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A Comparative Study on Immunomodulatory Activity of Polysaccharides from Two Official Species of *Ganoderma (Lingzhi)*

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Two *Ganoderma* species, *G. lucidum* and *G. sinense*, are listed as *Lingzhi* in *Chinese Pharmacopoeia* and they are considered to have the same therapeutic effects. Polysaccharides were the main immunomodulatory and anticancer components in *Ganoderma*. In this study, the chemical characters and the effects of polysaccharides from *G. lucidum* (GLPS) and *G. sinense* (GSPS) on macrophage functions were investigated and compared. Chemical studies showed that GLPS and GSPS were different, displaying various molecular weight distribution and ratio of monosaccharide components. In vitro pharmacological studies showed that both GLPS and GSPS had potent effects on macrophage functions, such as promoting macrophage phagocytosis, increasing their release of nitric oxide and cytokines interleukin (IL)-1α, IL-6, IL-10, and tumor necrosis factor-α. Generally, GLPS was more powerful than GSPS. This study is helpful to elucidate the active components and pharmacological variation between the 2 *Ganoderma* species. The structure-activity relationship of polysaccharides from *Ganoderma* needs further study.

INTRODUCTION

Ganoderma (*Lingzhi* in Chinese) is a genus of well-known medicinal mushrooms with multiple benefits to human health. It is also commonly used as a soup cooking material in daily life in southern China. Up to date, there are more than 80 *Ganoderma* species commonly found worldwide (1), but only 2 *Ganoderma* species, *G. lucidum* (GL) and *G. sinense* (GS), are listed as *Lingzhi* in *Chinese Pharmacopoeia* and they are considered to have the same therapeutic effects (Fig. 1) (2). Actually, chemical and partial pharmacological differences between GL and GS have been reported. In brief, the contents of triterpenoids (3–6), nucleosides and nucleobases (7), sterols (8) in GL were significantly higher than those in GS. Antiproliferative activity of GL extracts on tumor cells was much more potent than that of GS (4,9). *Cheng* also compared the effects of the 2 *Ganoderma* species on gene expression profile in human monocytic cells and found that about 25% of their target genes were mutual (10). In addition, aqueous extracts of GL and GS were shown similar potency to increase lymphocyte growth (9).

To date, anticancer activity, both in vitro and in vivo, of many *Ganoderma* species, such as GL (11), *G. atrum* (12, 13), *G. tsugae* (14), *G. applanation* (15,16) and *G. microsporum* (17–19), have been reported. Triterpenes in *Ganoderma* have been shown their directly suppression on the growth and invasive behavior of cancer cells (20,21), while polysaccharides from *Ganoderma* can stimulate immune system resulting in the production of cytokines and activation of anticancer activities of immune cells (1,22). Indeed, a great deal of work on therapeutic potential of polysaccharides from GL (GLPS) has been carried out, and more than 100 polysaccharides (including protein/peptide bound polysaccharides) have been isolated from the fruiting

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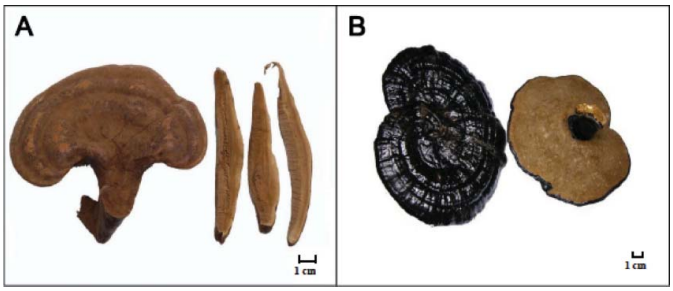

FIG. 1. The fruiting body of (A) *G. lucidum* and (B) *G. sinense*.

bodies, spores, mycelia, or cultivation broth of GL (23,24). These polysaccharides had extensive immunomodulatory effects, such as promoting the functions of antigen-presenting cells (25), mononuclear phygocyte system (26), humoral immunity (27,28) and cellular immunity (29). However, there were few reports on polysaccharides from GS (GSPS) (30–34). It is well known that macrophage activation plays an important role in the the defense mechanism against tumor cells (35). A number of studies demonstrated that *Ganoderma* polysaccharides exhibited anticancer effects via their ability to modulate macrophage function (12,22,32,33,36,37). In this study, effects of GLPS and GSPS on RAW 264.7 macrophage functions were investigated and compared. The bioactivity results combined with their chemical studies are helpful to elucidate their medical values and for understanding the quality control of the polysaccharides from *Ganoderma*.

