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Research article

Ginsenoside Rg_3 promotes inflammation resolution through M2 macrophage polarization

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ABSTRACT

Background: Ginsenosides have been reported to have many health benefits, including anti-inflammatory effects, and the resolution of inflammation is now considered to be an active process driven by M2-type macrophages. In order to determine whether ginsenosides modulate macrophage phenotypes to reduce inflammation, 11 ginsenosides were studied with respect to macrophage polarization and the resolution of inflammation.

Methods: Mouse peritoneal macrophages were polarized into M1 or M2 phenotypes. Reverse transcription-polymerase chain reaction, Western blotting, and measurement of nitric oxide (NO) and prostaglandin E_2 levels were performed *in vitro* and in a zymosan-induced peritonitis C57BL/6 mouse model.

Results: Ginsenoside Rg_3 was identified as a proresolving ginseng compound based on the induction of M2 macrophage polarization. Ginsenoside Rg_3 not only induced the expression of *arginase-1* (a representative M2 marker gene), but also suppressed M1 marker genes, such as *inducible NO synthase*, and NO levels. The proresolving activity of ginsenoside Rg_3 was also observed *in vivo* in a zymosan-induced peritonitis model. Ginsenoside Rg_3 accelerated the resolution process when administered at peak inflammatory response into the peritoneal cavity.

Conclusion: These results suggest that ginsenoside Rg_3 induces the M2 polarization of macrophages and accelerates the resolution of inflammation. This finding opens a new avenue in ginseng pharmacology.

1. Introduction

Pharmacognostic studies on ginseng, the root of *Panax ginseng* Meyer, have determined that ginsenosides, triterpenoid saponins, are the bioactive ingredients of ginseng [1,2]. Ginsenosides are believed to mediate most of the pharmacological effects of ginseng, which include anticancer, anti-inflammatory, and antidiabetic activities [1,3,4]. Moreover, of the ginsenosides, ginsenoside Rg_3 is one of the most effective steroidal saponins in steamed ginseng [5]. Ginsenoside Rg_3 exhibits a wide range of therapeutic and pharmacological properties, which include anti-inflammatory, anticancer, antioxidant, and antiobesity effects [4,6–10].

The resolution of inflammation is now considered to be an active process orchestrated by proresolving mediators and their receptors [11,12]. M2 polarized macrophages are supposed to play key roles during the resolution of inflammation [13,14]. Although anti-inflammatory effects of ginseng and ginsenosides have been studied at the molecular level, the effects of ginsenosides on macrophage polarization and on the resolution of inflammation have not been studied. Therefore, we aimed: (1) to find whether any ginsenoside could induce M2 polarization of macrophages; (2) to confirm induction of M2 polarization and suppression of M1 polarization at molecular and cellular levels *in vitro*; and (3) to verify M2 polarization effect on inflammation resolution in a mouse peritonitis model *in vivo*.

First, we investigated the effects of ginsenosides on macrophage polarization, in order to determine which ginsenoside could accelerate the resolution of inflammation [15]. Of the 11 ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_2, and Ro) tested, Rg_3 was found to inhibit M1 polarization and to induce M2 polarization in mouse peritoneal macrophages, and to promote the resolution of inflammation in a murine peritonitis model. The first report on

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ginsenoside Rg_3's effects on M2 polarization and resolution of inflammation would be the novelty of the present study.

2. Materials and methods

2.1. Materials

Ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_2, and Ro) were purified in Kang-ju Choi's laboratory, the Korea Ginseng and Tobacco Research Institute (Daejeon, Republic of Korea), and the purities were more than 99.9%. They were dissolved in absolute methanol and stored at −20°C. All other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

Eight- to ten-week-old male C57BL/6 (19–22 g) mice were purchased from Daehan Biolink (DBL; Seoul, Korea), housed in a laboratory animal facility at Pusan National University (Busan, Korea), and provided food and water *ad libitum*. The animal protocol used in this study was reviewed and approved beforehand by the Pusan National University-Institutional Animal Care Committee with respect to ethicality and scientific care.

2.3. Isolation and culture of mouse peritoneal macrophages

Mouse peritoneal macrophages were isolated from the peritoneal cavity of a 3% thioglycollate-treated C57BL/6 mouse 4 d after treatment and cultured at 37°C in a 5% CO_2 humidified incubator. Isolated macrophages were maintained in RPMI1640 containing 10% (v/v) heat-inactivated fetal bovine serum, 100 units/mL penicillin, 50 μg/mL streptomycin, 2 mM glutamine, and 1 mM sodium pyruvate for 18 h and then incubated in 0.5% fetal bovine serum-containing media for 24 h. RNA and protein samples were prepared after 5 h or 24 h of lipopolysaccharide (LPS) treatment (10 ng/mL or 100 ng/mL), respectively. Ginsenosides were added 1 h prior to adding LPS [15].

