotoxic substances such as NO in response to increased intracellular Ca^{2+} signaling [6]. We previously demonstrated that combined application of LPS/IFNγ robustly increases expression of inducible nitric oxide synthase (iNOS) mRNA and NO release from WT microglia, which were significantly attenuated in TRPM2-KO microglia [18]. Therefore, we next investigated the mechanism underlying the TRPM2-mediated LPS/IFNγ-induced NO accumulation. Simultaneous application of the extracellular Ca^{2+} chelator BAPTA (0.3 and 0.7 mM) resulted in suppression of LPS/IFNγ-induced NO production in WT microglia. However, BAPTA failed to inhibit NO accumulation in TRPM2-KO microglia (Fig. 2A and B). Moreover, we investigated the role of Pyk2 in NO production. Pyk2 is a Ca^{2+}-dependent tyrosine kinase that is activated by $[Ca^{2+}]_i$ elevation through TRPM2 and in turn activates MAPK signaling [16]. Treatment with the Pyk2 inhibitor AG17 (0.1 and 0.3 μM) significantly attenuated LPS/IFNγ-induced NO production only in WT microglia. AG17 treatment did not affect NO production in

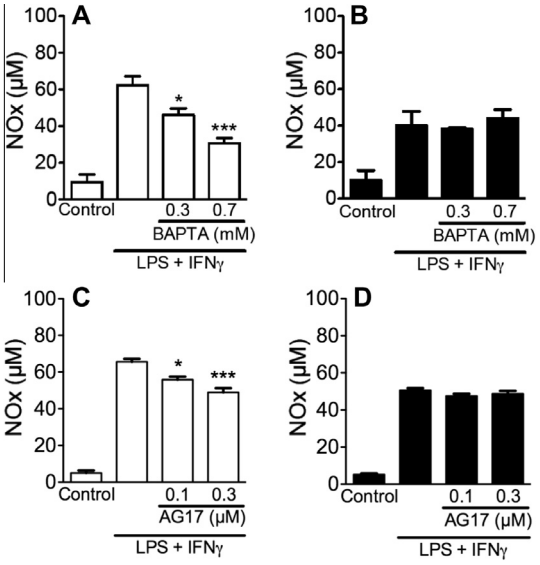

Fig. 2. The effects of Ca^{2+} chelators and Pyk2 inhibitors on LPS/IFNγ-induced NO production in microglia. Cultured WT (A, C) and TRPM2-KO (B, D) microglia were treated with LPS (100 ng/ml)/IFNγ (10 ng/ml) and different concentrations of the extracellular Ca^{2+} chelator BAPTA (A, B) or the Pyk2 inhibitor AG17 (C, D) for 48 h. The concentrations of NO in the culture supernatants were determined by the Griess reaction. Bars represent means ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ relative to LPS/IFNγ alone ($n = 3–5$).

TRPM2-KO microglia (Fig. 2C and D). These results indicate that TRPM2-mediated $[Ca^{2+}]_i$ elevation and subsequent Pyk2 activation are involved in LPS/IFNγ-induced NO production.

3.3. p38 MAPK and JNK inhibitors suppress TRPM2-mediated LPS/IFNγ-induced NO production

MAPKs, a family of serine/threonine kinases, are important for LPS/IFNγ-induced microglial activation [20]. Application of the p38 MAPK inhibitor SB203580 (3, 10 and 30 μM), JNK inhibitor SP600125 (10 and 30 μM), or ERK pathway inhibitor PD98059 (1, 3 and 10 μM) significantly suppressed LPS/IFNγ-induced NO production in WT microglia (Fig. 3A, C and E). However, only PD98059 significantly inhibited LPS/IFNγ-induced NO production in TRPM2-KO microglia (Fig. 3B, D and F). Taken together, these results suggest that TRPM2 recruits the p38 MAPK and JNK pathways, but not the ERK pathway, for LPS/IFNγ-induced NO production.