MATERIALS AND METHODS

Chemicals and Materials

GL was collected from Jinzhai (Anhui, China). GS was purchased from Li-sheng Herbal Drug Store (Macau, China). The identities of the 2 *Ganoderma* species were confirmed by Prof. Xiaolan Mao, Institute of Microbiology, Chinese Academy of Sciences. The voucher specimens of *Ganoderma* were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

Griess reagent, lipopolysaccharide (LPS) and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), penicillin/streptomycin (P/S) were obtained from Invitrogen Molecular Probes (Carlsbad, CA). Rainbow fluorescent particles (3.0–3.4 μm) were purchased from BD Biosciences (Bedford, MA), Milliplex Map kit were from Merck Millipore (Billerica, MA).

Preparation of Polysaccharides from *Ganoderma*

Powder of *Ganoderma* (100 g) was boiled with 30-fold volume of deionized water for 1 h at 100°C with stir at 70 rpm. The extract solution was collected by centrifugation (4000 × g for 10 min) (Allegra X-15R, Beckman Indianapolis, IN) and concentrated to 200 mL by a rotary evaporator (Büchi, Flawil, Switzerland). Then the extract solution was precipitated by addition of ethanol to final concentration of 80% (v/v) overnight (12 h) under 4°C. Precipitate was then collected by centrifugation and washed with 50 mL of 95% ethanol twice. After removal of ethanol on water bath (60°C), the residue was redissolved in 200 mL hot water (60°C) and then freeze dried (Coolsafe 110-4, Labogene ScanVac, Lynge, Denmark) to afford *Ganoderma* polysaccharides. One mg/mL *Ganoderma* polysaccharides was used for the phenol-sulfuric acid assay to determine the sugar content (38).

Molecular Weight Determination of Polysaccharides

Average molecular weight (Mw) and Mw distributions of the investigated polysaccharides were determined using an Agilent 1100 series LC system coupled with a DAWN EOS multi-angle laser light scattering photometer (MALLS, Wyatt Technology Co., Santa Barbara, CA) and refractive index (RI) detector (G1362A, Agilent Technologies Inc., Santa Clara, CA) (39). In brief, 50 μL sample solution (5 mg/mL) filtered through a 0.22-μm nylon syringe filter was injected into the system, and separated at 40°C on TSK G-6000PWXL (300 mm × 7.8 mm, i.d., 10 μm, Tosoh Bioscience, Tokyo, Japan) and TSK G-3000PWXL in series connected columns. Isocratic elution was performed with 15 mmol/L sodium chloride aqueous solution at a flow rate of 0.5 mL/min. The dn/dc value for the tested samples was given as 0.140 mL/g. The data and chromatograms were recorded and processed by using ASTRA software (Wyatt Technology Co.). The DWAN EOS photometer was calibrated by using HPLC grade toluene (Merck, Whitehouse Station, NJ) and normalized with bovine serum albumin standard (Sigma).

Compositional Monosaccharide Analysis of Polysaccharides

Compositional monosaccharide analysis of investigated polysaccharides was performed according to previous report (40). Briefly, dried samples after hydrolysis by trifluoroacetic acid were treated with hydroxylamine hydrochloride-pyridine solution (1 mL, ~20 mg/mL) in a sealed glass tube with a screw cap at 90°C for 30 min, and then acetic anhydride (1 mL) was added and heating continued for another 30 min. The analysis was performed on an Agilent 6890 gas chromatography instrument coupled with an Agilent 5973 mass spectrometer (Agilent Technologies). A HP-5MS capillary column (30 m × 0.25 mm, i.d.) coated with 0.25 μm film 5% phenyl methyl siloxane was used for separation. The selected ion monitoring (SIM) method (i.e. m/z 115 for ribose, arabinose, xylose, mannitol, m/z

129 for rhamnose and fucose, m/z 145 for mannose, glucose, galactose and m/z 168 for internal standard) was applied for accurate determination of the monosaccharides.

Cell Culture

RAW 264.7 mouse macrophage cells were purchased from American Type Culture Collection (Rockville, MD). RAW 264.7 cells were cultured in DMEM medium supplemented with 10% FBS, 1% P/S at 37°C in a humidified atmosphere of 5% CO_2.