2.4. Reverse transcriptase-polymerase chain reaction

To determine the expressions of marker proteins of M1 or M2 polarization in macrophages by reverse transcription-polymerase chain reaction (RT-PCR), first-strand cDNA was synthesized with total RNA isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Synthesized cDNA products and primers for each gene were used for PCR, which was conducted using Promega Go-Taq DNA polymerase (Promega, Madison, WI, USA). Specific primers for tumor necrosis factor-alpha (TNF-α) (sense 5′-GAC CCT CAC ACT CAG ATC AT-3′, antisense 5′-TTG AAG AGA ACC TGG GAG TA-3′), transforming growth factor-beta 1 (TGF-β1) (sense 5′-TTGCTTCAGCTCCACAGAGA-3′, antisense 5′-TGGTTGTAGAGGGCAAGGAC-3′), Ym-1 (sense 5′-ACT TTG ATG GCC TCA ACC TG-3′, antisense 5′-AAT GAT TCC TGC TCC TGT GG-3′), and interleukin (IL)-10 (sense 5′-CCAAGCCTTATCGGAAATGA-3′, antisense 5′-TTTTCACAGGGGAGAAATCG-3′) were used to amplify gene fragments. PCR was performed over 30 amplification cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s) in an Eppendorf Mastcycler gradient PCR machine (Eppendorf, Hamburg, Germany) [16]. Specific primers for arginase-1 (sense 5′-GTG AAG AAC CCA CGG TCT GT-3′, antisense 5′-CTG GTT GTC GGG GAG TGT T-3′), inducible nitric oxide synthase (iNOS) (sense 5′-ACC TAC CAC ACC CGA GAT GGC CAG-3′, antisense 5′-AGG ATG TCC TGA ACA TAG ACC TTG GG-3′), cyclooxygenase-2 (COX-2) (sense 5′-CCG TGG GGA ATG TAT GAG CA-3′, antisense 5′-CCA GGT CCT CGC TTA TGA TCT G-3′), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (sense 5′-TTCACCACCATGGAGAAGGC-3′, antisense 5′-GGCATGGACTGTGGTCATGA-3′) were used, and annealing was performed at 60°C. For IL-1β (sense 5′-GGAGAAGCTGTGGCAGCTA-3′, antisense 5′-GCTGATGTACCAGTTGGGGA-3′), annealing was undertaken at 57°C. Aliquots (7 μL) were electrophoresed in 1.2% agarose gels and stained with ethidium bromide [15].

2.5. Western blotting

Macrophages were harvested and resuspended in RIPA lysis buffer (GenDEPOT, Baker, TX, USA). Concentrations of proteins were determined using a BCA protein assay (ThermoScientific, Rockford, IL, USA). Proteins (30 μg) were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose. Membranes were blocked in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and 5% skim milk, incubated with specific primary antibodies recognizing β-actin, COX-2, iNOS, and arginase-1, and then incubated HRP-conjugated secondary antibodies (Cell Signaling Technology, Danvers, MA, USA). Signals were developed using an enhanced chemiluminescence system (Pierce Biotechnology Inc., Rockford, IL) [16].

2.6. Nitrites measurement

NO production was estimated by measuring the amount of nitrite (a stable metabolite of NO) in medium using Griess reagent, as previously described [17]. Cells were pretreated with different concentrations of ginsenoside Rg_3 for 1 h and subsequently stimulated with LPS (100 ng/mL) for 24 h. Nitrite concentrations in medium were determined using the Griess Reagent System (Promega).

2.7. Prostaglandin E_2 production

Peritoneal macrophages were incubated with ginsenoside Rg_3 for 1 h and subsequently stimulated with LPS (100 ng/mL) for 24 h. Macrophage culture supernatants were harvested and immediately assayed using a prostaglandin E_2 (PGE_2) EIA kit (Cayman Chemical, Ann Arbor, MI) [18].

2.8. Induction of peritonitis and peritoneal cell counting

Peritonitis was induced by injecting 30 mg/kg of zymosan (Sigma) intraperitoneally (i.p.), and 12 h later, mice were treated i.p. with vehicle, 1 mg/kg, or 5 mg/kg ginsenoside Rg_3. Peritoneal washing was performed 24 h after zymosan treatment using 4 mL of ice-cold RPMI1640. Total cell numbers in peritoneal washings were calculated by counting after trypan blue staining. The cells obtained were washed with 0.1M phosphate buffer (pH 6.8), which was prepared by mixing 153 mL of 0.2M NaH_2PO_4 (Monobasic; AMRESCO, Solon, OH) and 147 mL of 0.2M Na_2HPO_4 (Dibasic; AMRESCO) and adding distilled water to a final volume 900 mL. The cells were attached to slides using a Cellspin (Hanil, Anyang, Korea), which was operated at 500 rpm for 5 min. Slides were then dried at room temperature for 30 min and fixed in methanol for 30 s. Cells were then stained with May–Grünwald solution (Sigma) and Giemsa solution (Fluka, Buchs, Switzerland) to identify individual cell types [19].

