Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase-Dependent Activation of Phosphoinositide 3-Kinase and p38 Mitogen-Activated Protein Kinase Signal Pathways Is Required for Lipopolysaccharide-Induced Microglial Phagocytosis

Hu-Nan SUN,^{a,c} Sun-Uk KIM,^a Mi-Sook LEE,^b Sang-Keun KIM,^c Jin-Man KIM,^d Mijung YIM,^e Dae-Yeul YU,^{*a} and Dong-Seok LEE^{*f}

^a Disease Model Research Center, Biological Resource Center, KRIBB; Daejeon 305–806, Republic of Korea; ^b Department of Bioimaging, Korea Basic Science Institute; Chuncheon 200–701, Republic of Korea; ^c College of Veterinary Medicine, Chungnam National University; Daejeon 305–764, Republic of Korea; ^d Department of Pathology, College of Medicine, Chungnam National University; Daejeon 301–747, Republic of Korea; ^e College of Pharmacy, Sookmyung Women's University; Seoul 140–742, Republic of Korea; and ^f College of Natural Sciences, Kyungpook National University; Daegu 702–701, Republic of Korea. Received April 14, 2008; accepted June 16, 2008; published online June 20, 2008

The importance of microglial reactive oxygen species (ROS) signaling in neuroinflammatory processes has been well demonstrated; however, relatively little is known regarding the related mechanisms underlying these processes. Here, we show that ROS-dependent signal pathways that govern microglial phagocytosis are highly dependent upon nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) activation. Specifically, phagocytosis was greatly reduced by both antioxidant and Nox inhibitor treatments in lipopolysaccharide (LPS)-stimulated BV-2 microglia. Additionally, there was a marked reduction in intracellular ROS content. These results suggest that Nox is the main ROS source for LPS-induced microglial phagocytosis. More decisive evidence for the involvement of ROS in phagocytosis was obtained from an examination of phosphatidylinositol 3-kinase (PI3-K) and p38 mitogen-activated protein kinase (MAPK) signal pathway activation under reduced ROS levels. These two kinases were activated by LPS treatment and inhibited by ROS neutralization and Nox inhibition. We conclude that microglial phagocytosis requires ROS-dependent PI3-K and p38 MAPK activation and that Nox-derived ROS functions as an upstream regulator of both PI3-K and p38 MAPK. These findings will provide a fundamental basis for a therapeutic modality in inflammation-mediated neurodegenerative diseases.

Key words reactive oxygen species; microglial phagocytosis; phosphatidylinositol 3-kinase; nicotinamide adenine dinucleotide phosphate oxidase; p38 mitogen-activated protein kinase

Microglial cells are the resident immune cell population in the central nervous system (CNS). They are commonly activated in response to variety of neurodegenerative and neuroinflammatory conditions in conjunction with the release of proinflammatory factors, reactive nitrogen species, and reactive oxygen species (ROS). ROS has been characterized as an important secondary messenger and modulator for various mammalian intracellular signaling pathways,^{1–3} including neuroinflammatory processes.^{4,5} Therefore, both ROS generation and its respective neutralizing system may modulate the molecular mechanisms governing microglial function.

Phagocytosis is a representative microglial function for immune surveillance in the CNS. Microglia engulf unnecessary and pathogenic substances, including microbes, pathological proteins, apoptotic cells, and cellular debris, to separate them from normal tissues. During phagocytosis, phagosomes that contain a variety of ROS, including $\cdot\text{OH}$ and H_2O_2 that are derivatives of O_2^- generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox), actively participate in the modulation of signaling pathways involving microglial phagocytosis. Phosphatidylinositol 3-kinase (PI3-K) and mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK), are the representative ROS-related signal kinases and are activated during $\text{Fc}\gamma$ receptor-mediated phagocytosis.^{6,7} Although the kinases are proposed as a regulator of Nox activation pathway,^{8,9} most crucial evidences for involvement of

redox signaling cascades in microglial function was obtained from studies for the kinase activation by Nox-derived ROS.^{10,11} Therefore, Nox downstream redox signaling is considered to be a potent modulator of phagocytosis-related pathways in activated microglia. However, Nox-dependent redox signaling pathways for phagocytosis is largely unknown in microglia.