Cell Viability Assay

The viability of cells was measured using MTT assay. In brief, RAW 264.7 macrophages were seeded in 96-well microplates at 5×10^3 cells/well overnight and then exposed in serial concentrations of *Ganoderma* polysaccharides or 0.3 μg/mL LPS for 24 h. Equal volume of medium was used as vehicle control. MTT at final concentration of 0.5 mg/mL in PBS (pH 7.4) was added and coincubated for an additional 4 h in dark. Finally, the medium was discarded and replaced with 100 μL dimethyl sulfoxide to solubilize formazan crystals presented in cells. The absorbance was read at 570 nm using a microplate reader (1420 Multilabel counter victor^3, Perkin-Elmer, Waltham, MA). The results were expressed as ratio of absorbance between treatment and vehicle control groups.

Nitric Oxide Quantification

RAW 264.7 macrophages were seeded in 96-well microplates at 5×10^4 cells/well overnight and then stimulated with serial concentrations of *Ganoderma* polysaccharides or 0.3 μg/mL LPS for 24 h. The collected supernatants were mixed with an equal volume of Griess reagent and incubated at room temperature for 15 min. The absorbance was measured at 540 nm with a microplate reader. The nitric oxide (NO) productions were expressed as ratio of absorbance values between treatment groups and LPS treated-group.

Phagocytic Activity Assay

RAW 264.7 cells were seeded in 24-well plates at 20×10^4 cells/well in 500 μL medium overnight, and then exposed to serial concentrations of *Ganoderma* polysaccharides for another 18 h. Equal volume of 0.3 μg/mL LPS or medium was used as positive or vehicle control. Then 5 μL rainbow beads (about 1.0×10^7 particles/well) were added and continually incubated for additional 2 h in dark, and the cells were finally collected in 500 μL PBS. Flow cytometry (BD Biosciences, San Jose, CA) was used to test the percentage of beads ingested macrophages. The results were expressed as ratio of phagocytic rate between treated and vehicle control groups.

Determination of Cytokines

RAW 264.7 macrophages seeded in 96-well microplates at 5×10^4 cells/well overnight and then exposed to serial concentrations of *Ganoderma* polysaccharides or 0.3 μg/mL LPS for 24 h. The cell supernatants were collected by centrifugation at 1,000 × g for 10 min. The cytokines level (pg/mL) of IL-1α, IL-6, IL-10, and tumor necrosis factor (TNF)-α in the culture supernatant was measured by using a Luminex assay (Bio-Plex™ 200, Hercules, CA) with commercially available Millilex Map kits according to the manufacturer's instructions. In brief, 50 μL of standard of cytokines or test samples along with 50 μL mixed beads were added into the wells of a prewet 96-well filter plate and incubated overnight at 4°C. After washing, 25 μL detection antibodies were added and incubated for 1 h at room temperature. Subsequently, 25 μL streptavidin-PE were added and incubated for another 30 min, and then washed. Finally, the beads were suspended in 150 μL assay buffer and analyzed by using Bio-Plex 200 instrument (Bio-Rad, Hercules, CA). The data were analyzed using Bio-Plex Manager™ software 5.0 (Bio-Rad).

Determination of Endotoxin Contamination

The endotoxin concentration in the *Ganoderma* polysaccharides were tested by using Limulus Amebocyte Lysate assay (Lonza, Walkersville, MD) with a Glucashield reconstitution buffer formulated to block interference of (1→3)-β-D-glucans (Associates of Cape Cod, Falmouth, MA). In brief, the concentrations of endotoxin in the test samples were calculated from the absorbance values of solutions containing known amounts of endotoxin standard. An *E. coli* O113:H10 endotoxin (Associates of Cape Cod) was used as endotoxin standard. The results indicated that the *Ganoderma* polysaccharides contained less than 0.06 ng endotoxin/μg. That could exclude the possibility of endotoxin contamination in *Ganoderma* polysaccharides.

Statistical Analysis

Data were expressed as mean ± SEM of at least 3 independent experiments performed in quadruplicates for each sample. Differences between groups were analyzed using 1-way analysis of variance followed by Turkey post-hoc test (GraphPad Prism 5.0, San Diego, CA). Values of $P < 0.05$ were considered as statistical significant.

