

Critical Review

**Cellular and Molecular Mechanisms of Immuno-modulation
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Abstract. *Ganoderma lucidum* (Leyss. ex Fr.) Karst. (*Lingzhi* or *Reishi*) has been used for a long time in China to prevent and treat various human diseases. *G. lucidum* polysaccharides extracted from *G. lucidum* are one of efficacious ingredient groups of *G. lucidum*. A number of reports have demonstrated that *G. lucidum* polysaccharides modulate immune function both in vivo and in vitro. The immuno-modulating effects of *G. lucidum* polysaccharides were extensive, including promoting the function of antigen-presenting cells, mononuclear phagocyte system, humoral immunity, and cellular immunity. Cellular and molecular mechanisms, possible receptors involved, and triggered signaling cascades have also been studied in vitro. However, whole animal experiments are still needed to further establish the mechanism of the immuno-modulating effects by *G. lucidum*. Evidence-based clinical trials are also needed.

Keywords: *Ganoderma lucidum*, polysaccharide, immuno-modulation, mechanism

Introduction

Ganoderma lucidum (Leyss. ex Fr.) Karst. (*G. lucidum*: *Lingzhi* in Chinese, *Reishi* in Japanese) has been used for a long time in China to prevent and treat various human diseases. *Lingzhi* was classified as a drug of “high grade”, that is, a herb of medicinal value and without toxicity in the Shen Nong’s Materia Medica (*Shen Nong Ben Cao Jing*), which was published in the second century B.C. Li Shi-Zhen, a well-known ancient Chinese medicinal scientist, also described the efficacy and medical uses of *Lingzhi* in the world renown classic Compendium of Materia (*Ben Cao Gang Mu*) in the 16th century. Ancient Chinese medical scholars held the view that *G. lucidum* could strengthen body resistance and consolidate the constitution of patients, that is, “*Fuzheng Guben*”, which is one of the major principles in the therapeutics of traditional Chinese medicine (1). In the Chinese Pharmacopoeia (2000 ed., Vol. 1), both *G. lucidum* and *G. sinensis* are listed as *Lingzhi*. *G. lucidum* has been under modern pharmacological and

clinical research in the recent 30 years, and it has been reported to be effective in modulating immune functions, inhibiting tumor growth. Polysaccharides are one of efficacious ingredient groups of *G. lucidum*. A number of reports have demonstrated that *G. lucidum* polysaccharides modulate immune function both in vivo and in vitro. The immuno-modulating effects of *G. lucidum* polysaccharides are extensive, including promoting the function of antigen-presenting cells (APC), mononuclear phagocyte system, humoral immunity, and cellular immunity. Recently, the anti-tumor effects of *G. lucidum* polysaccharides have been deeply investigated and are believed to be going through immune mechanisms. Present review on cellular and molecular mechanisms of immuno-modulation by *G. lucidum* is built on the base of our research with references.

Effect of *G. lucidum* on macrophages

Macrophages are important immune cells, preferentially located near potential entry sites for microbial pathogens and specialized for the uptake of particulate material by phagocytosis. Most macrophages originate from peripheral blood monocytes and are able to leave

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the circulation following stimulation by chemotactic agents. A number of studies demonstrated that water extracts of the fruiting bodies of *G. lucidum* or *G. lucidum* polysaccharides could enhance phagocytosis of peritoneal macrophages in vivo and in vitro (1, 2). Further studies demonstrated that interleukin (IL)-1 and tumor necrosis factor- α (TNF- α) productions significantly increased in mouse peritoneal macrophages treated with *Ganoderma* polysaccharides (3). Berovic et al. also reported that a preparation of polysaccharides isolated from *G. lucidum*, which was mainly composed of β -D-glucanes, could induce TNF- α synthesis in primary cultures of human peripheral blood mononuclear cells (4). Our studies also showed that the addition of *G. lucidum* polysaccharides B (GL-B), which were extracted from fruiting body of *G. lucidum* with lower molecular weight of 6900–9100 (25–400 $\mu\text{g}/\text{mL}$), to the in vitro macrophages culture media resulted in a significantly increased TNF- α mRNA expression in a concentration-dependent manner (5). Following the administration of *G. lucidum* extract at 5, 10, or 20 g (crude material)/kg by forced stomach tube feeding, we found that TNF- α mRNA expression in the peritoneal macrophages was also increased markedly (6). These results indicate that the water extract and the polysaccharides fraction of *G. lucidum* could induce TNF- α expression in vivo and in vitro. The group of Tang and Zhang investigated the activation of mouse macrophages by the alkali-extracted polysaccharides from the spores of *G. lucidum* (LZSBS) in vitro. The result showed that LZSBS (500 mg/L) significantly increased the activation of mouse macrophages by 340%. LZSBS (200 mg/L) could promote IL-1 β and TNF- α secretion and nitric oxide (NO) production in mouse macrophages in vitro. The percentage of phagocytosis of latex granules by mouse macrophages was also significantly increased in the presence of LZSBS (200–500 mg/L) (7).