Lipopolysaccharide (LPS) is a common inflammogen used to activate microglia in several mammalian model systems. LPS stimulation triggers a diverse array of microglial responses such as inflammatory cytokine production and phagocytosis, which further lead to the activation of a variety of signaling cascades in phagocytes that pass through Nox and MAPKs.^{11,12} Accordingly, LPS is a useful stimulant for understanding the relationships between oxidative stress and phagocytosis in neuroinflammation. From the investigations for LPS signaling in phagocytes, reduction of oxidative stress or neutralization of ROS has been suggested as an essential process for reduction of inflammatory damages,^{13,14} including microglia-mediated neuroinflammation.¹⁵ However, relatively little is known about the coupled mechanisms between ROS-dependent signaling pathways and LPS-mediated phagocytosis in microglia.

Here, we demonstrate that ROS is a key modulator of microglial phagocytosis *via* Nox/PI3-K signal pathways. PI3-K activation was dependent on ROS generated by Nox. Furthermore, the neutralization of ROS ablated Nox/PI3-K-dependent phagocytosis in LPS-stimulated BV-2 microglial cells.

* To whom correspondence should be addressed. e-mail: lee1@knu.ac.kr; dyu10@kribb.re.kr

These findings provide a potential molecular mechanism for oxidative stress-related neuroinflammation and may be useful in designing therapeutic strategies.

MATERIALS AND METHODS

Chemicals Lipopolysaccharide (LPS, from *Escherichia coli* serotype 0111:B4) and NAC (*N*-acetyl-L-cysteine) were obtained from Sigma (St. Louis, MO, U.S.A.). Signaling inhibitors for p38 MAPK (SB203580), MEK (PD98059), JNK (SP600125), PI3-K (LY294002), Nox (apocynin), were from Calbiochem (U.S. and Canada).

Cell Culture BV-2 cells, which are immortalized microglial cells, were propagated in Dulbecco's modified Eagles medium (DMEM; Invitrogen) that contained 10% fetal bovine serum (FBS; HyClone, Logan, UT, U.S.A.) and antibiotics. Exponentially growing BV-2 cells were pretreated with 10 mM of *N*-acetyl-L-cysteine (NAC) or signal inhibitors, including 0.1–0.5 μ g/ml of apocynin, 5 μ g/ml of SB203580, 5 μ g/ml of SP600125, and 5 μ g/ml of PD98059, and followed by 1 μ g/ml of LPS. The BV-2 cells were kindly provided by Dr. Jau-Shyong Hong (NIEHS, NC, U.S.A.).

Fluorescent Microscopy for Microglial Phagocytosis BV-2 cells were grown on 0.1% gelatin coated-coverslip and treated with 1 μ g/ml of LPS. Alexa 488-conjugated *Escherichia coli* (Ec-A) BioParticles (Invitrogen) were sonicated, added into the culture medium without serum at the final time of LPS treatment, and incubated in 37 °C, 5% CO₂ for 15 min. The cells were washed with Ca²⁺ and Mg²⁺-free PBS (–) for 3 times and fixed with 2% paraformaldehyde for 30 min. After 2 times washing with PBS (–), the cells were mounted onto clean glass-slide and observed under fluorescence microscope (Zeiss).

Western Blotting Analysis Thirty micrograms of protein lysates were separated on 12% sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose membranes (Millipore, Bedford, MA, U.S.A.). The membranes were primarily blotted with polyclonal rabbit antibodies against pAKT (Santa Cruz), p-p38 MAPK (Santa Cruz) and α -tubulin (Sigma) at 4 °C overnight. The membranes were washed five times with 10 mM Tris-HCl (pH 7.5) containing 150 mM NaCl (Tris-buffered saline, TBS) and 0.2% Tween-20, and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma) or anti-mouse IgG (Sigma) for 1 h at room temperature. After the removal of excess antibodies by washing with TBS, specific binding was detected using a chemiluminescence detection system (Amersham, Berkshire, U.K.) according to the manufacturer's instructions.

Flow Cytometry for Phagocytosis Analysis and ROS Determination The microglia phagocytosis was analyzed with flow cytometry according to the method as previously reported.¹⁶⁾ Briefly, Alexa 488-conjugated *E. coli* (Ec-A) BioParticles (Invitrogen) were sonicated, added into the culture medium without serum at the final time of LPS treatment, and incubated in 37 °C, 5% CO₂ for 15 min. After incubation, the cells were washed with PBS for 3 times and suspended the cells with 500 μ l of PBS. The internalized fluorescence was immediately determined from 10000 cells by FACSscan (BD). For the determination of ROS level in microglia, BV-2 cells were incubated with 10 mM of CM-

H₂DCFDA (Invitrogen), a fluorescence-based ROS indicator, at 37 °C for 15 min at the final time of various treatments, and DCF fluorescence intensities were analyzed from 10000 cells by FACSscan.

