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Original Article

Comparative Analysis of Protein Tyrosine Phosphatases Regulating Microglial Activation

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Protein tyrosine phosphatases (PTPs) are key regulatory factors in inflammatory signaling pathways. Although PTPs have been extensively studied, little is known about their role in neuroinflammation. In the present study, we examined the expression of 6 different PTPs (PTP1B, TC-PTP, SHP2, MEG2, LYP, and RPTPβ) and their role in glial activation and neuroinflammation. All PTPs were expressed in brain and glia. The expression of PTP1B, SHP2, and LYP was enhanced in the inflamed brain. The expression of PTP1B, TC-PTP, and LYP was increased after treating microglia cells with lipopolysaccharide (LPS). To examine the role of PTPs in microglial activation and neuroinflammation, we used specific pharmacological inhibitors of PTPs. Inhibition of PTP1B, TC-PTP, SHP2, LYP, and RPTPβ suppressed nitric oxide production in LPS-treated microglial cells in a dose-dependent manner. Furthermore, intracerebroventricular injection of PTP1B, TC-PTP, SHP2, and RPTPβ inhibitors downregulated microglial activation in an LPS-induced neuroinflammation model. Our results indicate that multiple PTPs are involved in regulating microglial activation and neuroinflammation, with different expression patterns and specific functions. Thus, PTP inhibitors can be exploited for therapeutic modulation of microglial activation in neuroinflammatory diseases.

Key words: neuroinflammation, protein tyrosine phosphatase, microglia

INTRODUCTION

Microglia are the innate immune cells in central nervous system (CNS). These cells contribute to the homeostasis of the CNS by participating in immune responses as the first-line defense [1]. Microglia also play an important role in the pathophysiology of neuroinflammatory and neurodegenerative diseases. Hypertrophic microglia are one of the hallmarks of brain tissue from patients with Alzheimer's disease, Parkinson's disease, ischemic injury, or multiple sclerosis [2]. These reactive microglia can produce a variety of proinflammatory molecules such as nitric oxide (NO) and TNF-α, which amplify the inflammatory response and have neurotoxic effects. Therefore, inhibition of microglial hyperactivation might be a good strategy to develop therapeutics for neurodegenerative diseases [3].

Recently, we reported a novel role for protein tyrosine phosphatase 1B (PTP1B), which is a member of the protein tyrosine phosphatases (PTPs) family, as a positive regulator of neuroinflammation [4]. We showed that the level of PTP1B expression was increased by inflammatory stimuli, and microglial

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cells overexpressing PTP1B exhibited an enhanced production of NO and proinflammatory cytokines. In addition, a small-molecule inhibitor of PTP1B significantly suppressed the production of proinflammatory cytokines in the brain.

PTPs regulate the tyrosine phosphorylation of many important signaling molecules that are involved in key cellular processes such as cell growth and inflammation. Although more than 100 members of the PTP superfamily have been identified [5], the functional significance of PTPs in neuroinflammation remains largely unknown. Here, we examined the expression of 6 different PTPs (PTP1B, TC-PTP, SHP2, MEG2, LYP2, and RPTPβ) and their role in glial activation and neuroinflammation.

MATERIALS AND METHODS

Cell culture

The immortalized murine microglial BV-2 cell line [6], was maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% heat-inactivated fetal bovine serum (FBS) and 50 μg/ml gentamicin at 37°C. Mouse primary microglial cells were maintained in DMEM containing 10% heat-inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco) at 37°C in a humidified atmosphere with 5% CO_2. All animals and experimental procedures were approved by the Institutional Review Board of Kyungpook National University School of Medicine, and were carried out in accordance with the guidelines in the NIH Guide for the Care and Use of Laboratory Animals. The animals were maintained under temperature- and humidity-controlled conditions with a 12-h light/12-h dark cycle. The mouse primary microglial cultures were prepared using mild trypsinization as previously described, but with minor modifications [7]. In brief, the forebrains of 3~5 day-old C57BL/6 mice were chopped and dissociated through mechanical disruption using a nylon mesh. The cells were seeded into poly-L-lysine-coated flasks. After *in vitro* culture for 10~14 days, the microglial cells were isolated from the mixed glial cultures using mild trypsinization. The mixed glial cultures were then incubated with a trypsin solution (0.25% trypsin, 1 mM EDTA in Hank's balanced salt solution) diluted 1:4 in phosphate-buffered saline (PBS; 150 mM NaCl, 5 mM phosphate, pH 7.4) containing 1 mM $CaCl_2$ for 30~60 min. This procedure resulted in the detachment of an upper layer of astrocytes; the microglia remained attached to the bottom of the culture. The detached layer of astrocytes and the remaining microglia were treated with 0.25% trypsin separately and used for subsequent experiments. The purity of the cultures was greater than 95%, as determined by immunocytochemistry using a rabbit polyclonal anti-Iba-1 antibody (1:1000 dilution; Wako) or anti-GFAP antibody (1:1000 dilution).