2.9. Statistics

Results are expressed as the means ± standard errors of the indicated numbers of determinations. The statistical significances of differences were determined by analysis of variance using Tukey's *post hoc*, and statistical significance was accepted for p

values < 0.05. Analyses were performed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Effects of 11 ginsenosides on macrophage phenotypes

Initially, we tested the effects of the 11 ginsenosides on the M1 and M2 polarizations of peritoneal macrophages. As shown Fig. 1A, isolated peritoneal macrophages expressed M2 marker genes, such as *arginase-1*, *IL-10*, *TGF-β*, and *Ym-1*, but not M1 marker genes. However, after stimulation with LPS (a cell wall component of Gram-negative bacteria) macrophage phenotype changed, that is, they strongly expressed M1 marker genes, such as *COX-2*, *iNOS*, *IL-1β*, and *TNF-α*. Furthermore, the representative M2 marker gene, *arginase-1*, was downregulated after LPS stimulation (Fig. 1A) [15]. These observations show that the isolated peritoneal macrophages were M2 polarized, and that the LPS-stimulated macrophages were M1 polarized [15]. We used this polarization phenomenon to assess the effects of the 11 ginsenosides on macrophage polarization. Of these ginsenosides, Rg_3 most prevented LPS-induced loss of arginase-1 expression, which it did in a concentration-dependent manner (Fig. 1B). In addition, it slightly suppressed LPS-induced COX-2 induction. Based on these results, we focused on ginsenoside Rg_3 in the following experiments on macrophage polarization and the resolution of inflammation (the structure of ginsenoside Rg_3 is provided in Fig. 2A).

3.2. Effects of ginsenoside Rg_3 on M1 polarization

In mouse peritoneal macrophages, stimulation with 10 ng/mL LPS induced the expressions of M1 proinflammatory marker genes, such as *COX-2*, *iNOS*, *IL-1β*, and *TNF-α* (Fig. 2B). Of the four genes tested, iNOS and COX-2 mRNAs appeared to be reduced concentration-dependently by Rg_3, but not significantly so (data not shown). By contrast, the iNOS mRNA expression was much more affected than that of COX-2 mRNA. We also examined the inhibitory effects of Rg_3 at the protein level. As shown in Fig. 3A, the expressions of iNOS and COX-2 protein were obviously induced by LPS. Ginsenoside Rg_3 strongly and concentration-dependently inhibited iNOS protein induction, but its effect on COX-2 protein induction was not significant (Fig. 3B). At concentrations 5 μM and 10 μM, ginsenoside Rg_3 had significant inhibitory effects on iNOS protein induction. In addition, the inhibitory effects of ginsenoside Rg_3 on the expressions of iNOS and COX-2 were confirmed by measuring the products of iNOS and COX-2, that is, nitric oxide (NO) and PGE_2, respectively. As shown in Fig. 3C, Rg_3 significantly and concentration-dependently inhibited LPS-induced PGE_2 production (Fig. 3C), and as shown in Fig. 3D, Rg_3 markedly inhibited NO production in a concentration-dependent manner from a concentration of 1 μM.

3.3. Effects of ginsenoside Rg_3 on M2 polarization

After stimulating macrophages with LPS, the mRNA expression of arginase-1 (a representative M2 marker protein) disappeared, but its expression was retained by pretreating Rg_3 (Fig. 4A). By contrast, the mRNA expressions of other M2 markers were not much changed by 10 ng/mL LPS (Fig. 4A and B). The protective effect of ginsenoside Rg_3 on arginase-1 expression was also confirmed at the protein level (Fig. 4C and D). Therefore, ginsenoside Rg_3 not only inhibited LPS-induced M1 polarization, but also protected the M2 polarization of macrophages *in vitro*.

3.4. Ginsenoside Rg_3 accelerated the disappearance of polymorphonuclear lymphocytes in peritoneal cavities

The inhibitory effect of Rg_3 on LPS-induced M1 polarization and its protection of M2 polarization of macrophages *in vitro* were also