3.4. TRPM2-mediated signaling is important for LPS/IFNγ-induced p38 MAPK phosphorylation

To determine whether p38 MAPK is activated during TRPM2-mediated augmentation of LPS/IFNγ-induced NO production, we next assessed the phosphorylation of p38 MAPK by immunoblot analyses. Interestingly, LPS/IFNγ evoked p38 MAPK phosphorylation in cultured WT microglia (Fig. 4A). This phosphorylation peaked within 2 h of treatment and then declined. However, LPS/IFNγ did not induce p38 MAPK phosphorylation in TRPM2-KO microglia (Fig. 4A). By contrast, LPS/IFNγ-evoked ERK phosphorylation was observed in lysates from both WT and TRPM2-KO microglia (Fig. 4B). These results indicate that LPS/IFNγ-induced phosphorylation of p38 MAPK is selectively dependent on TRPM2 signaling and that LPS/IFNγ-induced phosphorylation of ERK is independent of TRPM2 signaling.

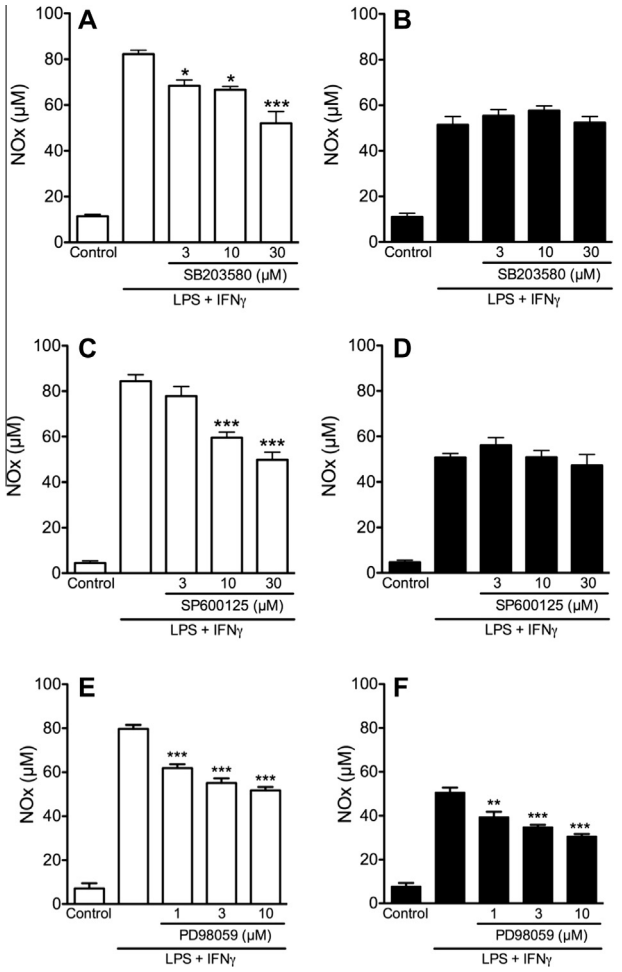

Fig. 3. The effects of p38 MAPK, JNK, and ERK inhibitors on LPS/IFNγ-induced NO production in microglia. Cultured WT (A, C, E) and TRPM2-KO (B, D, F) microglia were treated with LPS (100 ng/ml)/IFNγ (10 ng/ml) and different concentrations of the p38 MAPK inhibitor SB203580 (A, B), the JNK inhibitor SP600125 (C, D), or the MEK inhibitor PD98059 (E, F) for 48 h. NO production was measured by the Griess reaction. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ relative to LPS/IFNγ alone ($n = 3–5$).