RESULTS

Characters of Polysaccharides from *Ganoderma*

The yield of polysaccharides from two *Ganoderma* species was 0.7% (W/W, g/g), respectively, which were very low and similar. The sugar content determined by phenol-sulfuric acid method in GLPS and GSPS was 81.2% and 75.1% (W/W, g/g), respectively (Table 1).

TABLE 1
Yield, carbohydrate content, molecular weight, and compositional monosaccharide of *Ganoderma* polysaccharides

Polysaccharides	Yield (%, g/g)	Total carbohydrates (%, g/g)	Molecular weight		Compositional monosaccharide	
			Peak 1	Peak 2	Monosaccharide	Molar ration
GLPS	0.7	81.2	2.6×10^6	1.6×10^4	Glu: Man: Gal: Fuc	246:10: 43:6
GSPS	0.7	75.1	—	1.9×10^4	Glu: Man: Gal: Fuc	75:10: 23:4

GLPS = polysaccharides from *G. lucidum*; GSPS = polysaccharides from *G. sinense*; Glu = glucose; Man = mannose; Gal = galactose; Fuc = fucose; – = not detected.

Their profiles and Mw, as well as Mw distribution, were determined using HPSEC-MALLS-RI detection. The results showed that GLPS had 2 fractions with 2.6×10^6 and 1.6×10^4 of Mw (Fig. 2A), while GSPS only had 1.9×10^4 of Mw fraction (Fig. 2B). Moreover, the monosaccharide components of the *Ganoderma* polysaccharides were analyzed by GC-MS (Fig. 3). The results showed that 2 polysaccharides mainly contained glucose, mannose, galactose, and minor amount of fucose (Table 1). Especially, GLPS contained higher ratio of glucose.

Effect of Polysaccharides from *Ganoderma* on Macrophages Viability

The MTT results showed that 2 *Ganoderma* polysaccharides had no obvious cytotoxicity towards RAW 267.4 cells at all the concentration after 24 h culture. Actually, they might show a certain promotion of macrophage proliferation at the concentration of 19-37.5 μg/mL ($P < 0.05$, Fig. 4A).

Effect of Polysaccharides from *Ganoderma* on NO Production

NO is one of the smallest endogenous molecules involved in the macrophage functions. NO has been noted in cancer

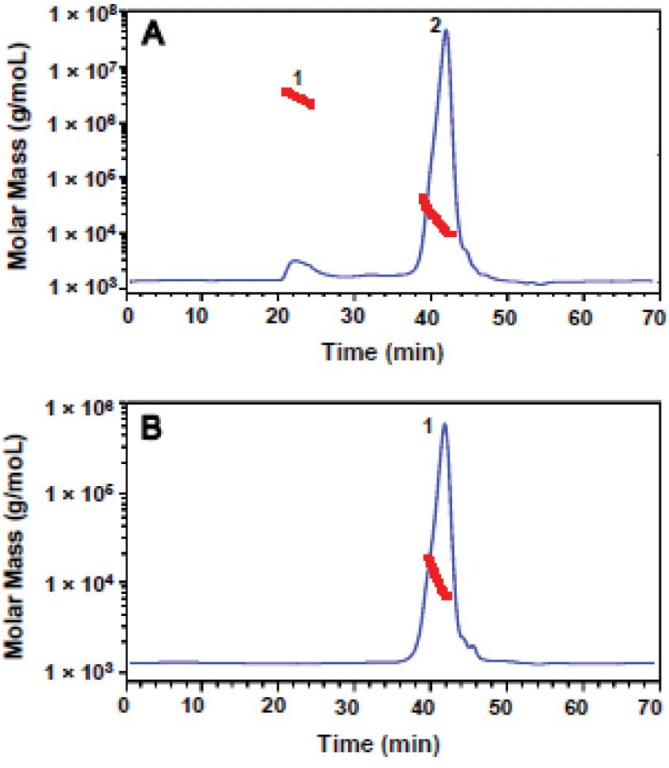

FIG. 2. HPSEC-RID profiles with molecular weight distribution of polysaccharides from (A) *G. lucidum* and (B) *G. sinense*. Blue (thin) lines represent refractive index signals and red (thick) lines represent Mw distribution.