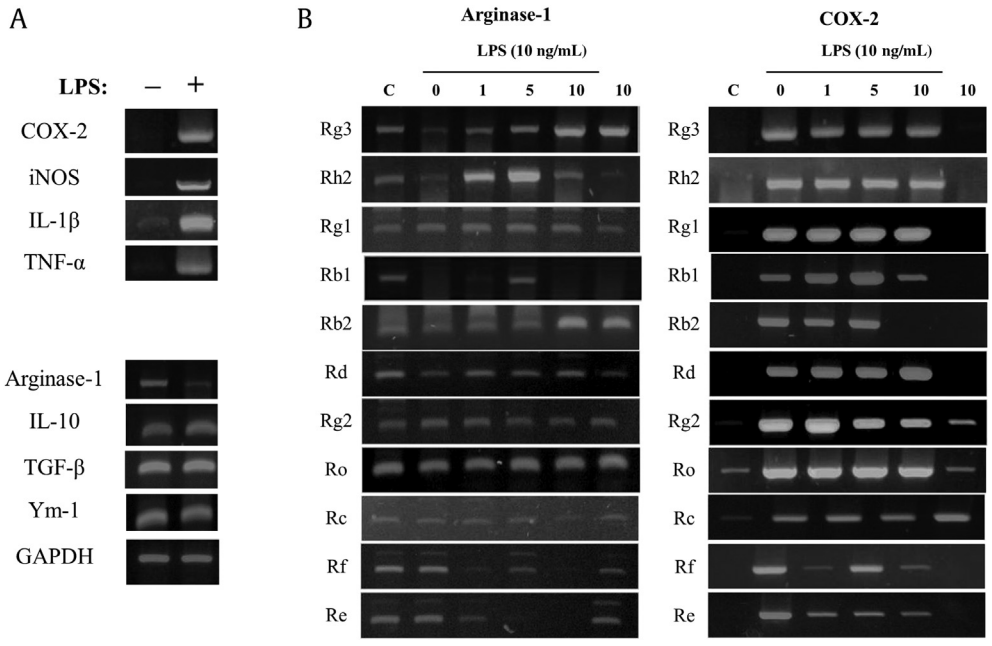

Fig. 1. Macrophage polarization and effects of ginsenosides on the expressions of M1 and M2 marker genes in peritoneal macrophages. (A) Mouse peritoneal macrophages were treated with vehicle or LPS 10 ng/mL for 5 h, and RT-PCR was then performed for proinflammatory and anti-inflammatory genes. The data shown are representative of three independent experiments. (B) Mouse peritoneal macrophages were treated with the indicated concentrations of each ginsenoside for 1 h, and then treated with vehicle or LPS 10 ng/mL for 5 h. RT-PCR for proinflammatory genes and anti-inflammatory genes was performed by targeting arginase-1 and COX-2. COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-10, interleukin 10; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; RT-PCR, reverse transcription-polymerase chain reaction; TGF, transforming growth factor; TNF, tumor necrosis factor.

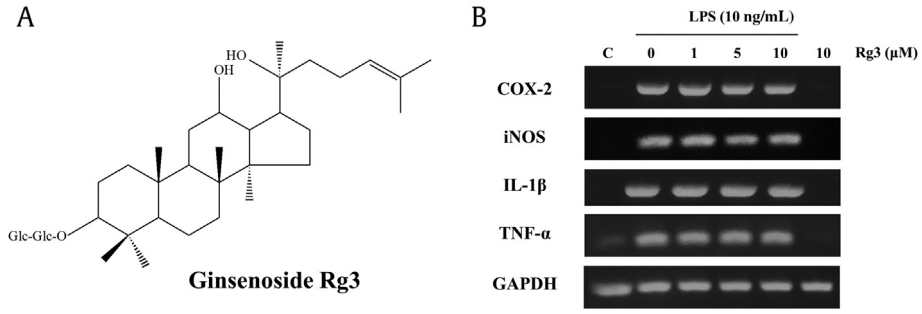

Fig. 2. Structure of ginsenoside Rg_3 and its effect on the expressions of M1 marker genes in macrophages. (A) The structure of ginsenoside Rg_3. (B) Mouse peritoneal macrophages were treated with the indicated concentrations of ginsenoside Rg_3 for 1 h, and then treated with vehicle or LPS 10 ng/mL for 5 h, and RT-PCR was performed for the proinflammatory genes, *COX-2*, *iNOS*, *IL-1β*, and *TNF-α*. The data shown are representative of three independent experiments. COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; RT-PCR, reverse transcription-polymerase chain reaction; TNF, tumor necrosis factor.

examined in a zymosan-induced mouse peritonitis model. Zymosan is a glucan found on the surfaces of fungi, such as yeast, and its injection into the peritoneal cavity induces inflammatory response, that is, the migration of immune cells (e.g., neutrophils) into the peritoneal cavity. In a preliminary experiment, we found that peak inflammatory response was observed 12 h after zymosan stimulation, and therefore, Rg_3 (1 mg/kg or 5 mg/kg) or vehicle was administered i.p. at this time. Peritoneal cells were stained and counted 24 h after zymosan administration. Cell numbers and cell population distributions were analyzed. As shown in Fig. 5A and B, total cell numbers increased by 161% in the zymosan-induced peritonitis group versus phosphate buffered saline-treated controls (Fig. 5A), and this increase was inhibited by 1 mg/kg or 5 mg/kg of Rg_3 by 60% and 93%, respectively (Fig. 5A).

