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Research Article

Immunomodulatory activities of *Gelidium amansii* gel extracts on murine RAW 264.7 macrophages

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ABSTRACT

The objective of this study was to investigate the effects of *Gelidium amansii*, a red algae cultivated in the northeastern coast of Taiwan, on immune regulatory activities in RAW 264.7 macrophages. *G. amansii* gel was prepared by boiling and filtering the dried *G. amansii* algae. Phosphate-buffered saline (PBS) and ethanol extracts of the freeze-dried gel were then used to treat the cells. The results showed that the PBS extracts of *G. amansii* gel activated the macrophage by increasing cell proliferation and by enhancing the production of nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6; the ethanol extracts did not show such effects. By contrast, neither PBS nor ethanol extracts of *G. amansii* gel affected NO production in lipopolysaccharide (LPS)-stimulated macrophages, although the ethanol extracts suppressed LPS-stimulated TNF-α, IL-1β, and IL-6 production. In conclusion, the PBS extracts of *G. amansii* gel activated the macrophage by enhancing the production of immune mediators, whereas the ethanol extracts showed anti-inflammatory activities by suppressing the cytokine production in LPS-stimulated RAW 264.7 macrophages.

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1. Introduction

Regulation of immune responses is closely associated with various physiological and pathological conditions, such as infection, cancer, and systemic lupus erythematosus (SLE), etc. Immune mediators released from macrophages play significant roles in these processes [1–3]. For example, once infected with bacterial endotoxin lipopolysaccharide (LPS), the macrophages can be stimulated to generate numbers of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, as well as other inflammatory mediators, such as nitric oxide (NO), prostaglandin E_2 (PGE_2), and adhesion molecules, to help eradicate the assaulted bacteria [4]. Thus, the activation of macrophages would be one of the self-defense mechanisms to protect the host against microbial pathogens. By contrast, dysregulated production of these immune mediators by macrophages during prolonged inflammation is associated with different pathological conditions, such as autoimmune diseases, cardiovascular diseases, and cancers [5]. Hence, agents that regulate the activation of macrophages leading to modulation of the production of the immune mediators may have protective roles in immune-associated diseases [6,7].

Lines of evidence indicated that various bioactive components from natural foods possess immune modulatory activities; such as resveratrol from grapes, organosulfuric compounds from garlic, and polysaccharides from mushrooms [6,8–10]. Other studies also showed that edible seaweeds exhibit immune modulatory effects [7,11,12]. *Gelidium amansii* is an edible red algae widely cultivated in northeastern Taiwan, and its gel product, prepared by boiling, filtering, cooling, and sweetening, is a popular dessert in the summer. Folk therapy indicates that the consumption of *G. amansii* gel may have health-promoting effects, such as lowering blood pressure, lowering blood glucose and lipid levels, preventing cancers and cardiovascular diseases, as well as enhancing immune activities [13]. Previously, Yan et al [14] indicated that extracts of *G. amansii* possess *in vitro* anti-oxidative activities. We also have reported that extracts of *G. amansii* gel inhibit the growth of cultured cancer cells [15]; however, the scientific evidence for other health promoting effects is limited. This study was aimed at examining the potential effects of *G. amansii* gel on immune regulatory activities in murine RAW 264.7 macrophages.

2. Methods

2.1. Chemicals and biochemicals

High glucose Dulbecco's Modified Eagle's Medium (DMEM), sodium bicarbonate, fetal bovine serum, trypan blue, and trypsin were purchased from GIBCO BRL (Grand Island, NY, USA). LPS, sulfanilamide, *N*-(1-naphthyl)ethylenediamine dihydrochloride, sodium nitrite, absolute ethanol, and dimethylsulfoxide (DMSO) were from Sigma Chemical (St. Louis, MO, USA).

2.2. Cell culture

Murine macrophage RAW 264.7 cell line was purchased from the Bioresource Collection and Research Center (BCRC #60001) in Taiwan. The cells were grown in monolayer in DMEM supplemented with 10% fetal bovine serum, and maintained at 37 °C in a 95% air and 5% CO_2 atmosphere and subcultured routinely.