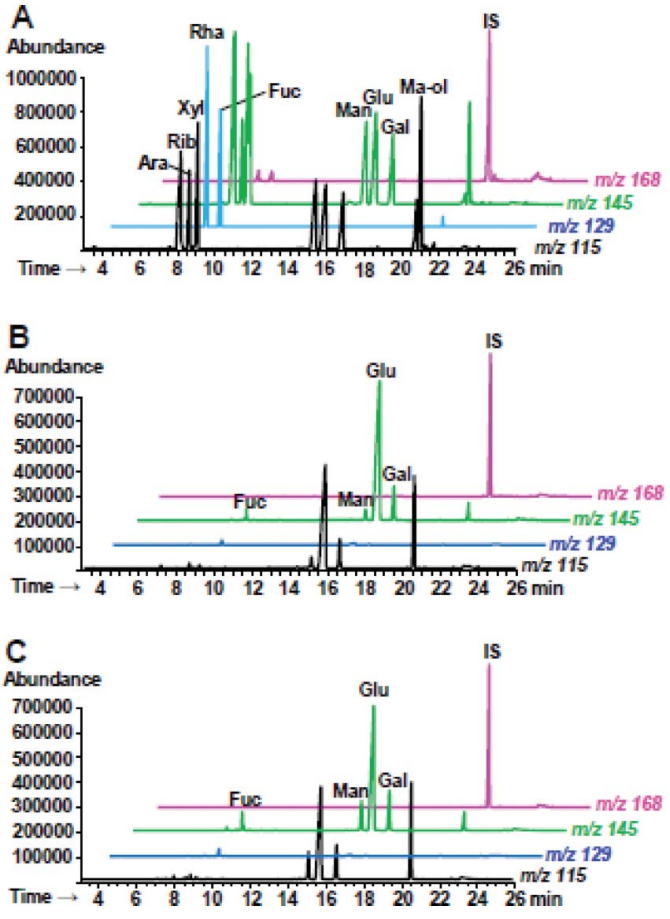

FIG. 3. Typical SIM chromatograms of (A) mixed standards and polysaccharides from (B) *G. lucidum* and (C) *G. sinense*. Rib = ribose; Ara = arabinose; Xyl = xylose; Ma-ol = mannitol; Rha = rhamnose; Fuc = fucose; Man = mannose; Glu = glucose; Gal = galactose; IS = internal standard.

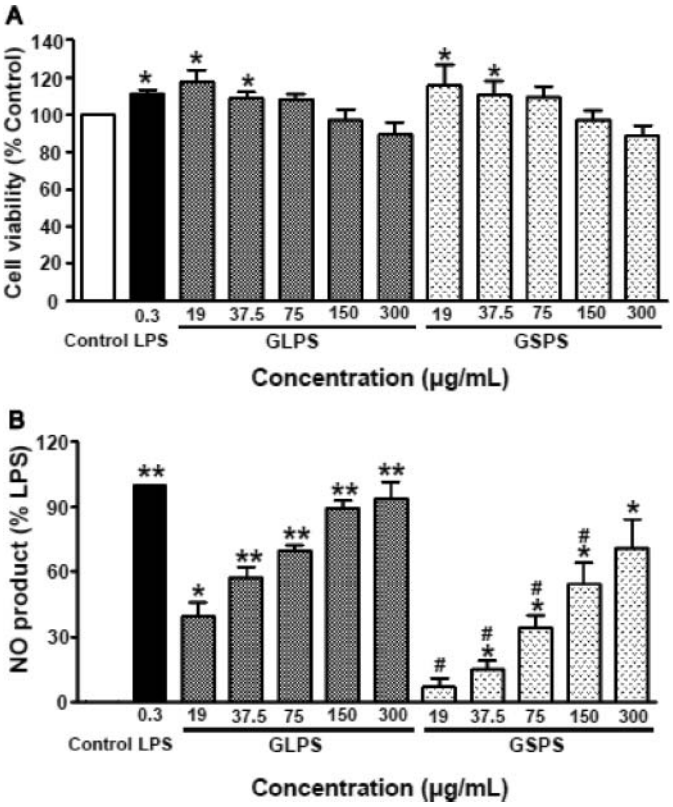

FIG. 4. A: Effect of *Ganoderma* polysaccharides on RAW 264.7 macrophages viability. The percentage of cell viability was calculated relative to the vehicle control group. B: Effect of *Ganoderma* polysaccharides on nitric oxide (NO) production from RAW 264.7 macrophages. The percentage of NO production was calculated relative to the lipopolysaccharide (LPS)-treated group. All data shown as mean ± SEM of 3 independent experiments. $*P < 0.05$. $**P < 0.001$ vs. control. $\#P < 0.05$ vs. polysaccharides from *G. lucidum* (GLPS). GSPS = polysaccharides from *G. sinense*.