Polymorph nuclear lymphocytes (PMNLs; neutrophils, eosinophils, and basophils), mononuclear lymphocytes (MNLs; T cells and B cells), and macrophages can be distinguished by their shapes and by May–Grünwald and Giemsa staining (Fig. 5B). PMNLs were found to be the main population during early peritonitis, and their disappearance was accelerated by Rg_3 (1 mg/kg or 5 mg/kg; Fig. 5B). The disappearance of MNLs from peritoneal cavities was also significantly accelerated by Rg_3 at 5 mg/kg (Fig. 5B).

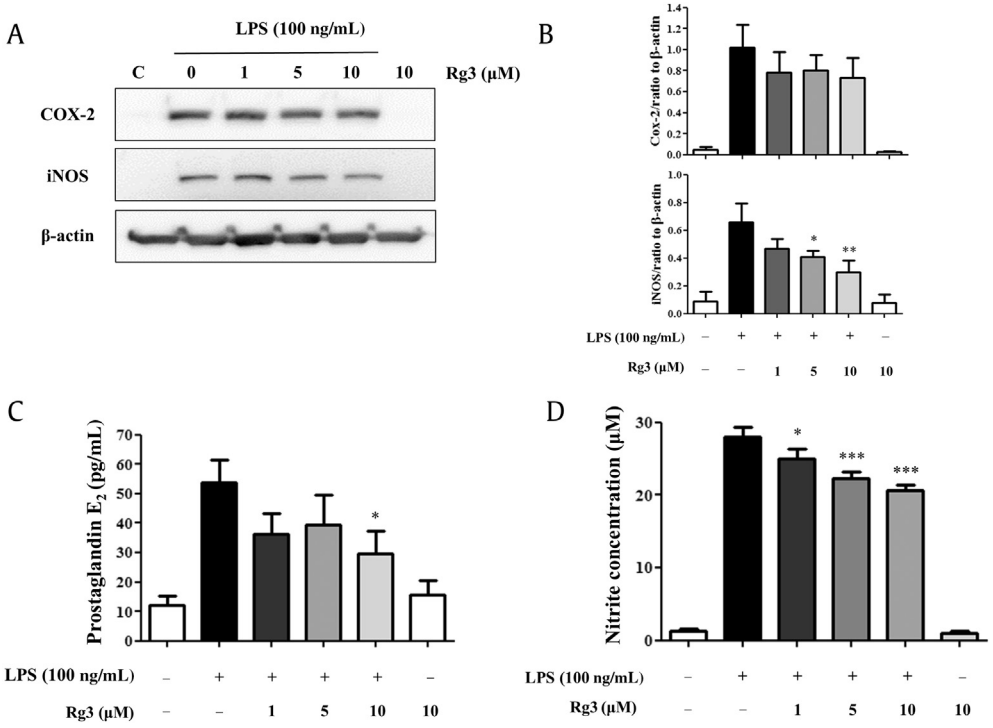

Fig. 3. Effect of ginsenoside Rg_3 on the protein expressions of COX-2 and iNOS and on NO and PGE_2 production in macrophages. Mouse peritoneal macrophages were treated with the indicated concentrations of ginsenoside Rg_3 for 1 h, and then treated with LPS 100 ng/mL for 24 h. Western blotting was conducted on cell lysates. (A) The data shown are representative of three independent experiments. (B) Relative protein levels of each protein versus β-actin are presented as histograms. (C) LPS-induced production of PGE_2. (D) LPS-induced production of nitrite. Ginsenoside Rg_3 (C) concentration-dependently inhibited PGE_2 production and (D) showed a tendency to inhibit nitrite production. Results are the means ± SEs of three independent experiments. Statistically significant at $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ levels versus the LPS-treated macrophages. COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NO, nitric oxide; PGE_2, prostaglandin E_2; SE, standard error; TNF, tumor necrosis factor.