In a recent study, we used *tert*-butylhydroperoxide (tBOOH) as an oxidant to produce oxidative damage stress on macrophages, and then we observed the effect of *G. lucidum* polysaccharide peptide (GLPP) on oxidative stress. GLPP isolated from fruiting body of *G. lucidum* was a hazel-colored powder with an average molecular weight of 5.13×10^5 and contained 16 kinds of amino acid. GLPP consisted of rhamnose, xylose, fructose, galactose, and glucose with molar ratio of 0.549:3.614:3.167:0.556:6.89 and linked together by β -glycosidic linkages. The result showed that GLPP could prevent tBOOH-induced oxidative injury of macrophages in vivo and in vitro. GLPP increased the survival rate of macrophages injured by tBOOH. The morphology change under the light microscope and electron

microscope showed that GLPP could protect the cell organelles such as mitochondria and endoplasmic reticulum (ERs) against injury (Figs. 1–3) (8). We observed further that the free radical scavenging activity of GLPP on mice peritoneal macrophages injured by alloxan or tBOOH in vivo and in vitro, respectively. 7,2-Dichlorodihydrofluorescein diacetate was used as a fluorescent probe. The fluorescence from cells was observed under the laser confocal microscope. Time series scan of confocal microscope was used to observe the changes of fluorescence by GLPP in mouse perito-

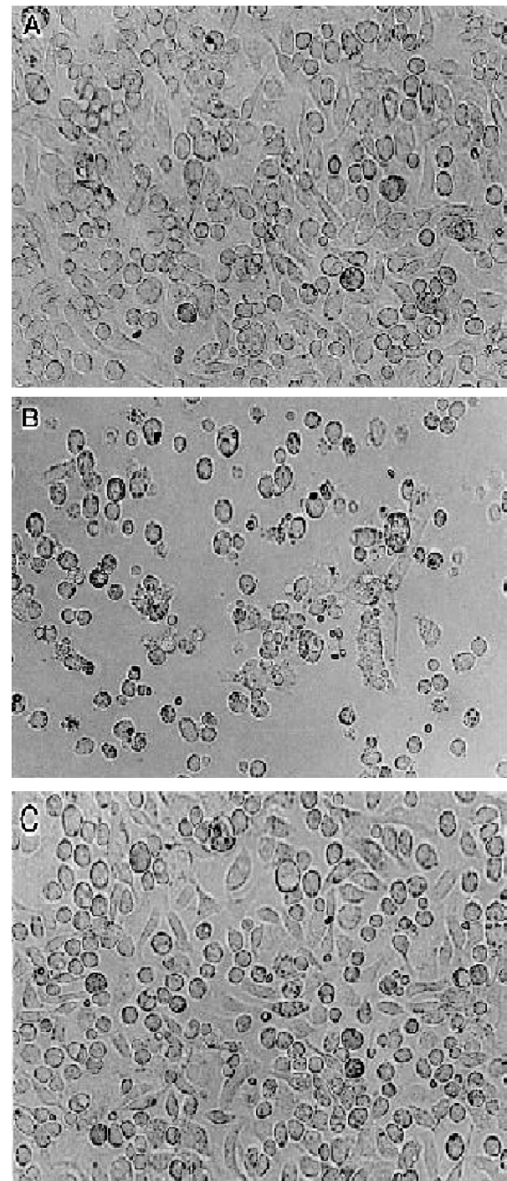


Fig. 1. Effect of GLPP on mouse macrophages injured by tBOOH. Light microscope, $\times 400$, A: Control (without treatment with tBOOH), B: treatment with tBOOH, C: treatment with tBOOH plus GLPP. Modified from Ref. 8 with permission.

neal macrophages over time. The results of confocal microscopy showed that GLPP (100 mg/kg, i.g. for 5 days) lowered fluorescence in the mice macrophages injured by alloxan (75 mg/kg, i.v.). GLPP (10 mg/L) also lowered fluorescence in the mice macrophages injured by tBOOH (7.76×10^{-5} mol/L) *in vitro*. Time series scan showed that GLPP (10 mg/L) lowered fluorescence in the mouse macrophages in the resting

state or during the respiratory burst induced by PMA (50 nmol/L) (9). To further study the protective effect of GLPP on macrophage mitochondrial membrane potential during free-radical-induced cell injury induced by tBOOH in mice, the mitochondrial membrane potentials were detected with a fluorescence marker, Rh123, using laser scanning technology. The results showed that oxidant tBOOH could cause mitochondrial membrane injury, decrease the membrane potential. Administration of GLPP (100 mg/kg, i.g.) for 5 days or 10 mg/L, *in vitro* could recover macrophage mito-