RESULTS

ROS Involvement in LPS-Mediated Microglial Phagocytosis ROS-dependent phagocytosis has been frequently studied in mammalian phagocytes, whereas microglial cells, especially BV-2 cells, were rarely used in the experiments. Thus, we primarily tried to examine whether LPS-mediated phagocytosis can be modulated by ROS in BV-2 microglial cells. For measurement of phagocytosis, Alexa 488-conjugated *E. coli* (Ec-A) BioParticles were incubated at the end of LPS treatment and analyzed by both fluorescence microscopy and flow cytometry (Fig. 1). In the current study, the microglial phagocytosis was efficiently measured from LPS treatment for 24 h (data not shown). Thus, the conditions for phagocytosis analysis were used throughout the experiments. Under a fluorescence microscope, we found that fluorescence of Ec-A BioParticles was internalized in the cytoplasm of BV-2 microglia and detected dominantly in LPS-stimulated microglia (Fig. 1A, right panels) as compared with control cells (Fig. 1A, left and middle panels), indicating LPS-dependent phagocytosis. For flow cytometry of phagocytosis, LPS-stimulated BV-2 cells were incubated with Ec-A BioParticles, trypsinized, and subjected to FACS analysis (Fig. 1B). The fluorescence intensity for Ec-A BioParticles was increased in BV-2 microglial cells by LPS treatment.

We also investigated whether ROS are involved in microglial phagocytosis. To reduce the intracellular ROS content during microglial activation, BV-2 cells were treated with LPS in the presence of *N*-acetyl-L-cysteine (NAC), which is a chemical antioxidant (Fig. 1C). The NAC treatment effectively reduced the ROS level within LPS-stimulated microglia. Under the more reduced condition facilitated by antioxidant treatment, BV-2 cells engulfed fewer fluorescent Ec-A BioParticles as compared with cells treated only with LPS (Fig. 1D). These results suggest the active involvement of ROS in microglial phagocytosis processes.

Participation of ROS Generated by NADPH Oxidase in Microglial Phagocytosis NADPH oxidase (Nox) has been characterized as the main source of intracellular ROS during microglial activation and is activated in a variety of microglial activation processes. Thus, we investigated whether ROS generated by Nox participates in the modulation of microglial phagocytosis. Apocynin is a well-known Nox inhibitor; treatment with apocynin effectively reduced intracellular ROS levels (Fig. 2A) in LPS-stimulated BV-2 cells. To further examine the effects of Nox inhibition and the resulting reduced ROS levels on microglial phagocytosis, BV-2 cells were treated with LPS in the presence or absence of either apocynin or NAC and then subjected to flow cytometry to measure the engulfed Ec-A BioParticles. Treatment with apocynin and NAC resulted in a reduction in phagocytosis in LPS-stimulated BV-2 microglia (Figs. 2B, C). Thus, microglial phagocytosis requires Nox-driven ROS generation.

The Essential Role of PI3-K and p38 MAPK Signal Pathways in Microglial Phagocytosis To examine PI3-K activation during microglial activation, BV-2 cells were

Fig. 1. Antioxidants Attenuate Phagocytosis in LPS-Stimulated BV-2 Microglia

(A) Fluorescence microscopy for the detection of phagocytosed fluorescent particles in LPS-stimulated microglia. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS for 24 h, incubated with 10 ng/ml Ec-A BioParticles for 15–30 min, and photographed under bright (upper panels) and fluorescence (middle panels) fields. Internalization is presented in merged images (lower panels). The scale bar is 20 μm . (B) Flow cytometry for the detection of phagocytosed fluorescent particles in LPS-stimulated microglia. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS for 24 h, incubated with 10 ng/ml Ec-A BioParticles for 15 min, and analyzed by FACSscan. The histogram is representative of three independent experiments. (C) Effects of antioxidants on ROS generation in LPS-stimulated microglia. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS in the presence or absence of 10 μM NAC for 24 h, incubated with 10 μM CM-H₂DCFDA for 15 min, and analyzed by flow cytometry. The histogram is representative of three independent experiments. (D) Effect of antioxidant on LPS-mediated phagocytosis. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS in the presence or absence of 10 μM NAC for 24 h and subjected to flow cytometry to measure engulfed Ec-A BioParticles.