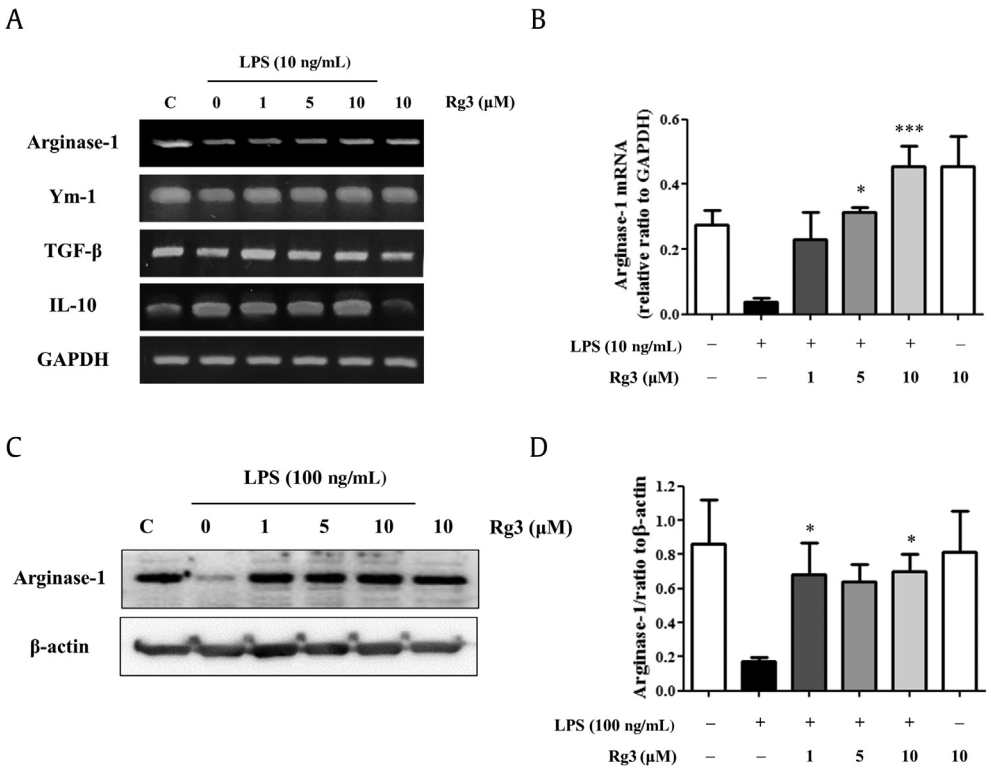

Fig. 4. Effect of ginsenoside Rg_3 on the expressions of M2 marker genes in macrophages. Mouse peritoneal macrophages were treated with the indicated concentrations of ginsenoside Rg_3 for 1 h, and then treated with vehicle or LPS 10 ng/mL for 24 h. RT-PCR was performed for the M2 marker genes, *arginase-1*, *IL-10*, *TGF-β*, and *Ym-1*. (A) The results shown are representative of three independent experiments. (B) Relative mRNA levels of each gene versus GAPDH are shown as histograms. Mouse peritoneal macrophages were treated with the indicated concentrations of ginsenoside Rg_3 for 1 h, and then treated with LPS 100 ng/mL for 24 h. Western blotting was conducted on cell lysates. (C) The results shown are representative of three independent experiments. (D) Relative protein levels of each protein versus β-actin are presented as histograms. The values shown are means ± SEs ($n = 3$). Statistically significant at $^{*}p < 0.05$ and $^{***}p < 0.001$ levels versus LPS-treated macrophages. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-10, interleukin 10; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; RT-PCR, reverse transcription-polymerase chain reaction; SE, standard errors; TGF, transforming growth factor.

4. Discussion

In the present study, ginsenoside Rg_3 was shown to suppress the LPS-induced expressions of the M1 marker gene, *iNOS*, and the production of NO. In addition, LPS-induced COX-2 expression and PGE_2 production were also marginally suppressed by ginsenoside Rg_3. Previously, the anti-inflammatory effect of ginsenoside Rg_3 has been demonstrated by its suppressions of iNOS expression and PGE_2 production, that is, ginsenoside Rg_3 suppressed LPS-induced iNOS expression and NO, reactive oxygen species (ROS), and PGE_2 production in RAW264.7 macrophages in a concentration-dependent manner [4,20]. Also, topically administered ginsenoside Rg_3 pretreatment of the dorsal skins of female ICR mice significantly inhibited 12-*O*-tetradecanoylphorbol-13-acetate-induced ornithine decarboxylase activity [21]. Furthermore, in $A\beta_{42}$-treated BV-2 microglial cells, ginsenoside Rg_3 reduced inflammatory cytokine expression by inhibiting NF-κB p65 binding to its DNA consensus sequences [22]. In an LPS-induced mouse model of acute lung injury, ginsenoside Rg_3 inhibited increases in the levels of proinflammatory cytokines, including those of TNF-α, IL-1β, and IL-6, inhibited increases of neutrophils in bronchoalveolar lavage fluid, and protected lungs from histopathological changes [23]. These effects were supposed to be mediated by the inhibition of NF-κB p65 phosphorylation and downstream COX-2 expression by ginsenoside Rg_3 [23]. However, the induction of M2 polarization and acceleration of the resolution process have not been previously proposed as action mechanisms of ginsenoside Rg_3.

The present study shows for the first time that ginsenoside Rg_3 protects the expression of *arginase-1* (the M2 marker gene) in LPS-treated mouse peritoneal macrophages *in vitro*. In addition, this protective effect on M2 polarization was confirmed *in vivo* by the accelerated disappearance of neutrophils after peak inflammatory response in the zymosan-induced peritonitis model. Furthermore, because in the *in vivo* experiment ginsenoside Rg_3 was administered after peak inflammatory response had been reached, the observed accelerated disappearance demonstrated a proresolving effect, which differs from its anti-inflammatory effects in zymosan or LPS *in vivo* models. We do not know how ginsenoside Rg_3 accelerates the resolution process, although it is possible that the productions of proresolving mediators, such as lipoxins, resolvins, and protectins, by ginsenoside Rg_3 are responsible [11,12]. Because M2 polarized macrophages have been reported to play key roles in the resolution phase of inflammation [13,14], ginsenoside Rg_3-induced M2 polarization may contribute to inflammation resolution. Further studies are required to determine the nature of the of action mechanisms involved. We hope our findings regarding the effects of ginsenoside Rg_3 on macrophage polarization and on the resolution of inflammation can provide a means of devising proresolving drugs based on ginseng pharmacology.