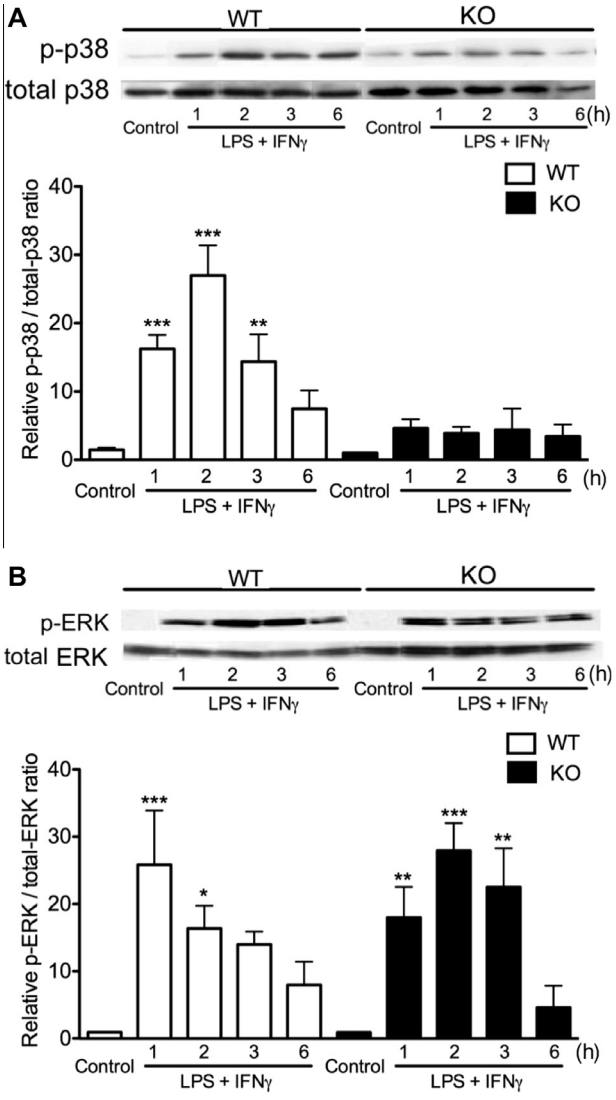

Fig. 4. p38 MAPK and ERK activation are differentially regulated by LPS/IFNγ-induced activation in cultured microglia from WT and TRPM2-KO mice. Cultured microglia were treated with LPS (100 ng/ml) and IFNγ (10 ng/ml) for 1, 2, 3, or 6 h, and were then collected and lysed. Western blot analyses for phosphorylation of p38 MAPK (A) and ERK (B) were performed by using both total and phospho-specific antibodies. The quantitative analyses indicate the ratio of phosphorylated-p38 MAPK or ERK to total p38 MAPK or ERK, respectively. Representative immunoblots are shown in the top panels. Note that phosphorylation of p38 MAPK was markedly suppressed in LPS/IFNγ-treated TRPM2-KO microglia. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ relative to each control ($n = 3–8$).

4. Discussion

In this study, we demonstrated that TRPM2 functions as a non-selective cation channel and that LPS/IFNγ stimulation leads to

TRPM2-mediated extracellular Ca^{2+} influx in cultured microglia. We also determined that activation of TRPM2 results in Pyk2-mediated activation of p38 MAPK and JNK signaling and increased NO production in microglia.

Excessive NO release from activated microglia contributes to systemic brain inflammation and neurodegenerative diseases, such as ischemia, Parkinson's disease and nociception [5]. We previously reported that TRPM2 deficiency in microglia inhibits release of the pro-inflammatory mediators CXCL2 and NO, and subsequently decreases neuropathic pain [18]. Therefore, it is conceivable that TRPM2-KO mice might be resistant to other neurodegenerative diseases that involve excessive NO release.