Measurement of nitric oxide production

The production of nitric oxide (NO) was estimated by measuring the amount of nitrite, a stable metabolite of NO. The cells were treated with lipopolysaccharide (LPS from E. *coli* 055: B5; Sigma) in the presence or absence of the inhibitors for PTP1B [8, 9], TC-PTP, SHP2, MEG2, LYP, and RPTPβ. At the end of a 24 h incubation period, 50 μl of the cell culture media was mixed with an equal volume of a Griess reagent (0.1% naphthylethylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid) in a 96-well microtiter plate. The light absorbance was read at 540 nm and sodium nitrite was used for a standard curve.

Assessment of cell viability

Cell viability was assessed using a modified 3-(4,5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, as previously described [10]. After LPS treatment for 24 h, either in the presence or absence of pharmacological inhibitors, the culture media was aspirated. MTT (0.5 mg/ml in PBS) was added to cells, which were then incubated at 37°C for 4 h. The resulting formazan crystals were dissolved in DMSO. The absorbance was determined at 570 nm using a microplate reader.

RT-PCR

Total RNA was extracted from the brain tissues or treated cells using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. The reverse transcription (RT) was conducted using the Superscript II reverse transcriptase (Invitrogen) and an oligo (dT) primer. Traditional polymerase chain reaction (PCR) amplification was performed using specific primer sets at an annealing temperature of 55~60°C for 20~30 cycles. PCR was performed using a C1000 Touch Thermal Cycler (Bio-Rad, Richmond, CA, USA). For the PCR product analysis, 10 μl of each PCR reaction was electrophoresed on a 1% agarose gel and detected under ultraviolet light following ethidium bromide staining. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primer sequences were designed based on published cDNA sequences (Table 1).

Immunostaining

For histochemical analysis, mice were transcardially perfused with saline and whole brains were fixed in 4% paraformaldehyde for 72 h. The fixed brains were incubated in 30% sucrose for 72 h, embedded in Optimal Cutting Temperature (O.C.T.) compound (Tissue-Tek), and then cut into 12-μm-thick sagittal sections. The

Table 1. Primers used for RT-PCR

Target genes	Accession number	Forward primer (5'-->3')	Reverse primer (5'-->3')
PTP1B	NM_011201.3	AAGACCCATCTTCCGTGGAC	ACAGACGCCTGAGCACTTTG
TC-PTP	NM_008977.3	GCTGGCAGCCGTTATACTTG	TGGCCAGGTGGTATAATGGA
SHP2	NM_011202.3	TGGTTTCACCCCAACATC	CGTGGGTCACTTTGGACTTG
MEG2	NM_019651.2	CCTGGAATGTGGCTGTCAAG	ATGCTCCCTTCAGCAGGTTT
LYP	NM_008979.2	TTCCTGAACAAAGCCTCACG	GGGAGTTGATTTGGTCCGTT
RPTPβ	NM_001311064.1	AGATCAAGGGTGGGCATT	ATGGGACTATCCGGATTTGG
GAPDH	NM_008084	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

sections were permeabilized with 0.3% Triton X-100 and blocked with 1% BSA and 5% normal donkey serum for 1 h at room temperature. The brain sections were incubated with primary antibodies (rabbit polyclonal anti-Iba-1 (1:500 dilution) at 4°C overnight, followed by an incubation for 1 h at room temperature with secondary antibodies (Cy3-conjugated donkey anti-rabbit IgG; Jackson ImmunoResearch Laboratories). The anti-fade mounting medium containing DAPI (VECTASHIELD, Vector laboratories) was used for mounting and counterstaining. Tiled images of each section were captured with a CCD color video camera (Olympus D70) through a 63x objective lens attached to a microscope (Olympus BX51).