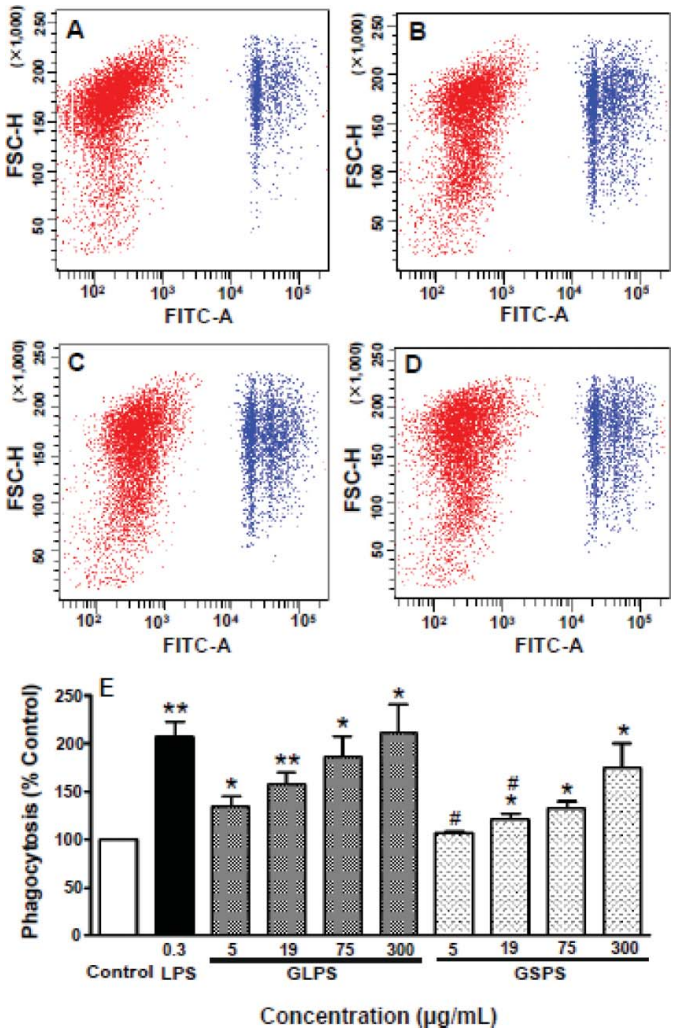

FIG. 5. Typical flow cytometry profiles with normal cells (red [left]) and phagocytosed beads (blue [right]) of phgocytosis of RAW 264.7 macrophages treated with (A) culture medium, (B) lipopolysaccharide (LPS), and both (C) polysaccharides from *G. lucidum* (GLPS) and (D) polysaccharides from *G. sinense* (GSPS) at 300 μg/mL, as well as (E) quantitative analysis of RAW 264.7 macrophages phagocytosis. Data were expressed as mean ± SEM of 3 independent experiments. $*P < 0.05$. $**P < 0.001$ vs. control. $\#P < 0.05$ vs. GLPS.

biology associated with cancer cell apoptosis, cancer cell cycle, cancer progression and metastasis, cancer angiogenesis, cancer chemoprevention, and modulator for chemo/radio/immunotherapy (35,41). The release of NO from RAW 264.7 macrophages upon incubation with polysaccharides from *Ganoderma* was detected. As shown in Fig. 4B, both GLPS and GSPS stimulated NO production released from macrophages, which respectively at most induced 94.2 ± 7.2%, 74.2 ± 10.1% NO production of LPS (0.3 μg/mL) ($P < 0.05$). GLPS (19–150 μg/mL) was more potent than GSPS at the same concentration ($P < 0.05$).