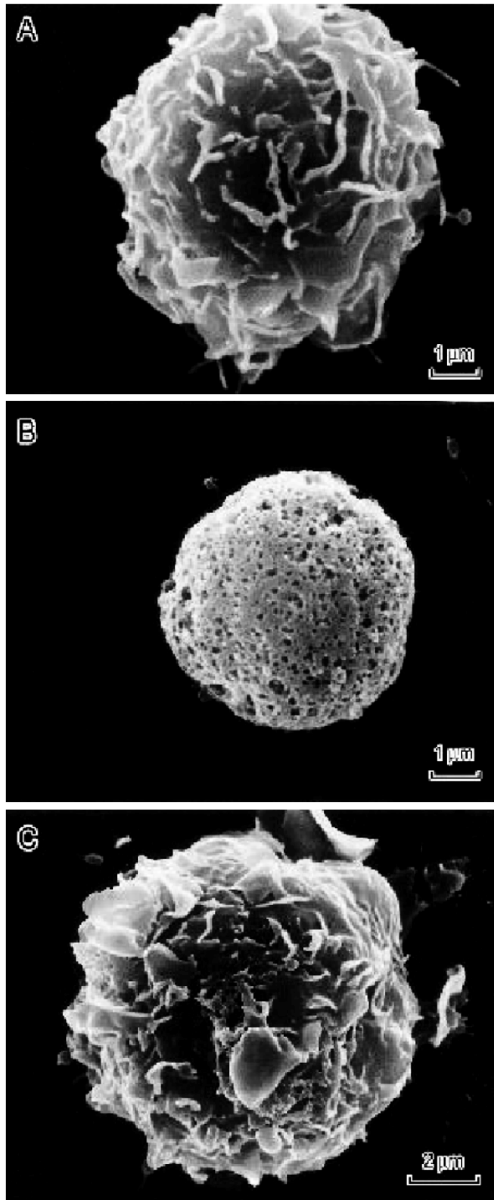


Fig. 2. A macrophage incubated with tBOOH (0.1 mmol/L) for 24 h was observed under a scanning electron microscope. A: Long microvilli were observed in the control group ($\times 11000$), B: membrane of macrophage became smooth in the tBOOH-treated group ($\times 11000$), C: a few microvilli of the macrophage were slightly shorter in the GLPP-treated group ($\times 8000$). Modified from Ref. 8 with permission.

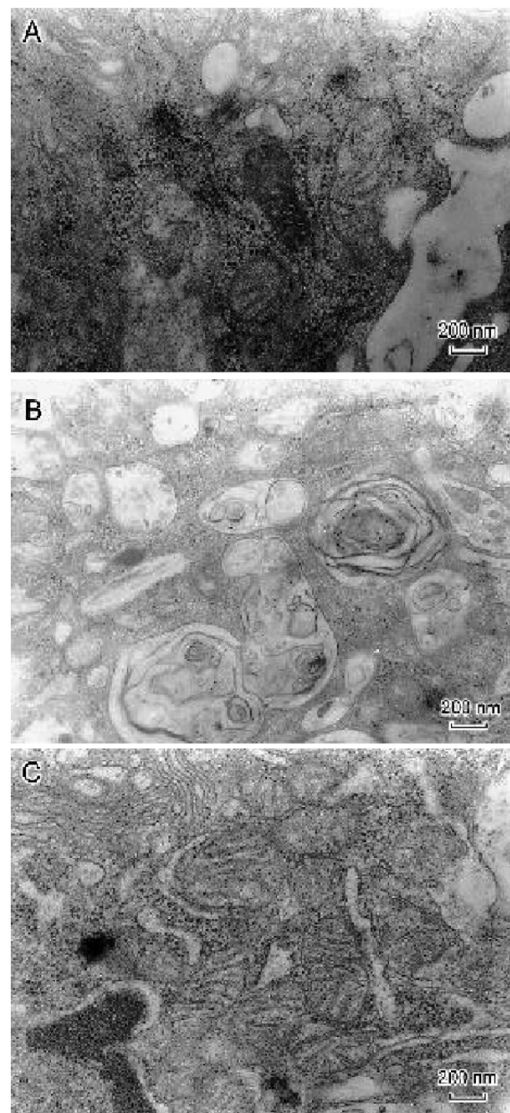


Fig. 3. Structure of macrophage incubated with tBOOH (0.01 mmol/L) for 24 h was observed under a transmission electron microscope. A: The structure of mitochondria was normal in control group ($\times 30000$), B: structure of mitochondria became stratified in the tBOOH-treated group ($\times 25000$), C: the cristae of mitochondria were slightly disorganized or unchanged in the GLPP-treated group ($\times 30000$). Modified from Ref. 8 with permission.

chondrial membrane potential. It means GLPP could protect macrophage mitochondrial membrane and alleviate the membrane injury by free radicals in vivo and in vitro (10). The mitochondria, which were the major site producing reactive oxygen species (ROS) and also subjected to great injury by ROS, were significantly protected by GLPP. It suggests that GLPP has potential scavenging ROS and antioxidant effects. Mitochondrial membrane may be the site where GLPP exerts its effect.