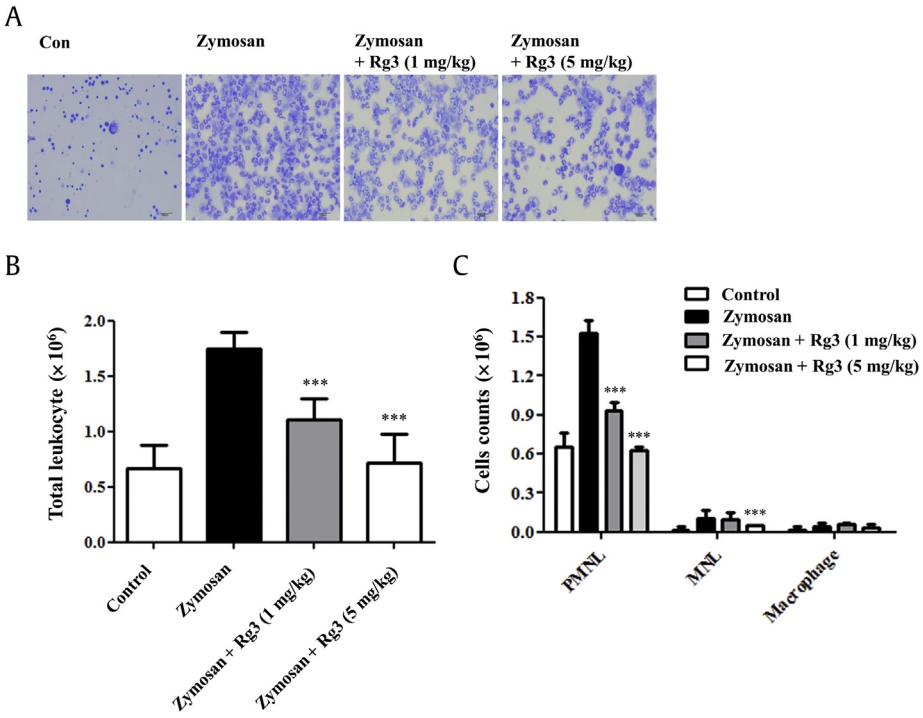

Fig. 5. Effect of ginsenoside Rg_3 on zymosan-induced peritonitis in mice. C57BL/6 mice were treated with zymosan (30 mg/kg, i.p.), and 12 h later treated with vehicle ($n = 5$) or ginsenoside Rg_3 (1 mg/kg or 5 mg/kg; $n = 5$) by intraperitoneal injection. After a further 12 h, mouse peritoneal cells were collected, May–Grünwald and Giemsa stained, and counted. (A) Representative photos of peritoneal cells. (B) Total cell counts in peritoneal fluids of mice with zymosan-induced peritonitis (zymosan), and ginsenoside Rg_3 (1 mg/kg or 5 mg/kg) treated mice with zymosan-induced peritonitis (zymosan + ginsenoside Rg_3). (C) Cells counts of polymorph nuclear lymphocytes (PMNLs), mononuclear lymphocytes (MNLs), and macrophages in the peritoneal cavity fluids of zymosan or zymosan+ ginsenoside Rg_3 (1 mg/kg or 5 mg/kg) treated mice. The values shown are means ± SEs ($n = 5$). Statistically significant at $^{***}p < 0.001$ level versus mice in the zymosan-treated group. SE, standard error; i.p., intraperitoneally.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