Effect of Polysaccharides from *Ganoderma* on Phagocytosis

Fluorescent beads are commonly used in phagocytosis assays because they are easy to manipulate and can be readily determined by flow cytometry for their exceptional intense fluorescence after laser excitation (42). Flow cytometry could not only count cell numbers, but also detect the intensity of fluorescence to evaluate phagocytosis activity of macrophages. Typical flow cytometric profiles of beads ingested macrophages in different groups were shown in Fig. 5A–5D. The left area indicated normal cells, whereas right one showed macrophages with phagocytosed beads. The stronger intensity of FITC meant more beads captured by cells. The results indicated that 2 *Ganoderma* polysaccharides (19–300 μg/mL) and LPS (0.3 μg/mL) could significantly enhance macrophages phagocytosis, respectively ($P < 0.05$, Fig. 5E). The maximum effect of GLPS was similar to that of 0.3 μg/mL LPS, which was a more than twofold increase compared to the vehicle control group ($P < 0.05$). Moreover, GLPS exhibited more potent stimulation

on macrophage phagocytosis than GSPS at the concentration of 5–19 μg/mL ($P < 0.05$, Fig. 5E).

Effect of Polysaccharides from *Ganoderma* on Cytokines Release

Cytokines play a critical role in the defense against tumor development. For instance, IL-1 can inhibit the proliferation and induces death of various cancer cells (43,44). IL-6 synergizes with IL-1α/β promotes T cell proliferation, T helper cell differentiation, and development of T cell-mediated cytotoxicity (45,46). Although IL-10 is commonly regarded as an anti-inflammatory cytokine, a wealth of evidence is accumulating that IL-10 also possesses some immunostimulating properties (47). Moreover, IL-10 is needed for T-helper cell functions, T-cell immune surveillance, and suppression of cancer-associated inflammation (47,48). TNF-α is the most important mediator directly involved in tumor cell killing of macrophages, which can induce programmed cell death, while leaving normal cells unscathed (35,49). The effects of *Ganoderma* polysaccharides on the release of IL-1α, IL-6, IL-10, and TNF-α from RAW 264.7 macrophages were determined. The data showed that they could significantly increase the production of IL-1α, IL-6, IL-10, and TNF-α from macrophages ($P < 0.05$, Table 2). Generally, at the same concentration, GLPS were more potent than GSPS on cytokines release from macrophages. Comparing to vehicle control (37.7 $\pm$ 3.7 pg/mL), GLPS (300 μg/mL) greatly increased release of IL-6 from macrophages to 14965.5 $\pm$ 831.9 pg/mL ($P < 0.001$), whereas GSPS only induced that to 2471.8 $\pm$ 94.8 pg/mL ($P < 0.001$) at the same concentration. Similarly, GLPS at most induced release of IL-10 from macrophages to 12806.6 $\pm$ 114.3 pg/mL, which was greatly more than that of vehicle control 47.0 $\pm$ 5.0 pg/mL ($P < 0.001$), whereas GSPS only mostly increased that to 2722.5 $\pm$ 195.1 pg/mL at the same concentration ($P < 0.05$, Table 2).

DISCUSSION

Ganoderma have been used as food and for therapeutic purpose for decades, and various compounds derived from these have potential biological activities. Many studies indicated that GLPS enhanced the immune response of the human body to exhibit the antitumor effect (11,22,28,50). As 1 of 2 *Ganoderma* species used as *Linzhi*, few studies focused on GSPS (31,33,34). Macrophages, which play an important role in the initiation of adaptive immune responses, are involved in the defensive line against microbial invasion and cancer growth (35). Activated macrophages can inhibit the growth of a variety of tumor cells and microorganisms in a direct manner, involving the increase of phagocytosis (51) and release of cytotoxic substances, such as reactive oxygen species, NO, and TNF-α (35). On the other hand, indirect antimicrobial or antitumor activities also derived from the secretion of cytokines and chemokines, such as IL-1, IL-6, and IL-12 (52). The results in this study showed that polysaccharides from GL and GS enhanced macrophages functions, such as increasing macrophage phagocytic activity, promoting their release of NO production and cytokines IL-1α, IL-6, IL-10, and TNF-α. These results indicated that GLPS and GSPS had potent stimulatory effects on macrophage functions, which is accordance with the previous reports (9). In addition, this study offered the concentration of GLPS (19–300 μg/mL) which might have immune-enhancing effects in vivo, which was consistent with the common dose of GL (50–300 g per day) in clinical use and previous human report (50).