2.3. Preparation of G. amansii gel extracts

Dried *G. amansii* was purchased from a local store on the northeastern coast of Taiwan. The gel extracts were prepared according to the method described previously [11]. Briefly, 100 g dry *G. amansii* was boiled with 2 L water for 2 hours. After filtering, cooling, sonicating, and freeze-drying, an amount of 4.78 g gel powder was obtained. The powder was then mixed with phosphate buffered saline (PBS) or absolute ethanol in a platform shaker for 24 hours. After centrifugation and filtration, the PBS or ethanol extracts were ready to use in the experiments, in which the PBS or ethanol served as a control, respectively. The concentrations expressed in this study are micrograms of freeze-dried gel powder/milliliter of medium for PBS or ethanol extracts, and the final concentrations of PBS and ethanol added to the medium were 1%.

2.4. Cell proliferation assay

The effects of the extracts on cell proliferation were measured using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay Kit (Promega, Madison, WI, USA). Basically, 10^4 cells/well were seeded in a 96-well plate and treated with the PBS or ethanol extracts of *G. amansii* gel in the presence and absence of LPS (100 ng/mL) for 24 hours. The rate of proliferation was determined by converting 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to a colored formazan product by live cells as detected by absorbance at 492 nm on a micro-plate reader.

2.5. Assays for NO and cytokine production and secretion

To understand the effects of the *G. amansii* gel extracts on the production and secretion of immune mediators, macrophages were treated with the extracts in the presence and absence of LPS (100 ng/mL) for 24 hours, and the medium was collected for analysis. The nitrite concentration was quantified after its reaction with a Griess reagent (1% sulfanilamide/0.1% naphthylethylenediaminedihydrochloride in 5% H_3PO_4) using sodium nitrite as a standard. The production and secretion of TNF-α, IL-1β, and IL-6 were determined by enzyme linked immunosorbent assay (ELISA) commercial kits (R&D System, Minneapolis, MN, USA).

2.6. Statistical analysis

Values are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Fisher's least significant difference test were employed to determine the statistical differences between groups using SAS software

version 9.1 (SAS Institute, Cary, NC, USA). Statistical significance of mean differences was based on p value less than 0.05.

3. Results

3.1. *Effects of G. amansii on activation of RAW 264.7 macrophages*

Because activation of macrophages is a critical event in the host self-defense mechanism to protect against pathogen infection, the effects of *G. amansii* on the activation of macrophages were examined. After the cells were treated with various concentrations of the PBS extracts of *G. amansii* gel for 24 hours, the cell proliferation was significantly enhanced at all concentrations applied, and even produced 203% increase at a concentration of 150 μg/mL. However, the ethanol extracts did not exert such effect on the proliferation of the macrophages (Fig. 1).

The activation of the macrophages is usually associated with the increased production of immune mediators. To understand whether the *G. amansii* extracts affect the production of immune mediators, the condition mediums from the treated cells were collected for analysis. As shown in Fig. 2, the production of NO was significantly stimulated by the PBS extracts in a concentration-dependent manner, whereas the ethanol extracts did not show any effect on NO production in macrophages (Fig. 2). As shown in Fig. 3, the PBS extracts stimulated the TNF-α production at all concentrations applied, and concentration-dependently enhanced the levels of IL-1β and IL-6 in the cultured medium. Correspondingly, none of the cytokines was affected by the treatment of the ethanol extracts of *G. amansii* (data not shown).

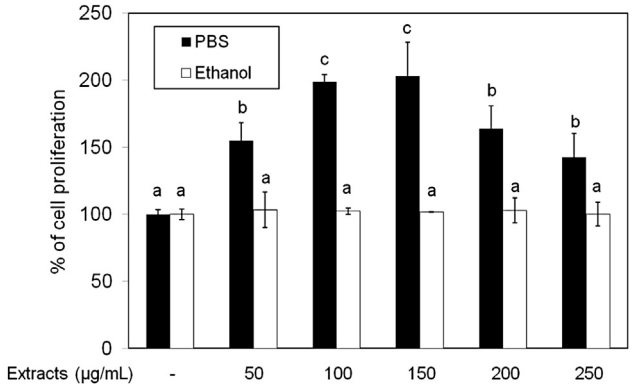

Fig. 1 – Effects of extracts of *Gelidium amansii* gel on proliferation of RAW 264.7 macrophages. Cells were treated with various concentrations of PBS or ethanol extracts of *G. amansii* gel for 24 hours. Cell proliferation was determined by an MTS assay kit. Values represent the mean ± SD from three measurements. The experiment was repeated three times, and similar results were obtained. Data in the same treatment with different letters significantly differ ($p < 0.05$). MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PBS = phosphate buffered saline; SD = standard deviation.