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References

[1] Choi KT. Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C A Meyer. Acta Pharmacol Sin 2008;29:1109–18.
[2] Im DS, Nah SY. Yin and Yang of ginseng pharmacology: ginsenosides vs gintonin. Acta Pharmacol Sin 2013;34:1367–73.
[3] Nah SY, Kim DH, Rhim H. Ginsenosides: are any of them candidates for drugs acting on the central nervous system? CNS Drug Rev 2007;13:381–404.
[4] Shin YM, Jung HJ, Choi WY, Lim CJ. Antioxidative, anti-inflammatory, and matrix metalloproteinase inhibitory activities of 20(*S*)-ginsenoside Rg_3 in cultured mammalian cell lines. Mol Biol Rep 2013;40:269–79.
[5] Jovanovski E, Bateman EA, Bhardwaj J, Fairgrieve C, Mucalo I, Jenkins AL, Vuksan V. Effect of Rg_3-enriched Korean red ginseng (*Panax ginseng*) on arterial stiffness and blood pressure in healthy individuals: a randomized controlled trial. J Am Soc Hypertens 2014;8:537–41.
[6] Qiu XM, Bai X, Jiang HF, He P, Wang JH. 20-(*S*)-ginsenoside Rg_3 induces apoptotic cell death in human leukemic U937 and HL-60 cells through PI3K/Akt pathways. Anticancer Drugs 2014;25:1072–80.
[7] Park D, Bae DK, Jeon JH, Lee J, Oh N, Yang G, Yang YH, Kim TK, Song J, Sun HL. Immunopotentiation and antitumor effects of a ginsenoside Rg(3)-fortified red ginseng preparation in mice bearing H460 lung cancer cells. Environ Toxicol Pharmacol 2011;31:397–405.
[8] Choi SY, Cho CW, Lee Y, Kim SS, Lee SH, Kim KT. Comparison of ginsenoside and phenolic ingredient contents in hydroponically-cultivated ginseng leaves, fruits, and roots. J Ginseng Res 2012;36:425–9.
[9] Hwang JT, Lee MS, Kim HJ, Sung MJ, Kim HY, Kim MS, Kwon DY. Antiobesity effect of ginsenoside Rg_3 involves the AMPK and PPAR-gamma signal pathways. Phytother Res 2009;23:262–6.
[10] Kim HS, Lee EH, Ko SR, Choi KJ, Park JH, Im DS. Effects of ginsenosides Rg_3 and Rh_2 on the proliferation of prostate cancer cells. Arch Pharm Res 2004;27:429–35.
[11] Serhan CN, Krishnamoorthy S, Recchiuti A, Chiang N. Novel anti-inflammatory–pro-resolving mediators and their receptors. Curr Top Med Chem 2011;11:629–47.
[12] Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: novel pro-resolving lipid mediators in resolution. Semin Immunol 2015;27:200–15.
[13] Ramon S, Dalli J, Sanger JM, Winkler JW, Aursnes M, Tungen JE, Hansen TV, Serhan CN. The protectin PCTR1 is produced by human M2 macrophages and enhances resolution of infectious inflammation. Am J Pathol 2016;186:962–73.
[14] Ariel A, Serhan CN. New lives given by cell death: macrophage differentiation following their encounter with apoptotic leukocytes during the resolution of inflammation. Front Immunol 2012;3:4.
[15] Park SJ, Lee KP, Kang S, Lee J, Sato K, Chung HY, Okajima H, Im DS. Sphingosine 1-phosphate induced anti-atherogenic and atheroprotective M2 macrophage polarization through IL-4. Cell Signal 2014;26:2249–58.
[16] Ryu YK, Lee JW, Moon EY. Thymosin beta-4, actin-sequestering protein regulates vascular endothelial growth factor expression via hypoxia-inducible nitric oxide production in HeLa cervical cancer cells. Biomol Ther (Seoul) 2015;23:19–25.
[17] Lee JW, Kim NH, Kim JY, Park JH, Shin SY, Kwon YS, Lee HJ, Kim SS, Chun W. Aromadendrin inhibits lipopolysaccharide-induced nuclear translocation of NF-κB and phosphorylation of JNK in RAW 264.7 macrophage cells. Biomol Ther 2013;21:216–21.
[18] Sohn YA, Hwang SA, Lee SY, Hwang IY, Kim SW, Kim SY, Moon A, Lee YS, Kim YH, Kang KJ, et al. Protective effect of liriodendrin isolated from *Kalopanax pictus* against gastric injury. Biomol Ther (Seoul) 2015;23:53–9.
[19] Lee HN, Kundu JK, Cha YN, Surh YJ. Resolvin D1 stimulates efferocytosis through p50/p50-mediated suppression of tumor necrosis factor-alpha expression. J Cell Sci 2013;126:4037–47.

[20] Yoon SJ, Park JY, Choi S, Lee JB, Jung H, Kim TD, Yoon SR, Choi I, Shim S, Park YJ. Ginsenoside Rg_3 regulates *S*-nitrosylation of the NLRP3 inflammasome via suppression of iNOS. Biochem Biophys Res Commun 2015;463:1184–9.
[21] Keum YS, Han SS, Chun KS, Park KK, Park JH, Lee SK, Surh YJ. Inhibitory effects of the ginsenoside Rg_3 on phorbol ester-induced cyclooxygenase-2 expression, NF-kappaB activation and tumor promotion. Mutat Res 2003;523–4:75–85.
[22] Joo SS, Yoo YM, Ahn BW, Nam SY, Kim YB, Hwang KW, Lee DI. Prevention of inflammation-mediated neurotoxicity by Rg_3 and its role in microglial activation. Biol Pharm Bull 2008;31:1392–6.
[23] Cheng Z, Li L. Ginsenoside Rg_3 ameliorates lipopolysaccharide-induced acute lung injury in mice through inactivating the nuclear factor-kappaB (NF-kappaB) signaling pathway. Int Immunopharmacol 2016;34:53–9.