Mouse model of neuroinflammation

LPS was administered intraperitoneally (i.p.) to induce neuroinflammation in mice, as previously described [11]. All experiments were carried out on 9~11-week-old male C57BL/6 mice (25~30 g) supplied by Koatech (Pyongtaec, Korea). To evaluate the expression of PTPs in the brain under an inflammatory condition, LPS was injected i.p. at a dose of 5 mg/kg. The brains were collected 48 h after LPS administration. To assess the effect of PTP inhibitors on neuroinflammation, animals were divided into 8 experimental groups. Group 1 was treated with saline and 0.5% DMSO, group 2 was treated with LPS and 0.5% DMSO, and groups 3~8, were treated with LPS and each inhibitor of PTP1B, TC-PTP, SHP2, MEG2, LYP, and RPTPβ diluted in saline containing 5% propylene glycol. DMSO was included in the vehicle because PTP inhibitors were dissolved in DMSO. LPS (5 mg/kg) was administered i.p. for a single challenge. Inhibitors or vehicle were administered intracerebroventricularly (i.c.v.). For histological analysis, the mice were anesthetized 48 h after the LPS injection, and then transcardially perfused with saline and then with 4% paraformaldehyde. Microglial activation was assessed using Iba-1 staining. At least 3 animals were used for each experimental group. Immunohistological intensity analysis of Iba-1 staining was performed using Image J software (NIH, Bethesda, MD, USA) as previously described [12]. The image was set with a binary threshold of 50% of the background level, and then the particles were converted to a subthreshold image area with a size of 100 to 1000 pixels, which shows the Iba-1-positive activated cells. To count the Iba-1 positive cells, 5 squares (300 × 300 μm) were placed around the injection site in the subthreshold image of 6 independent sections, and the cells in the 5 squares were counted and statistically analyzed. Then, the counted cell number was expressed as cell number per square millimeter (cells/mm^2).

Statistical analysis

All data are presented as mean±SE from 3 or more independent experiments, unless stated otherwise. The statistical comparisons between the different treatments were performed using either a Student's *t*-test or a one-way ANOVA in GraphPad PRISM (Graphpad Software. San Diego, CA, USA) and Excel. To determine the statistical significance of more than 2 groups, the values were compared using a one-way ANOVA followed by a Tukey's multiple comparison test (parametric test) or a one-way ANOVA with a Dunn's test (non-parametric test). For the comparison of 3 groups, the unpaired two-tailed Student's *t*-test was used, followed by a Mann-Whitney correction for non-parametric data.

RESULTS

Expression of PTPs in the inflamed brain

To investigate the role of PTPs in the mouse brain under an inflammatory condition, we first analyzed the mRNA expression of several PTPs. Five non-receptor type of PTPs (subtype NT1-NT4) and 1 receptor type of PTPs were selected in this study (Table 2) because these PTPs were well studied and the specific inhibitors have been developed. For an animal model of neuroinflammation, we used LPS-injected mice. Whole brains were collected 48 h after an i.p. injection of LPS (5 mg/kg). The gene expression levels of PTP1B (also known as PTPN1), TC-PTP (also known as PTPN2, a phosphatase that is highly homologous to PTP1B), SHP2 (also known as PTPN11), MEG2 (also known as PTPN9), LYP (also

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Table 2. The list of PTPs inhibitors used in this study

Chemical name of inhibitors	Target PTPs	Subtype (synonyms)	References
Non-receptor PTP			
(S)-4-(((S)-1-(l2-azanyl)-3-(4-(difluoro(phosphono)methyl)phenyl)-1-oxopropan-2-yl)amino)-3-((S)-3-(4-(difluoro(phosphono)methyl)phenyl)-2-pentadecanamidopropanamido)-4-oxobutanoic acid	Protein tyrosine phosphatase type 1B (PTP1B)	NT1 (PTP-1, PTPN1)	[9]
((4-((S)-3-(((S)-1-amino-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1R,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid	T-cell phosphatase (TC-PTP)	NT2 (PTP-2, PTPN2)	[16]
((4-((S)-3-(((S)-1-(((S)-1-amino-3-(2-(4-hydroxy-3-methoxyphenyl)acetamido)-1-oxopropan-2-yl)amino)-5-(3-iodobenzamido)-1-oxopentan-2-yl)amino)*-6-hydroxy-3-iodo-1-methyl-2-(3-(2-oxo-2-((4-(thiophen-3-yl)phenyl)amino)acetamido)phenyl)-1H-indole-5-carboxylic acid*	Src homology domain2-containing PTP2 (SHP2)	NT3 (SH-PTP2, PTPN11)	[33]
((4-((S)-3-(((S)-1-(((S)-1-amino-3-(2-(4-hydroxy-3-methoxyphenyl)acetamido)-1-oxopropan-2-yl)amino)-5-(3-iodobenzamido)-1-oxopentan-2-yl)amino)-2-(3-bromo-4-methylbenzamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid	Megakaryocyte-PTP2 (MEG2)	NT3 (PTPN9)	[15]
3-((3-Chlorophenyl)ethynyl)-2-(4-(2-(cyclopropylamino)-2-oxoethoxy)phenyl)-6-hydroxybenzofuran-5-carboxylic Acid	Lymphoid specific-tyrosine phosphatase (LYP)	NT4 (LYP)	[14]
Receptor PTP			
2-(3-(2-(3-bromo-5-iodobenzamido)acetamido)phenyl)-6-hydroxy-3-iodo-1-methyl-1H-indole-5-carboxylic acid	Receptor-type tyrosine protein phosphatase beta (RPTPβ)	(PTPRZ1 VE-PTP)	[13]