Recently, it was found that NO production was increased by administration of GLPP (25–200 mg/kg, i.g.) for 5 days in mice or GLPP (3.125–200 mg/L) in vitro. The expression of inducible nitric oxide synthase (iNOS) was also increased by administration of GLPP (25–200 mg/kg, i.g.) for 5 days in mice or GLPP (3.125–200 mg/L) in vitro. The result indicates that the mechanism of increasing NO production by GLPP may be related to enhancement of iNOS synthesis in mouse peritoneal macrophages (11).

Possible receptor in macrophage and trigger a signaling cascade

It is plausible to assume that the polysaccharides bind to a certain receptor in macrophages and trigger a signaling cascade, which is involved in the regulation of function, cytokine synthesis, and release in macrophages. The group of Li and Lei conducted a series of investigations to determine the effects of *Ganoderma* polysaccharides B₇ (GL-B₇) on involved signaling events in macrophages. Intracellular calcium concentration ($[Ca^{2+}]_i$) in a single mouse peritoneal macrophage was determined by laser scanning confocal microscope imaging of the calcium fluorescent indicator dye Fluo3/AM. GL-B₇ (20 μg/mL) induced an increase in $[Ca^{2+}]_i$ of about 248%. By the addition of EGTA (5 mmol/L) to chelate the extracellular calcium and pretreatment of the cells with verapamil to block the calcium channel, the increase of $[Ca^{2+}]_i$ induced by GL-B₇ was markedly decreased to about 78%, but not completely abolished. In calcium-free medium, GL-B₇ triggered a weak increase in $[Ca^{2+}]_i$ and then a plateau appeared. Addition of calcium-containing buffer at the plateau caused a further increase in $[Ca^{2+}]_i$, resulting in another higher plateau. The data indicate that GL-B₇ induced both influx of extracellular calcium and the release of calcium from intracellular calcium stores (12). ER is considered to be the intracellular calcium stores. Inositol triphosphate (IP₃) and ryanodine receptors on the membrane of ER mediate the release of calcium from the intracellular calcium stores into the cytosol. Actually, GL-B₇ could induce IP₃ formation in macrophages. It suggests that

the cytosolic calcium increase triggered by the polysaccharide might be, at least in part, mediated by the IP₃ pathway. In addition, GL-B₇ also stimulated the generation of diacylglycerol (DAG) which is the activator of protein kinase C (PKC) (13). As expected, treatment of the cells with GL-B₇, PKC activity was rapidly increasing, reaching a peak value at 30 min, and the subcellular translocation of PKC activity from the cytosol to the membrane was also observed (14). Moreover, cyclic adenosine monophosphate (cAMP), a second messenger involved in many physiological processes, accumulated along with the stimulation of GL-B₇ to the macrophages (15). A recent study revealed that exposure of human neutrophils to *G. lucidum* polysaccharides time-dependently caused increases in PKC, p38 mitogen-activated protein kinase (MAPK), hematopoietic cell kinase (HCK), and other tyrosine kinase Lyn activities, these may be the action that corresponded to an enhanced unspecific immune function (16). Hsu et al. recently reported that *G. lucidum* was able to enhance phagocytic activity and migration of human primary neutrophils and inhibit spontaneous and Fas-induced neutrophil apoptosis in vitro primarily relies on activation of Akt-regulated signaling pathways (17).

Toll-like receptors are part of a family of related receptors that mediate innate immunity against a variety of microbes. Activation of toll-like receptors leads to secretion of immuno-stimulatory cytokines, leading to an enhanced immune response biased to a cytotoxic T-cell response. As well known, toll-like receptor 4 (TLR4) is one of the main pattern recognition receptors expressed by macrophages. In a recent study, our collaborators showed that fluorescence-labeled APS (polysaccharides from *Astragalus membranaceus*; fl-APS) positively stained human and mouse macrophages in a TLR4-dependent manner. Interestingly, *G. lucidum* polysaccharides (GL-PS) isolated from *G. lucidum* with an average molecular weight of 584900 were able to competitively inhibit the binding of fl-APS with mouse peritoneal macrophages as determined by flow cytometric analysis, implying that GL-PS is able to bind directly with TLR4 on the macrophage surface. Furthermore, GL-PS induced significant IL-1β production by peritoneal macrophages from BALB/c but not C3H/HeJ mice, also suggesting that GL-PS activated macrophages via TLR4 (18). Hsu et al. also demonstrated that TLR4, but not complement receptor type 3, is a putative receptor of the extract of *G. lucidum* (*Reishi*) polysaccharides (EORP), mediating the consequent immuno-modulating events associated with IL-1 gene expression. They have found that the EORP differentially modulates the protein kinase (PK)-mediated signal transduction pathways associated with inflammatory cytokine IL-1.