Fig. 2. Nox Generates Intracellular ROS Involved in Microglial Phagocytosis

(A) Effect of a Nox inhibitor on intracellular ROS generation in LPS-stimulated microglia. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS in the presence or absence of 0.1–0.5 $\mu\text{g}/\text{ml}$ apocynin for 24 h, incubated with either 10 ng/ml Ec-A BioParticles or 10 μM CM-H₂DCFDA for 15 min, and analyzed by flow cytometry. The histogram is representative of three independent experiments. (B) Effects of a Nox inhibitor on LPS-mediated microglial phagocytosis. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS in the presence or absence of 0.1–0.5 $\mu\text{g}/\text{ml}$ apocynin for 24 h, incubated with 10 ng/ml Ec-A BioParticles for 15 min, and analyzed by flow cytometry. The histogram is representative of three independent experiments. (C) Quantitative analysis of the effects of antioxidants and a Nox inhibitor on LPS-mediated microglial phagocytosis. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS in the presence or absence of 100 ng/ml apocynin or 10 μM NAC for 24 h, incubated with 10 ng/ml Ec-A BioParticles for 15 min, and analyzed by flow cytometry. The intensity of phagocytosed fluorescent particles was analyzed densitometrically. The results were from three independent experiments (* p < 0.5 and ** p < 0.01).

Fig. 3. Inhibition of the PI3-K Signaling Pathway Ablates Microglial Phagocytosis

(A) Time course of PI3-K activation in LPS-stimulated microglia. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS, harvested at the indicated time, and subjected to Western blotting with anti-phospho-AKT (pAKT) antibody. The band intensity of pAKT was analyzed densitometrically. The error bars indicate the mean $\pm$ S.D., n = 3. (B) Time course of MAPK activation in LPS-stimulated microglia. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS, harvested at the indicated time, and subjected to Western blotting with anti-phospho-antibodies for p38, ERK1/2, and JNK. (C) Effects of p38 MAPK and PI3-K inhibitors on LPS-mediated microglial phagocytosis. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS in the presence or absence of either 5–10 $\mu\text{g}/\text{ml}$ LY294002 or 3–5 $\mu\text{g}/\text{ml}$ SB203580 for 24 h, incubated with 10 ng/ml Ec-A BioParticles for 15–30 min, and analyzed by flow cytometry. The histogram is representative of three independent experiments. (D) Quantitative analysis of the effects of signaling inhibitors on LPS-mediated microglial phagocytosis. BV-2 microglia were stimulated with 1 $\mu\text{g}/\text{ml}$ LPS in the presence or absence of signaling inhibitors (5–10 $\mu\text{g}/\text{ml}$ LY294002, 3–5 $\mu\text{g}/\text{ml}$ SB203580, 10 $\mu\text{g}/\text{ml}$ SP600125, or 10 $\mu\text{g}/\text{ml}$ PD98059) for 24 h and subjected to flow cytometry. The intensity of phagocytosed fluorescent particles was analyzed densitometrically. The results were from three independent experiments (* p < 0.5 and ** p < 0.01).

Fig. 4. The Activation of the PI3-K and p38 MAPK Signal Pathways is Dependent on Nox-Driven ROS

(A) Effects of ROS reduction and Nox inhibition on the activation of PI3-K signaling pathways. BV-2 microglia were stimulated with 1 μ g/ml LPS, harvested at the indicated time, and subjected to Western blotting with anti-phospho-antibodies for AKT and p38. (B) Quantitative analysis of the effects of antioxidant and Nox inhibitors on PI3-K and p38 MAPK activation in LPS-stimulated microglia. The band intensities of pAKT and p-p38 MAPK shown in (A) were analyzed densitometrically. The results were from two independent experiments (* $p < 0.05$).

treated with LPS, harvested, and subjected to Western blotting with anti-phosphorylation antibodies for AKT, p38 MAPK, ERK1/2, and JNK. Phosphorylation of AKT (pAKT) was increased by LPS treatment for 6 h in a time-dependent manner. This increase disappeared by 12 h post-treatment in BV-2 cells (Fig. 3A). Phosphorylations of p38 MAPK, ERK1/2, and JNK were also increased by LPS treatment; the most prominent activation was found between 1 and 3 h after LPS treatment.