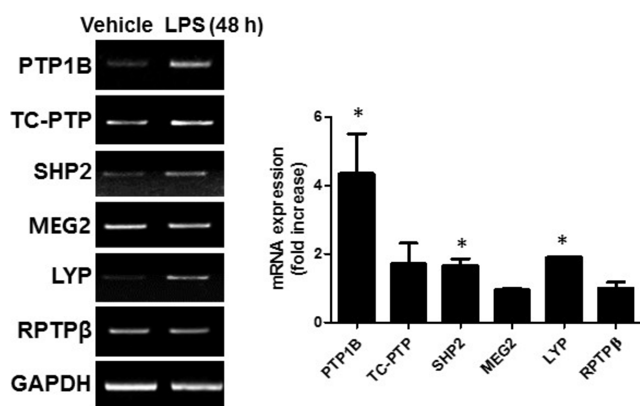

Fig. 1. PTP expression in the mouse brain after LPS injection. The mRNA expression of PTPs in the brain 48 h after LPS injection (5 mg/kg) were measured using RT-PCR. The band intensity from 3~4 independent experiments was measured and normalized to GAPDH expression. *p<0.05 vs. vehicle control.

known as PTPN22) and RPTPβ (also known as RPTPz) were assessed by RT-PCR using gene-specific primers. We found that all PTPs were expressed in the mouse brain. PTP1B expression was increased after 48 h (Fig. 1), which is consistent with our previous report [4]. SHP2 and LYP were slightly increased compared to vehicle control, but LPS stimulation did not alter the expression of the other PTPs (Fig. 1).

Enhancement of PTP1B, TC-PTP, and LYP expression in glial cells following LPS treatment

Because microglia are the resident immune cells in the CNS and participate in the initiation and propagation of an inflammatory response, we examined PTPs expression in primary microglia using RT-PCR. PTP1B, TC-PTP, and LYP, but not MEG2, SHP2, or RPTPβ mRNA levels were increased after 24 h of LPS stimulation (100 ng/ml) (Fig. 2). Similarly, PTP1B, TC-PTP, and LYP mRNA were upregulated after LPS and IFNγ stimulation in primary astrocytes (Fig. 2). Taken together, our results indicate that inflammatory stimuli upregulate several PTPs in glia.

Inhibition of PTPs suppresses microglial inflammatory activation

Microglia are the resident immune cells of the brain, and are part of an important defense mechanism. However, uncontrolled inflammatory activation of microglia has been observed in many neurodegenerative diseases [2]. Activated microglia play a central role in neuroinflammation by secreting various neurotoxic factors, such as nitric oxide (NO). Next, we explored the possibility that PTP inhibitors could reduce microglial activation. To test this hypothesis, we used specific inhibitors of PTPs that we previously developed [9, 13-16]. PTPs share a conserved catalytic domain for phosphatase enzyme activity. Nevertheless, specific inhibitors for