In human macrophages and murine macrophage J774A.1 cells, EORP was found to up-regulate IL-1 secretion and precursor of IL-1 (pro-IL-1) as well as IL-1-converting enzyme expression. Specifically, EORP rapidly stimulates protein tyrosine kinase-mediated phosphorylation, followed by induction of PKs and activation of MAPKs: ERK, JNK, and p38. These findings establish that the extract of *Reishi* polysaccharides induces cytokine expression via TLR4-modulated PK signaling pathways (19).

Although we have some information about the receptor-signaling events related to the roles of *Ganoderma* polysaccharides, we still do not know the exact signaling pathways involved in given functions of macrophages affected by the polysaccharides. It is, therefore, necessary to further investigate the involved signaling pathways by linking them to some given macrophage functions.

***G. lucidum* polysaccharides promote maturation and function of dendritic cells**

Dendritic cells (DC), a kind of important professional APC, are crucial for the initiation of primary immune response of both helper and cytotoxic T lymphocytes (CTL). We established the culture of murine bone marrow derived DC in vitro and further explored whether *Gl*-PS have regulatory effects on maturation and function of DC. The result showed that *Gl*-PS at the concentration of 0.8, 3.2, and 12.8 $\mu\text{g}/\text{mL}$ could increase the co-expression of CD11c and I-A/I-E molecules on the DC surface, promote mRNA expression of cytokine IL-12 p40 in DC, and augment protein production of IL-12 P40 in culture supernatants. Our research showed that *Gl*-PS up-regulated the coexpression of I-A/I-E and CD11c on DC surface, mRNA expression and protein secretion of IL-12 p40 unit, which indicated that *Gl*-PS could promote the maturation of DC in the presence of lipopolysaccharide (LPS). On the other hand, the up-regulation of co-expression of I-A/I-E and CD11c on the DC surface also indicated the mechanism by which *Gl*-PS promotes the maturation of DC may be related to its effect on I-A/I-E expression. The lymphocyte proliferation of mixed lymphocyte culture (MLC) induced by mature DC was also enhanced by *Gl*-PS. These data demonstrate that *Gl*-PS promotes not only the maturation of cultured murine bone marrow derived DC in vitro, but also the immune response initiation induced by DC (20). Further data show that *Gl*-PS are able to promote the cytotoxicity of specific CTL induced by DC during the stage of antigen presentation mainly through interferon (IFN)- γ and granzyme B pathways (21).

Recently, Lin et al. investigated the effects of the polysaccharide component with a branched (1 \rightarrow 6)- β -D-glucan moiety of *G. lucidum* (PS-G) on human monocyte-derived DC. Treatment of DC with PS-G (10 $\mu\text{g}/\text{mL}$) resulted in the enhanced cell-surface expression of CD80, CD86, CD83, CD40, CD54, and human leukocyte antigen (HLA)-DR, as well as the enhanced production of IL-12 p70, p40, and IL-10 and also IL-12 p35, p40, and IL-10 mRNA expression, and the capacity for endocytosis was suppressed in DC. In addition, treatment of DC with PS-G resulted in enhanced T cell-stimulatory capacity and increased T cell secretion of IFN- γ and IL-10. Neutralization with antibodies against TLR4 inhibited the PS-G-induced production of IL-12 p40 and IL-10, suggesting a vital role for TLR4 in signaling DC upon incubation with PS-G. Further study showed that PS-G was able to augment inhibitor of κB ($\text{I}\kappa\text{B}$) kinase and nuclear factor (NF)- κB activity and also $\text{I}\kappa\text{B}\alpha$ and p38 MAPK phosphorylation. Furthermore, inhibition of NF- κB by helenalin and p38 MAPK by SB98059 prevented the effects of PS-G in the expression of CD80, CD86, CD83, CD40, CD54, and HLA-DR and production of IL-12 p70, p40, and IL-10 in various degrees. Taken together, these data demonstrate that PS-G can effectively and rapidly induce the significant activation and maturation of human DC by the NF- κB and p38 MAPK pathways (22).

Effect of *G. lucidum* on the nature killer cells

Natural killer (NK) cells are large granular lymphocytes, not belonging to either the T- or B-cell lineages. NK cells are considered to be part of the innate defense system since, in contrast to cytotoxic T-cells, they are able to kill certain tumor cells in vitro without prior sensitization. The basal activity of NK cells increases dramatically following stimulation with interferons. In addition, NK cells display Fc-receptors for IgG and are important mediators of antibody-dependent-cell mediated-cytotoxicity. A number of reports indicated that water extracts of the fruiting bodies of *G. lucidum* or *G. lucidum* polysaccharides could enhance activity of NK cells in in vivo experiments (1). Recently, Chien et al. reported that a fucose-containing glycoprotein fraction (F3), isolated from the water-soluble extracts of *G. lucidum*, could increase the presence of the NK cells (CD56(+) marker) significantly from 1.1% to 3.2% in human umbilical cord blood mononuclear cells, indicating that F3 quantitatively influenced NK cell activities. They also found that F3 is not harmful to human cells in vitro; and after F3 treatment, NK-cell-mediated cytotoxicity was significantly enhanced by 31.7% at effector/target cell ratio (E/T) 20:1, but was

not altered at E/T 5:1 (23).