To determine the effects of PI3-K and MAPK inhibition on LPS-mediated microglial phagocytosis, BV-2 cells were treated with LPS in the presence or absence of the signaling inhibitors LY294002 for PI3-K, SB203580 for p38 MAPK, SP600125 for ERK1/2, and PD98059 for JNK. The internalization of Ec-A BioParticles was analyzed by flow cytometry. The LPS-mediated internalization of Ec-A BioParticles was reduced by LY294002 and SB203580 (Figs. 3C, D), but not by SP600125 or PD98059. The most notable inhibition of phagocytosis was found using LY294002. Thus, phagocytosis in LPS-treated BV-2 cells is mediated largely by the PI3-K signaling pathway and more mildly by the p38 MAPK signaling pathway.

PI3-K and p38 MAPK Act as Downstream Regulators of Activated Nox-Dependent Microglial Phagocytosis

We have demonstrated that Nox, PI3-K, and p38 MAPK are involved in LPS-induced microglial phagocytosis. To determine the specific relationships between Nox-mediated ROS generation and signaling pathways passing through PI3-K and p38 MAPK, BV-2 cells were stimulated by LPS in the presence or absence of either NAC or apocynin and subjected to western blotting with anti-pAKT and p-p38 antibodies. The intensities of immunoreactive bands for pAKT and p-p38 MAPK were greatly reduced by co-treatment with either NAC or apocynin as compared with cells treated with LPS only (Fig. 4). These results suggest that Nox-driven ROS generation is essential for LPS-mediated microglial phagocytosis *via* the activation of the PI3-K and p38 MAPK signaling pathways.

DISCUSSION

During microglia activation, a variety of signaling pathways are triggered and actively participate in the modulation

of inflammatory processes.^{17,18)} With regard to the mechanisms of neuroinflammation, ROS-dependent signal cascades have been characterized, and many researchers have attempted to determine these complex networks for the identification of potential therapeutic targets. We demonstrated that Nox-dependent PI3-K and p38 MAPK activation is essential for LPS-induced phagocytosis in BV-2 microglial cells. Although the importance of PI3-K and p38 MAPK signal pathways in phagocytes is well known,^{19–21)} their respective roles in microglial phagocytosis were previously unquantified.

The inhibition of PI3-K and p38 MAPK attenuates Nox activity in phagocytes; for example, p38 MAPK and PI3-K inhibitors interrupt signaling pathways involved in Nox-mediated O_2^- generation and phagocytosis.^{20,22,23)} However, Nox-generated ROS modulate a variety of inflammatory signal pathways, including MAPKs.¹⁰⁾ The inhibition of Nox activation ablates microglial activation processes *via* the blockage of ROS-related signaling pathways, resulting in the inhibition of inflammatory mediator production and phagocytosis.^{10,11,24)} Thus, microglia-mediated inflammatory processes may be highly dependent on the balance between the Nox activation pathway and its downstream signal cascades. Although we did not characterize the effects of PI3-K and p38 MAPK inhibitors on Nox activation, we observed that Nox inhibition resulted in the inactivation of both kinase pathways and phagocytosis. This is the first demonstration that Nox-dependent phagocytosis requires the activation of the PI3-K and p38 MAPK pathways in LPS-stimulated BV-2 microglial cells.

ERK1/2 and JNK are important kinases in microglial redox signaling.^{25,26)} Their inhibition results in the modulation of various pathways, including the production of inflammatory mediators. Although bacterial phagocytosis activates ERK1/2 and JNK, as well as p38 MAPK, the most notable inhibition of phagocytosis is obtained with p38 MAPK inhibition.⁹⁾ Our results are in accordance with these findings (Fig. 3D). We also found that the intracellular ROS content was significantly modulated only by treatment with SB203580, and not with SP600125 or PD98059 (data not shown). These data are indicative of the cooperative action of ROS with MAPK signal pathways in BV-2 microglial cells. Therefore, the complex signaling cascades that regulate microglial function are in need of more elaborate inspection.

We demonstrated that the PI3-K and p38 MAPK pathways are essential for Nox-dependent microglial phagocytosis. BV-2 cells with reduced ROS levels fail to activate the redox-signaling pathways governing the phagocytosis process. Our findings are good evidence for why ROS should be controlled to obtain the successful cure or alleviation of neuroinflammatory progression.