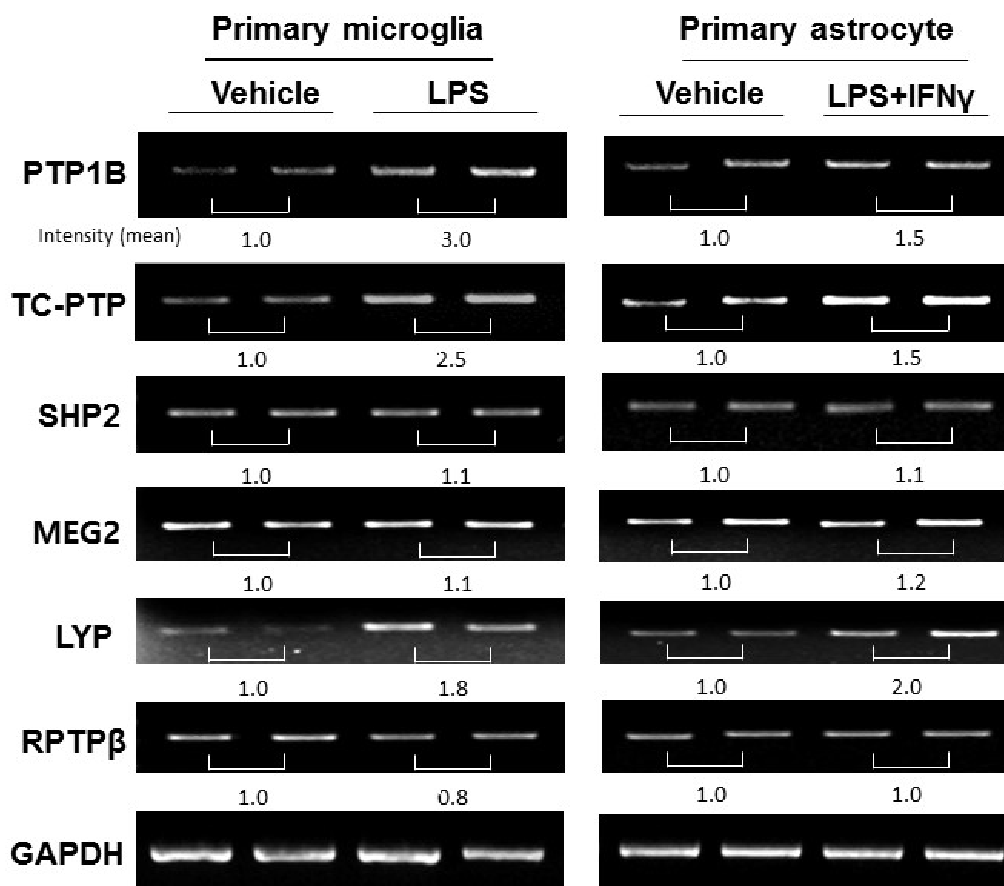

Fig. 2. PTP expression in primary microglia and astrocytes after LPS treatment. The mRNA expression of PTPs in primary microglia and astrocytes 24 h after inflammatory stimulation (LPS treatment (100 ng/ml) for microglia; LPS and IFN-γ (10 U/ml) treatment for astrocytes) were measured using RT-PCR. The quantification of each PTP band intensity is normalized by the band intensity of GAPDH.

PTP1B, TC-PTP, SHP2, MEG2, LYP, and RPTPβ have been shown to be highly specific [8, 9]. Table 2 shows the chemical name and the target protein of the inhibitors used in our study. The levels of NO production were determined in the culture media of the LPS-treated microglial cells that were co-treated with inhibitors at different concentrations. LPS-induced NO levels were decreased by inhibitors for PTP1B, TC-PTP, SHP2, LYP, and RPTPβ in a dose-dependent manner. The IC_{50} value of each PTP inhibitor is listed in Table 3. PTP inhibitors themselves did not alter the basal levels of NO production. No significant cytotoxicity was observed for any of the PTP inhibitors, except that for SHP2 at concentrations of 10 μM, as determined in a MTT assay (Fig. 3 right). It will be interesting to evaluate the effects of these PTP inhibitors on microglial iNOS expression and neuronal loss in LPS-injected mice in the future.

Table 3. Inhibition of LPS-stimulated NO production by PTP inhibitors in microglial cells (IC50, μM)

Target PTPs	NO reduction IC50 (μM)
PTP1B	10.6
TC-PTP	17.68
SHP2	6.08
MEG2	22.92
LYP	1.87
RPTPβ	15.1

BV-2 microglial cells were treated with LPS (100 ng/ml) in the presence of 1, 2, 5 and 10 μM of PTP inhibitors for 24 h. The nitrite content was measured using the Griess reaction.

The PTP inhibitors limit microglial activation in an in vivo neuroinflammation model

Finally, we examined whether the PTPs inhibitors influence microglial activation *in vivo*, which is a hallmark of neuroinflammation [17-21]. The brain tissues were collected and stained with anti-Iba-1 antibody, which is a microglia marker, to evaluate the intensity of Iba-1 staining and microglial morphological changes 48 h after i.p. injections of LPS (Fig. 4A). LPS significantly increased the number of Iba-1-positive activated microglia (Fig. 4D). The inhibition of PTP activity via i.c.v. injection of PTP1B, and RPTPβ inhibitors significantly reduced LPS-induced microglial activation in the hippocampus and cortex (Fig. 4C and D). TC-PTP inhibitors reduced LPS-induced microglial activation in the cortex, and SHP2 reduced microglial activation in the