Effect of *G. lucidum* on T lymphocytes and its possible mechanism

A series of investigations from our laboratory demonstrated that the cell-mediated immune function was also enhanced by *G. lucidum*, as suggested by the observations that *G. lucidum* promoted the MLC reaction (24, 25). It also exerted an increasing effect on the induction of delayed hypersensitivity to protein antigen. BN3A, BN3B, and BN3C, three kinds of *G. lucidum* polysaccharides, significantly increased lymphocyte proliferation induced by concanavalin A (ConA) and IL-2 production in the normal mice, as well as in the aged mice in vitro. BN3A and BN3C also could antagonize the suppressive effect of hydrocortisone on the proliferation of mouse spleen cells (1, 26). Further study showed that *G. lucidum* polysaccharides increased the DNA synthesis of spleen cells in MLC through the enhancement of DNA polymerase α induction in the young and aged mice (1, 25). It was found that *G. lucidum* polysaccharides not only increased the contents of nuclear DNA and RNA but also remarkably changed the cell ultrastructure and mean cross section areas of nucleus and cytoplasm and reduced the ratio of the nucleus to cytoplasm in murine splenocytes (27). Moreover, *G. lucidum* increased the production of IFN- γ and significantly increased IFN- γ mRNA expression in the T-lymphocytes (5). Chen et al. reported that *G. lucidum* was effective in repairing the damage of subset T-cells in the spleen of γ -irradiated mice (28). Wang et al. used RT-PCR to identify the cytokines expression in mouse spleen cells after treatment with an F3 isolated from *G. lucidum*. Among six tested cytokines IL-1, IL-2, IFN- γ , TNF- α , IL-4, and IL-6, the first three were observed to express significantly in the presence of F3 (10 μ g/mL) when compared to the expression of a house keeping gene (hypoxanthine phosphoribosyl transferase) (29). Kohguchi et al. studied the immuno-potentiating effects of the antler-shaped fruiting body of *G. lucidum* (*Rokkaku-Reishi*, RR) in mice. These authors analyzed the glycosyl linkage of β -glucan contained in RR. BALB/c mice were administered orally with RR for 3 days at a dose of 50 or 500 mg/kg, and IFN- γ production by splenocytes in response to LPS was examined on day 4. The oral administration of 500 mg/kg of RR resulted in a significant increase in IFN- γ production. Stimulation of splenic adherent cells from these mice with LPS also resulted in a significant increase in IL-12 production compared with that from the control mice, suggesting that splenic macrophages were activated by RR admin-

istration. Furthermore, 500 mg/kg of RR administered for 14 days resulted in a significant increase in IFN- γ production by splenocytes in response to both LPS and ConA. These results suggest that not only splenic macrophages but also T cells were activated by the long-term treatment with RR in vivo. On the other hand, the production of IL-4, which is known as an allergic disease-related cytokine, was not affected by the long-term treatment with RR. The results suggest that the oral administration of RR resulted in Th1-associated immuno-potentiating activities in vivo (30). Recently, the effect of *G. lucidum* on complete blood count (CBC) and blood biochemistry and immuno-competence was studied in horses. Cellular-mediated immunity was monitored by flow cytometry to survey the percentage changes of CD5+, CD4+, CD8+ T-lymphocytes, and B-lymphocytes in the peripheral blood lymphocytes (PBLs). The effect of *G. lucidum* on humoral immunity was examined by the fast plate agglutination test to survey the change and manifestation of the titer of specific anti-egg albumin antibodies in the serum after egg albumin injection. The findings on CBC and blood biochemistry indicated that *G. lucidum* was quite safe to horses. The results on cell-mediated immunity and humoral immunity showed, respectively, that *G. lucidum* could increase the percentage of CD5+, CD4+, and CD8+ T-lymphocytes in PBLs and promote production of specific antibodies in horses (31).

Preliminary studies of the effects of *G. lucidum* polysaccharides on T cell signaling pathways have been carried out in vitro. The group of Li and Lei investigated the effects of GL-B₇ on IP₃ and DAG in murine T cell using radio-immunological assay, anion-exchange columns, and thin-layer chromatography in vitro. The result indicated that GL-B₇ increased the production of IP₃ in resting T cells. The peak of IP₃ was approached at 30 s. Pertussis toxin (PTX) pre-incubated with T cells could inhibit the production of IP₃ by GL-B₇. However, GL-B₇ could promote DAG production in resting T cells, and two DAG peaks were found: the first peak was rapid and transitory, and the second was delayed. However, GL-B₇ did not influence the production of IP₃ and DAG in ConA-active T cells. The result suggests both pathways of signal transduction of IP₃/Ca²⁺ and DAG/PKC may be involved in the immuno-modulation of GL-B₇ to T cells (32). This group further found that GL-B₇ could markedly increase the activities of protein kinase A (PKA) and PKC in murine T cells in a dose-dependent manner. The peak time was at 5 and 20 min and the activities of PKA and PKC returned to basic level at 20 min and 1.5 h, respectively. GL-B₇ could induce translocation of PKC and antagonize the inhibitory effect of staurosporine (10 μ mol/L) on PKC

in T cells. The immuno-potentiating and antitumor effects of *Ganoderma* polysaccharides may be associated with its activation on PKA and PKC in murine T cells (33).