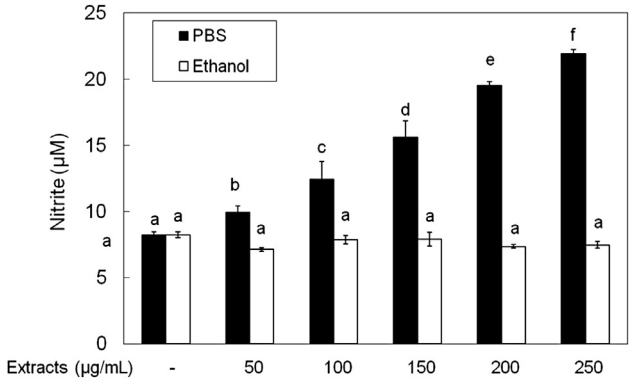

Fig. 2 – Effect of extracts of *Gelidium amansii* gel on NO release into the medium by RAW 264.7 macrophages. Cells were treated with various concentrations of PBS or ethanol extracts of *G. amansii* gel for 24 hours. The nitrite content in the medium was determined by a Griess reagent. Values represent the mean ± SD from three measurements. The experiment was repeated three times, and similar results were obtained. Data in the same treatment with different letters significantly differ ($p < 0.05$). NO = nitric oxide; PBS = phosphate buffered saline; SD = standard deviation.

3.2. *Effects of G. amansii on immune mediators in LPS-stimulated RAW 264.7 macrophages*

Prolonged inflammation is linked to various pathological conditions, so the effects of *G. amansii* on the proliferation and the production of immune mediators from LPS-activated macrophages were also examined. The results showed that the PBS extracts stimulated cell proliferation in macrophages activated by LPS at certain concentrations, and the ethanol extracts increased the proliferation only at a concentration of 200 μg/mL (Fig. 4). As expected, LPS stimulated the production of all the immune mediators we measured in RAW 264.7 macrophages, whereas the ethanol extracts significantly inhibited, up to 62% and 50%, the production of the stimulated TNF-α and IL-6, respectively, and even completely blocked the enhanced IL-1β production (Figs 5 and 6). The PBS extracts of *G. amansii* gel, on the other hand, further enhanced the production of TNF-α at concentrations greater than 150 μg/mL, but slightly suppressed the productions of IL-1β and IL-6 in LPS-activated macrophages (Fig. 6). Furthermore, neither PBS nor ethanol extracts considerably affected the LPS-activated NO production, although the NO production slightly increased with certain concentrations (Fig. 5).

4. Discussion

This study provides evidence that the extracts from *G. amansii* gel possessed *in vitro* immune modulatory activities, in which the PBS extracts enhanced the macrophage activities through increasing cell proliferation and production of immune mediators, whereas the ethanol extracts inhibited LPS-stimulated activities of macrophages by suppressing the