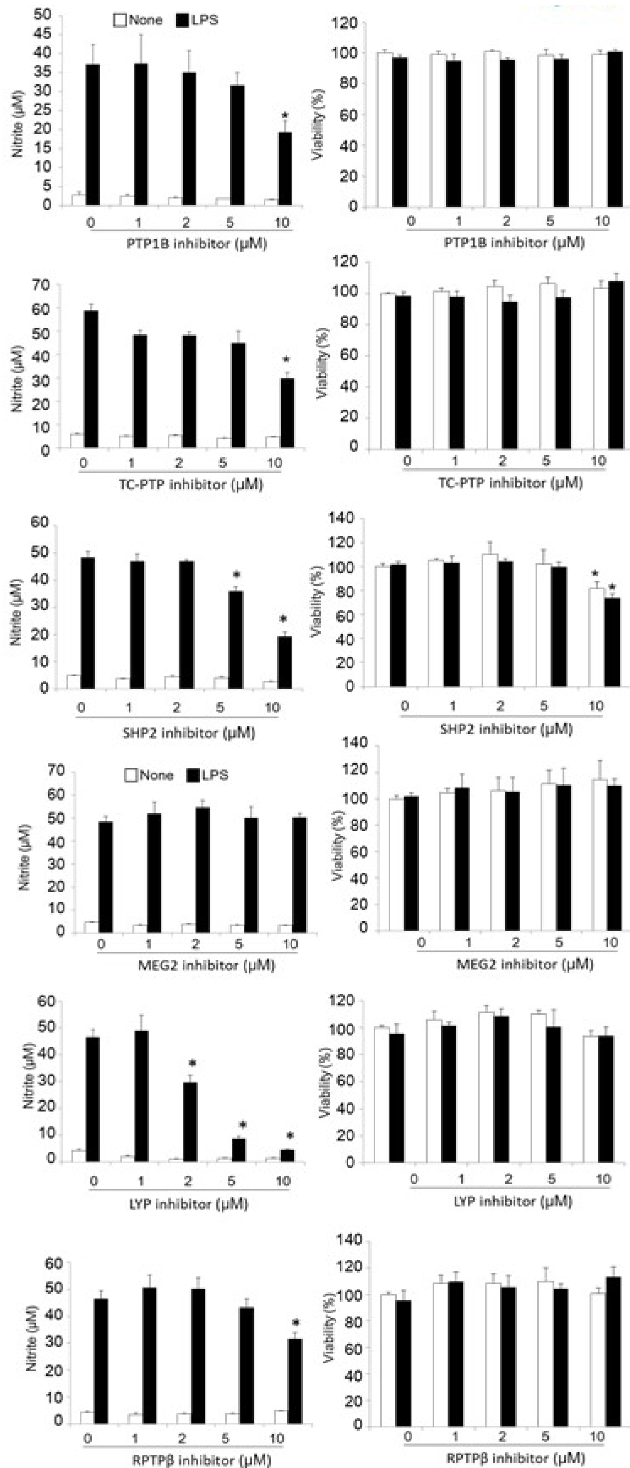

Fig. 3. PTP inhibitors suppress LPS-induced NO production in microglial cells. BV-2 microglial cells were treated with LPS (100 ng/ml) in the presence of the indicated concentration of a PTP inhibitors for PTP1B, TC-PTP, SHP2, MEG2, LYP, and RPTPβ for 24 h. The nitrite content was measured using the Griess reaction (left panel), and the cytotoxicity of the PTP inhibitors was assessed in a MTT assay (right panel). The data were expressed as the mean±SEM (n=3). *p<0.05 vs. LPS only; analyzed using one-way ANOVA with Tukey's multiple comparison test.

hippocampus. As shown in Fig. 4B, microglial morphological change was observed after LPS injection. The number of activated microglia was quantified after delivery of the PTP inhibitors. Taken together, our results show that inhibiting PTP1B, TC-PTP, SHP2, and RPTPβ activity under inflammatory conditions attenuated microglial inflammatory activation *in vitro* and *in vivo*.

DISCUSSION

We demonstrated that all of the PTPs that were examined in this study were expressed in microglia, and the inhibition of the activity of several PTPs decreased LPS-induced microglial activation in an *in vitro* cellular model. We further showed that i.c.v. administration of small-molecule inhibitors of PTP1B, TC-PTP, SHP2, and RPTPβ attenuated the LPS-induced microglial activation in a mouse neuroinflammatory model.

Neuroinflammation is thought to be a promising therapeutic target for acute brain injury and chronic neurodegenerative diseases such as traumatic brain injury and Alzheimer's disease. The quiescent and resting microglia can be activated by different inflammatory stimuli. Microglial activation is often divided into 2 functional states of polarization, namely, a M1-type (classical/inflammatory activation) and a M2-type (alternative/anti-inflammatory activation) [22]. In the present study, we focused on the classical activation state of microglia, which might be induced by a proinflammatory response such as LPS exposure. Excessive production of NO and proinflammatory cytokines in activated microglia that are stimulated by inflammatory signals have been implicated with the pathogenesis of several neurodegenerative diseases [23]. The activation of microglia also involves changes in their morphology (reviewed in [2]). Inflammatory reactions result in a thicker, less branched, and hypertrophic morphology of microglia. These morphological changes, which occur during the transition from a typical resting state to an activated phenotype, are associated with an increased production of neurotoxic proinflammatory mediators such as NO and TNFα [1, 24]. In our neuroinflammatory model, the number of activated microglia was dramatically increased by LPS, and this LPS-induced microglial activation was attenuated by PTP inhibitors in the hippocampus and cortex.