TRPM2 is a well understood redox-sensitive, Ca^{2+}-permeable cation channel that is directly gated by ADPR [10], nicotinamide adenine dinucleotide (NAD^+) [11], intracellular Ca^{2+} [12]. However, TRPM2 is also gated by mild heat [21,22], H_2O_2 [22] and increases in intracellular Cl^- concentration [23], which indicates that TRPM2 is a multimodal sensor channel. In this study, Ca^{2+} imaging experiments demonstrated that LPS/IFNγ induced a 30 or 60 min delayed and gradual $[Ca^{2+}]_i$ elevation in WT microglia that was absent from TRPM2-KO microglia. These data suggest that TRPM2 is activated downstream of LPS/IFNγ derived signaling. Previous studies showing that TRPM2 functions as a Ca^{2+}-permeable channel downstream of LPS-derived signaling in human monocytes [17] and in mouse bone marrow derived macrophages [24] support the results presented here. Moreover, we found that the NADPH oxidase inhibitor DPI completely suppressed the LPS/IFNγ-elicited increase in $[Ca^{2+}]_i$ in WT microglia, which is reminiscent of previous findings showing that DPI inhibits LPS/IFNγ-induced ROS release and iNOS induction [25]. Therefore, these results suggest that NADPH oxidase-mediated ROS signaling is important for iNOS gene expression. Furthermore, a previous study clearly demonstrated that aggregated amyloid1–42 activates NADPH oxidase in microglia, which in turn results in superoxide release, PGE_2 formation, iNOS expression, and NO production [26]. Therefore, the NO production observed in our experiments may have been caused by released ROS that were derived from NADPH oxidase. Moreover, the multifunctional ectoenzyme CD38, which is widely expressed in hematopoietic cells such as microglia and catalyzes both the formation of cADPR from NAD^+ and the hydrolysis of cADPR to ADPR, helps promote LPS/IFNγ-induced microglial activation [27]. Taken together, it is conceivable that TRPM2 is opened by ROS that are produced by NADPH oxidase, such as H_2O_2, or by oxidative stress that produces NAD^+ in mitochondria, which is then changed into ADPR via CD38 [28]. Based on this hypothesis, we examined whether exogenous H_2O_2 could induce $[Ca^{2+}]_i$ elevation and found that application of H_2O_2 induced a subtle $[Ca^{2+}]_i$ increase that was mediated by TRPM2. H_2O_2 treatment did not induce a massive Ca^{2+} influx similar to what we observed in HEK293 cells stably expressing TRPM2 (data not shown). These results imply the possibility that the native expression levels of TRPM2 protein in the plasma membranes of microglia were relatively low and that TRPM2 may be translocated to plasma membranes in response to LPS/IFNγ stimulation. This hypothesis is supported by a previous report that observed a similar phenomenon when studying chloride intracellular channel 1 (CLIC1). CLIC1 is a microglia chloride channel that is translocated from the cytosol to the plasma membrane within 1 h of amyloid beta treatment [29]. We were unable to test this possibility due to the lack of a commercially available antibody for mouse TRPM2 that is capable of detecting the cellular localization of endogenous TRPM2 protein. Future studies are necessary to determine the detailed cytosolic mechanisms that explain how LPS/IFNγ signaling opens TRPM2 channels in microglia.

In this study, we found that LPS/IFNγ treatment induced TRPM2 activation, which led to Pyk2 activation, and ultimately to p38 MAPK and JNK activations. The contribution of ERK to LPS/IFNγ-induced NO production is crucial but was found to occur independent of the TRPM2-mediated cascade. Yamamoto et al. previously reported that H_2O_2-induced TRPM2 activation, and resultant Pyk2 and Ras activations, lead to specific ERK activation and not to p38 MAPK and JNK activations. In addition, Wehrhahn et al. [17] reported that TRPM2 is required for LPS-induced production of several cytokines and chemokines including TNFα and IL6 in THP-1 cells. We previously demonstrated that microglial TRPM2 was involved in LPS/IFNγ-induced iNOS and CXCL2 mRNA upregulation, but not in IL1β, IL6, and TNFα upregulation [18]. The reasons for these discrepancies are unknown. However, Lee et al. [30] reported that a specific inhibitor of gamma-glutamyl-cysteine synthetase, buthionine sulphoximine (BSO) induces NO production in monocytic THP-1 cells, but not in primary human microglia. These data imply that there are different pathways leading to iNOS expression in monocytes and in microglia. The other possibility is that there may be some differences in the methods. We used primary cultured mouse microglia, while others used macrophages.

In conclusion, this study demonstrated that TRPM2-mediated Ca^{2+} signaling increases LPS/IFNγ-induced NO production through a mechanism involving Pyk2-mediated p38/JNK activation in microglia. Based on these results, TRPM2 is a potential therapeutic target to inhibit excessive microglial activation and neuroinflammation.

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