Effect of *G. lucidum* on B lymphocytes

The plaque forming cells (PFC) response is a specific method to examine the effect of medicine on the animal's humoral immune function. *Ganoderma* polysaccharides (BN₃C) i.p. injection promoted the PFC response to the sheep red blood cells not only in the normal mice but also in the aged mice (26). In vitro, *G. lucidum* polysaccharides also significantly increased the lymphocyte proliferation induced by LPS (34, 35). A bioactive fraction GLIS, isolated from the fruiting body of *G. lucidum* could stimulate the activation, proliferation, and differentiation of B lymphocytes. The B lymphocytes were enlarged, expressed CD71 and CD25 on the cell surface, and showed an increase in the secretion of immunoglobulin. Furthermore, the activation of B lymphocytes by GLIS did not depend on the activation of T lymphocytes; it was associated with stimulating the expression of PKC α and PKC γ in B lymphocytes by GLIS directly. However, GLIS did not influence the [Ca²⁺]_i of lymphocytes. According to these results, it showed that GLIS is a new B cell-stimulating factor (36). It has been indicated that *G. lucidum* polysaccharides (in particular, active β -D-glucans) can bind to lymphocyte surfaces through specific receptors or serum-specific proteins, leading to alteration of the activities of macrophages, T-helper cells, NK cells, and other effector cells. These perhaps gave some explanation for the phenomenon of why the immuno-modulatory effects of *G. lucidum* were so extensive (37).

Five-week-old female C57BL/6J mice were fed an AIN-93G diet containing 0, 0.5%, 1%, 3%, and 5% (wt/wt) *G. lucidum* mycelium for 4 weeks. Mice were orally immunized with 5 μ g CT on days 7 and 21 of the feeding period. The total IgA and specific anti-CT IgA level in luminal washes of small intestine, serum, and fecal pellets as well as anti-CT IgG response in serum were determined by ELISA. The total IgA level generally was not affected by *G. lucidum* mycelium except in serum samples. By contrast, the anti-CT IgA antibody response was reduced in all the samples from mice fed diets containing *G. lucidum* mycelium except in the small intestine luminal washes of mice fed the diet containing 5% *G. lucidum* mycelium. The serum anti-CT IgG response was only decreased in mice fed 3% *G. lucidum* mycelium. Thus, *G. lucidum* mycelium reduced the mucosal specific IgA response of young adult mice orally immunized with CT (38).

Other immuno-modulatory effect of *G. lucidum*

Most of the studies demonstrated that *G. lucidum* possessed immune-enhancing action, while some other studies showed that *G. lucidum* also could down-regulate the excessive immune function. It appears that the cytokines-modulating effect of *G. lucidum* polysaccharides would be tissue-specific. It was found that *G. lucidum* at 600 mg/kg, i.p. for 3 days significantly inhibited Forssman cutaneous vasculitis (FCV) in guinea pigs and alleviated their general symptoms of Forssman systemic shock (FSS) in guinea pigs. The results also showed that *G. lucidum* at 300 or 600 mg/kg, i.p. for 3 days could decrease the skin swelling of reversed cutaneous anaphylaxis (RCA) in rats; thus even by the oral administration, *G. lucidum* at 800 mg/kg, i.g. for 6 days appeared to exert the same effect. In another experiment, authors measured the blood pressure, heart rate, and respiration during FSS in guinea pigs and found that *G. lucidum* could prevent the blood pressure suddenly rising that caused by a sublethal dose of anti-serum, but had no significant effect on both heart rate and respiration when compared with the control group (39). *G. lucidum* polysaccharides had potent healing effect on indomethacin-induced gastric lesions in the rat due partly to the suppression of gene expression of TNF- α (40). Application of *G. lucidum* polysaccharides also significantly mitigated hepatic tumefaction, decreased ALT enzyme release and NO production in serum or supernatant, and improved the pathological changes of chronic and acute inflammation in the BCG-induced immune liver injury in mice. Moreover, the immuno-histochemical result showed that *G. lucidum* polysaccharides inhibited iNOS protein expression in the BCG-immune hepatic damage model (41). The triterpenoids isolated from *G. lucidum* also showed significant protective effects against immunological liver damage induced by BCG plus LPS in mice both in vivo and in vitro (42). Recently a study from our laboratory demonstrated that *G. lucidum* polysaccharides i.p. injection could decrease the serum glucose level and the prevalence of diabetes in the multiple low dose streptozotocin-induced autoimmune diabetes (43). Kino et al. reported that LZ-8, an immuno-modulatory lectin isolated from *G. lucidum*, has been shown to have immuno-suppressive activity in vivo. Intraperitoneal administration of LZ-8, twice weekly into the mice (8 and 12 mg/kg) greatly prevented the production of antibody to the hepatitis B surface antigen (HBs Ag) with the inhibition rates of 83.3% and 96.8%, respectively, in C57BL/10 and C57BL/10BR mice (44). Similarly, a polysaccharide with a molecular weight of 1.26×10^5 , obtained from