Acknowledgments The first authors, H.-N. Sun and S.-U. Kim, contributed equally to this work. This work was supported by the Research Program for New Drug Target Discovery Grant (NBM3300711) Grant from the Ministry of Science & Technology, by the International Collaboration Program of Science and Technology of MOST (FGM0600712), by the KRIBB Research Initiative Program Grant (KGM2130813), and by a Grant (Code# 20080401-034-050-008-01-00) from BioGreen21 Program, Rural Development Administration, Republic of Korea.

REFERENCES

- May J. M., de Haen C., *J. Biol. Chem.*, **254**, 2214—2220 (1979).
- Finkel T., *J. Leukoc. Biol.*, **65**, 337—340 (1999).
- Maulik N., *Antioxid. Redox Signal.*, **4**, 805—815 (2002).
- Block M. L., Hong J. S., *Biochem. Soc. Trans.*, **35**, 1127—1132 (2007).
- Floyd R. A., *Proc. Soc. Exp. Biol. Med.*, **222**, 236—245 (1999).
- Ninomiya N., Hazeki K., Fukui Y., Seya T., Okada T., Hazeki O., Ui M., *J. Biol. Chem.*, **269**, 22732—22737 (1994).
- Rose D. M., Winston B. W., Chan E. D., Riches D. W., Gerwins P., Johnson G. L., Henson P. M., *J. Immunol.*, **158**, 3433—3438 (1997).
- Ding J., Vlahos C. J., Liu R., Brown R. F., Badwey J. A., *J. Biol. Chem.*, **270**, 11684—11691 (1995).
- McLeish K. R., Klein J. B., Coxon P. Y., Head K. Z., Ward R. A., *J. Leukoc. Biol.*, **64**, 835—844 (1998).
- Pawate S., Shen Q., Fan F., Bhat N. R., *J. Neurosci. Res.*, **77**, 540—551 (2004).
- Qin L., Liu Y., Wang T., Wei S. J., Block M. L., Wilson B., Liu B., Hong J. S., *J. Biol. Chem.*, **279**, 1415—1421 (2004).
- Nagai H., Noguchi T., Takeda K., Ichijo H., *J. Biochem. Mol. Biol.*, **40**, 1—6 (2007).
- Peristeris P., Clark B. D., Gatti S., Faggioni R., Mantovani A., Mengozzi M., Orencole S. F., Sironi M., Ghezzi P., *Cell. Immunol.*, **140**, 390—399 (1992).
- Wiser J., Alexis N. E., Jiang Q., Wu W., Robinette C., Roubey R., Peden D. B., *Free Radic. Biol. Med.*, **45**, 40—49 (2008).
- Kim E. J., Kwon K. J., Park J. Y., Lee S. H., Moon C. H., Baik E. J., *J. Neurosci. Res.*, **70**, 97—107 (2002).
- Liu Y., Hao W., Letiembre M., Walter S., Kulanga M., Neumann H., Fassbender K., *J. Neurosci.*, **26**, 12904—12913 (2006).
- Ji R. R., Strichartz G., *Sci. STKE*, **2004**, reE14 (2004).
- Schwab J. M., Schluesener H. J., *Cell Death Differ.*, **11**, 1245—1246 (2004).
- Lowry M. B., Duchemin A. M., Coggeshall K. M., Robinson J. M., Anderson C. L., *J. Biol. Chem.*, **273**, 24513—24520 (1998).
- Yamamori T., Inanami O., Nagahata H., Cui Y., Kuwabara M., *FEBS Lett.*, **467**, 253—258 (2000).
- Zelck U. E., Gege B. E., Schmid S., *Dev. Comp. Immunol.*, **31**, 321—331 (2007).
- Cui Y. D., Inanami O., Yamamori T., Niwa K., Nagahata H., Kuwabara M., *Inflamm. Res.*, **49**, 684—691 (2000).
- Alblas J., Honing H., de Lavalette C. R., Brown M. H., Dijkstra C. D., van den Berg T. K., *Mol. Cell. Biol.*, **25**, 7181—7192 (2005).
- Wang T., Qin L., Liu B., Liu Y., Wilson B., Eling T. E., Langenbach R., Taniura S., Hong J. S., *J. Neurochem.*, **88**, 939—947 (2004).
- Bhat N. R., Zhang P., Lee J. C., Hogan E. L., *J. Neurosci.*, **18**, 1633—1641 (1998).
- Hidding U., Mielke K., Waetzig V., Brecht S., Hanisch U., Behrens A., Wagner E., Herdegen T., *Biochem. Pharmacol.*, **64**, 781—788 (2002).