Recently, PTP1B has been reported to be an important proinflammatory molecule in the brain [4]. In the present study, we showed that other PTPs also have important roles in NO production in microglia. In addition, specific inhibitors of PTP1B, TC-PTP, and RPTPβ have an inhibitory effect on microglial activation in a mouse neuroinflammatory model. PTPs represent a super-family of enzymes that play essential roles in normal

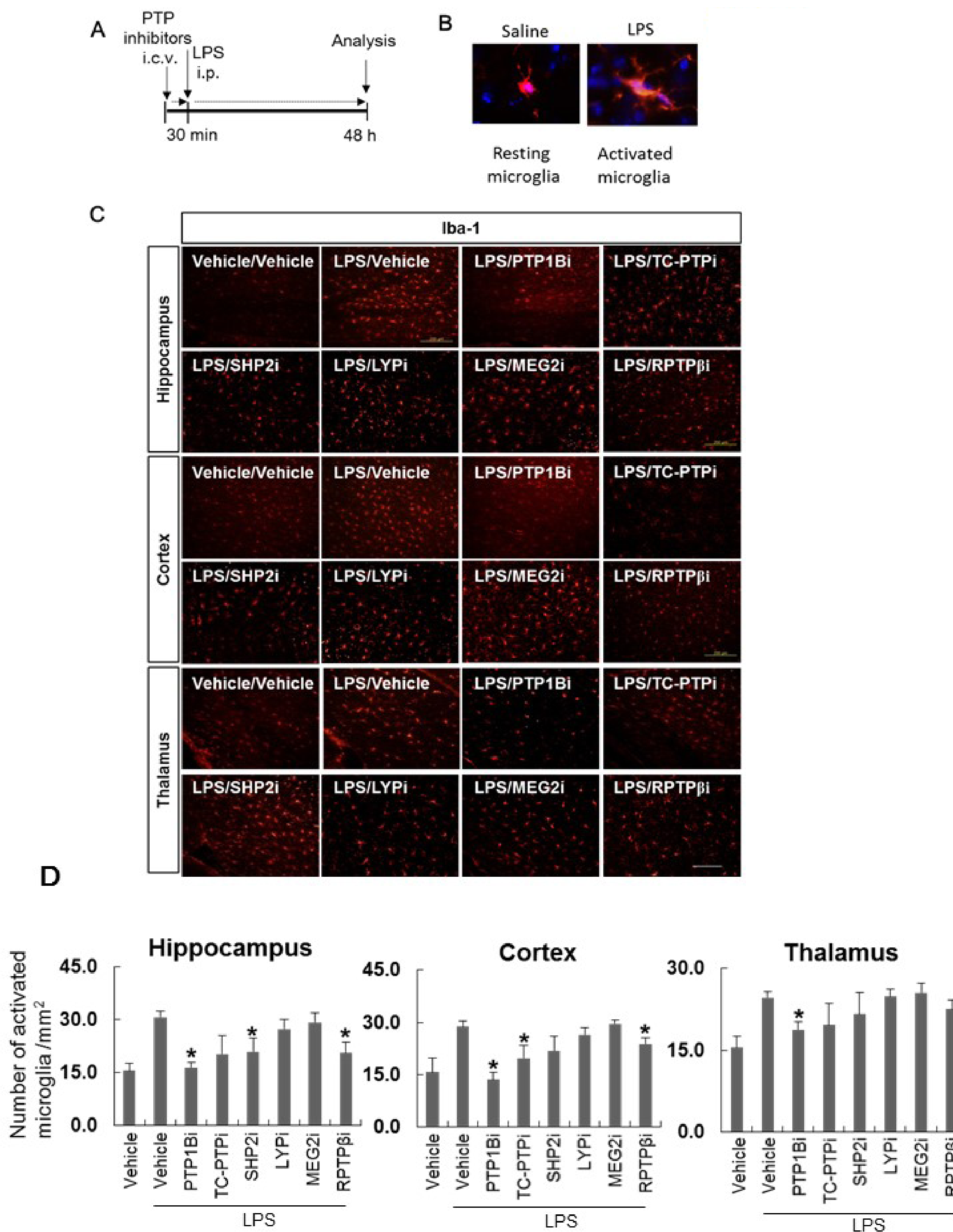

Fig. 4. PTP inhibitors suppress microglial activation in a mouse neuroinflammation model. (A) C57BL/6 mice were injected i.c.v. with vehicle (saline containing 0.5% DMSO and 5% propylene glycol) or PTP inhibitors (diluted in saline containing 5% propylene glycol). At 30 min after the injection of inhibitors for PTP1B, TC-PTP, SHP2, MEG2, LYP, and RPTPβ, mice were injected i.p. with LPS (5 mg/kg). The mice were anesthetized and transcardially perfused with ice-cold saline 48 h after the LPS injection. (B) Representative pictures of resting microglia and activated microglia stained with anti-Iba-1 antibody (a marker for microglia). (C) The brains were removed and the sections were stained with Iba-1 antibody. Iba-1-positive cells were observed in the mouse hippocampus, cortex, and thalamus. Inhibitors were indicated as PTP1Bi, TC-PTPi, SHP2i, MEG2i, LYPi, and RPTPβi. Scale bar, 200 μm. (D) The graph shows activated microglial cell number per mm^2. *p<0.05 vs. LPS and vehicle. More than three brain sections were examined for each experimental group.