the sporoderm-broken spores of *G. lucidum* was found to have a strong effect on suppressing the antibody production and the ConA- or LPS-induced lymphocyte proliferation in mice (45). In a pilot study, New Zealand Black/White (B/W) F1 lupus mice were fed with *G. tsugae* extract (The major components consisted of polysaccharide, nucleotide, tripenoids, and Ling-Zhi-8 identified by HPLA analysis) in an equivalent way to that used by patients for systemic lupus erythematosus. It was found *G. tsugae* alone showed a therapeutic advantage compared with the lupus control. *G. tsugae* improved the survival rate of lupus mice, increased body weight, decreased the amount of proteinuria, and decreased serum levels of anti-dsDNA autoantibody in B/W F1 mice. Pathological findings in lung, kidney,

and liver tissues showed that *G. tsugae* can decrease perivascular and parenchyma mononuclear cell infiltration (46).

In advance of future research

As mentioned above, it is believed that *G. lucidum* possesses extensive immuno-modulating activities including promoting the function of APC, mononuclear phagocyte system, humoral immunity, and cellular immunity. The mechanism involves cellular and molecular regulation of *G. lucidum* on immune components. These data are summarized in Table 1. The pharmacological results, as preclinical data, from in vivo experiments are more important than these from in vitro

Table 1. Effects of *Ganoderma lucidum* polysaccharides on immune cells

Immune cells	Effects	References
Macrophages	Enhance phagocytosis	1, 2, 7
	Promote IL-1, TNF α production and TNF α mRNA expression	3 – 6
	Prevent oxidant tBOOH-induced oxidative injury	8 – 10
	Protect mitochondrial membrane and alleviate membrane injury by free radicals	10
	Increase [Ca ²⁺] _i	12
	Induce IP ₃ and DAG formation	13
	Increase PKC activity	14
	Activate macrophages via TLR4 and TLR4-modulated protein kinase signaling pathways	18, 19
Neutrophils	Increase in PKC, MAPK, HCK and tyrosine kinase Lyn activities	16
	Inhibit spontaneous and Fas-induced apoptosis by activation of Akt-regulated signaling pathways	17
Dendritic cells	Promote maturation and immune response initiation induced by DC	20
	Promote cytotoxicity of specific CTL induced by DC	21
	Increase IFN γ and granzyme B production and mRNA expression	21
	Induce activation and maturation of human DC by the NF- κ B and p38 MAPK pathways	22
Natural killer cells	Enhance activity of NK cells	1
	Increase NK-cell-mediated cytotoxicity	23
T lymphocytes	Increase lymphocyte proliferation induced by ConA and MLC	24, 25
	Promote IL-2, IFN- γ production	5, 25
	Increase DNA synthesis and enhance activity of DNA polymerase α	25
	Increase the percentage of CD5 ⁺ , CD4 ⁺ and CD8 ⁺ T-lymphocytes	31
	Increased production of IP ₃ and DAG	32
	Increase activities of PKA and PKC	33
B lymphocytes	Increase lymphocyte proliferation induced by LPS	34, 35
	Increase in secretion of immunoglobulin	36
	Stimulate expression of PKC	36
	Reduced mucosal specific IgA response of young adult mice orally immunized with cholera toxin	38

experiments. Some cellular and molecular results from in vitro experiment usually are false positive ones, so whole animal experiments are still needed to further establish the mechanism of immuno-modulating effects by *G. lucidum*. Particularly, a double-blind, randomized controlled clinical trial with placebo is needed.

A number of studies indicate that polysaccharides isolated from *G. lucidum* are main active components for immuno-modulating and antitumor activities. It is well known that polysaccharides having immuno-modulating and antitumor action differ greatly in their chemical composition and configuration and physical properties. These are exhibited in a wide range of glycan extending from homopolymers to highly complex heteropolymers. Although it is difficult to correlate the structure and activity of complex polysaccharides, some possible relationships can be inferred. It has been reported that most of the *Ganoderma* polysaccharides show the same basic β -glucan structure with different types of glycosidic linkages. Therefore it is obvious that some structural features such as β -1,3-linkages in the main chain of the glucan and further β -1,6-branch points are needed for immuno-modulating and antitumor activities. The β -glucan containing mainly 1,6-linkages has less activity. Glucan with high molecular weight appear to be more effective than those with low molecular weight. However, a study of the relationship between structure and efficacy of *G. lucidum* is needed in the future (47–51).

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