Antiproliferative activity of ethanolic extracts from GL and GS was compared, and the former was found to be more potent

TABLE 2
Effects of *Ganoderma* polysaccharides on the secretion of cytokines from RAW 264.7 macrophages

Samples	Concentration (μg/mL)	Levels of cytokines (pg/mL)			
		IL-1α	IL-6	IL-10	TNF-α
Control	0	41.4 $\pm$ 12.9	37.7 $\pm$ 3.7	47.0 $\pm$ 5.0	70.2 $\pm$ 3.2
LPS	0.3	139.3 $\pm$ 17.1**	10204.1 $\pm$ 331.5**	6688.9 $\pm$ 467.5**	11312.9 $\pm$ 399.8**
GLPS	19	149.4 $\pm$ 28.5*	1352.9 $\pm$ 188.8*	708.4 $\pm$ 58.3**	5357.1 $\pm$ 474.5*
	75	183.1 $\pm$ 14.7*	7055.7 $\pm$ 612.9**	4156.6 $\pm$ 186.6**	6638.1 $\pm$ 13.5**
	300	194.4 $\pm$ 12.2**	14965.5 $\pm$ 831.9**	12806.6 $\pm$ 114.3**	8088.3 $\pm$ 640.9**
GSPS	19	105.4 $\pm$ 9.0*	99.6 $\pm$ 18.9*#	123.8 $\pm$ 10.0*#	3109.7 $\pm$ 395.4**
	75	137.1 $\pm$ 17.3*	671.5 $\pm$ 78.2*#	642.7 $\pm$ 51.1**#	7247.9 $\pm$ 1598*
	300	190.2 $\pm$ 33.3**	2471.8 $\pm$ 94.8**#	2722.5 $\pm$ 195.1**#	10693.5 $\pm$ 1032.4**

Data were shown as mean $\pm$ SEM of 3 independent experiments. LPS = lipopolysaccharide; GLPS = polysaccharides from *G. lucidum*; GSPS = polysaccharides from *G. sinense*; IL = interleukin; TNF-α = tumor necrosis factor-α.
*$P < 0.05$. **$P < 0.001$ vs. control. #$P < 0.05$ vs. GLPS group.

than the latter due to the higher content of triterpenes (4,9). In this study, GLPS showed more potent regulation of macrophages than GSPS, such as release of NO and cytokines (IL-6 and IL-10). Actually, GLPS also showed stronger ability to enhance macrophages phagocytosis than GSPS. But significant differences were not found at the high concentrations (75–300 μg/mL), which might because of the big standard deviations. IL-1 and TNF-α are currently assumed to be the primary mediators involved in the macrophage killing of tumor cells (36). Previous studies showed that GLPS could activate bone marrow-derived macrophages from tumor-bearing mice and produce immunomodulatory substances, such as IL-1β, TNF-α, and NO (36). GSPS was also found to significantly induce the release of TNF-α, IL-1β in human peripheral blood mononuclear cell (33). In this study, GLPS and GSPS showed similar effects on the secretion of IL-1α and TNF-α from macrophages. This might due to the feedback regulation of NO production in the culture supernatant of macrophages (53–55). It has been known that the biological activities of polysaccharides are related to their chemical composition, configuration, and chain conformation, as well as their physicochemical properties. The molecular size of polysaccharides is an important physico-chemical parameter that may correlate with their biological activity, for example, the activity of (1→3)-β-glucans is strongly dependent on their Mw (56). GLPS have been deeply investigated, and the major bioactive polysaccharides are glucans with (1→3)-β-linkages of main chain and (1→6)-β-linkage for branch (23,28). (1→3)-β glucans with anti-tumor activity were also found from GS (30). We previously found that some chemical characteristics, namely HPTLC profiles of trifluoroacetic acid (39), 1,3-β-D-glucosidic, and 1,4-α-D-galactosiduronic linkages (33), were similar in polysaccharides from GL and GS. However, they had different pectinase treated saccharide mapping (57). The similar compositional sugars were found in GL and GS (39), nevertheless, they existed in various molar ratios. In the present study, varied effects of GLPS and GSPS on macrophage functions might attribute to their different Mw, ratio of compositional monosaccharides and glycosidic linkages. Purified polysaccharides are necessary to clearly understand the different immunomodulation of GL and GS. Recently, Han et al. reported that GSPS characterized with backbones of 1→6-linked-β-D-glucopyranosyl residues (32), 1→4-linked-β-D-glucopyranosyl, and 1→6-linked-β-D-glucopyranosyl (33). More structural features of GSPS are needed to study in future.

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