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development and physiology. The reversible phosphorylation of a protein is one of the most powerful ways to orchestrate the function of proteins in a cellular system. PTPs have been suggested as important regulators of inflammatory pathways [25, 26]. Imbert et al. reported that pervandate, which is a PTP inhibitor, stimulated the downstream events of the T-cell activation process, including the induction of NF-kB activity [27]. Their study demonstrated a direct connection between PTPs and NF-kB activation through tyrosine phosphorylation of IkB. Among PTPs, PTP1B has emerged as a key player that regulates LPS-induced NF-kB signaling. Our group previously reported that overexpression of PTP1B in cell cultures increases the level of LPS-induced proinflammatory cytokine production, while an siRNA-induced reduction in PTP1B levels inhibits the LPS-induced microglial activation [4] Moreover, a PTP1B-specific inhibitor reduced neuroinflammation in a mouse model. These data implicate PTP1B (and possibly other PTPs) as an attractive target for the treatment of inflammatory brain diseases.

Other PTPs are also associated with inflammation. Levels of TC-PTP peak in response to IFN-γ treatment in THP-1 monocyte cells. The loss of TC-PTP potentiates the IFN-γ-induced phosphorylation of both of the STATs and p38 [28]. The overexpression of SHP2 was observed in rheumatoid arthritis, and knockdown of SHP2 reduced the invasion and adhesion of fibroblast-like synoviocytes. In addition, a signaling pathway that responds to inflammatory cytokines was impaired after SHP2 knockdown, implicating SHP2 as a candidate therapeutic target for inflammatory diseases [29]. A gain-of-function mutation for LYP is related to rheumatoid arthritis, suggesting its function in inflammation, which is consistent with our results [30]. Moreover, clinical use of the newly discovered LYP inhibitor has been proposed for treating a wide range of autoimmune disorders [14].

MEG2 was originally cloned from a megakaryocytic cell line. MEG2 binds to several phosphoinositides and to phosphatidylserine. In addition, MEG2 is implicated in the regulation of homotypic vesicle fusion in hematopoietic cells and events leading to phagocytosis. Based on these known functions of MEG2, we expected it to play an important role in microglia. However, the MEG2 inhibitor showed no protective effects against LPS-induced inflammation *in vitro* or *in vivo*.

The role of PTPs in neuronal development and CNS diseases has not been thoroughly studied. Nevertheless, RPTPβ is known to play a negative a role in oligodendrocyte differentiation in demyelinating CNS diseases [31]. Recently, it was shown that inactivation of RPTPβ by an inhibitory ligand promotes remyelination through activation of oligodendrocyte precursor differentiation [32]. Moreover, the RPTPβ inhibitor AKB9785 decreased inflammation in a sepsis model.

There are currently few examples of a direct association of PTPs with neuroinflammatory diseases, and clearly, the credibility of these enzymes as therapeutic targets requires substantial and continued validation. As a prerequisite for the clinical development of therapeutics targeted to PTPs, it is important to precisely determine the cellular and *in vivo* effects of small-molecule PTPs inhibitors. Unfortunately, as a consequence of the conserved nature of the PTP active sites (i.e., pTyr binding sites), there are currently only a few PTP-specific inhibitors that exhibit the potency and specificity required for biological and pharmacological investigation. On the basis of the observation that PTP substrate recognition requires both pTyr and its adjacent flanking residues [9, 14], we have focused on a strategy for developing bidentate PTP inhibitors that bind to both the active site and a unique adjacent peripheral site. Using this approach, we have obtained several small-molecule inhibitors, which were tested using the LPS-induced neuroinflammation model and microglia cell lines in the present study. Our results suggest that small-molecule inhibitors for PTP1B, TC-PTP, SHP2, and RPTPß can serve as anti-inflammatory agents, validating the notion that small-molecule inhibitors can be used as anti-neuroinflammation therapeutics. Since PTP1B and several PTPs are also involved in the regulation of microglial activation pathways, these potent and specific PTPs inhibitors should be useful reagents in helping to define the role of PTPs in the normal physiology of microglia as well as neuroinflammation.

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