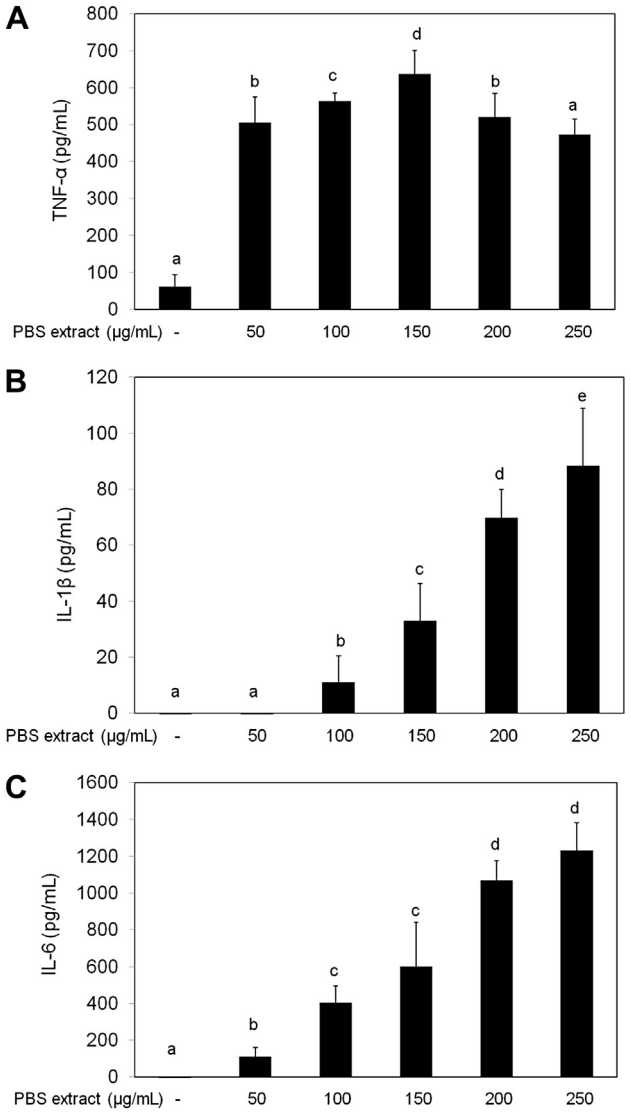

Fig. 3 – Effect of PBS extracts of *Gelidium amansii* gel on TNF-α (A), IL-1β (B), and IL-6 (C) released into the medium by RAW264.7 macrophages. Cells were treated with various concentrations of PBS extracts from *G. amansii* gel for 24 hours. The concentrations of cytokines in the medium were then examined by commercial ELISA kits. Values represent the mean ± SD from three measurements. The experiment was repeated three times, and similar results were obtained. Data with different letters significantly differ ($p < 0.05$). ELISA = enzyme linked immunosorbent assay; IL-1β = interleukin-1β; IL-6 = interleukin-6; PBS = phosphate buffered saline; SD = standard deviation; TNF-α = tumor necrosis factor-α.

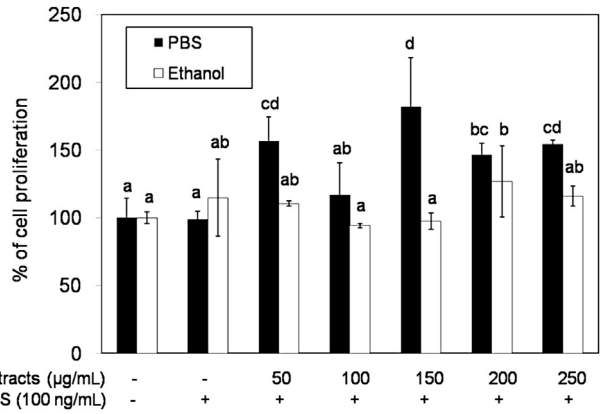

Fig. 4 – Effects of extracts of *Gelidium amansii* gel on LPS-induced proliferation of RAW 264.7 macrophages. Cells were treated with various concentrations of PBS or ethanol extracts of *G. amansii* gel and LPS (100 ng/mL) for 24 hours. Cell proliferation was determined by an MTS assay kit. Values represent the mean ± SD. from three measurements. The experiment was repeated three times, and similar results were obtained. Data in the same treatment with different letters significantly differ ($p < 0.05$). LPS = lipopolysaccharide; MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PBS = phosphate buffered saline; SD = standard deviation.

production of TNF-α, IL-1β, and IL-6. Macrophage activation has been noted to play significant roles on both physiological and pathological conditions. Because the ethanol extracts showed no significant stimulating effect on macrophage activation, and the PBS extracts showed less effect on anti-inflammation, the consumption of *G. amansii* gel from our ordinary diet seems to be notable for regulating immune functions. However, *in vivo* experiments are required to confirm this possibility.

A variety of foods have been indicated to possess immune-enhancing activities through the activation of macrophages because the increased production of NO and cytokines, such as TNF-α and IL-1β, by the activated macrophages helps clear microbes from the body. For example, *Ganoderma lucidum* is a widely used medicinal mushroom shown to have immune-enhancing activities, and such activities are in concordance with the increased production of NO and cytokines in macrophages [9]. Yoshizawa et al [16] reported that marine algae *Porphyra yezoensis* extracts possess macrophage-activating activities by increasing immune mediators and phagocytic activities both *in vivo* and *in vitro*. Consistently, the increased macrophage proliferation, enhanced NO, and enhanced cytokine production by PBS extracts of *G. amansii* gel suggest the potential roles of *G. amansii* on activation of macrophages, thus protecting the host against microbial infections. Various bioactive components in algae extracts, such as polysaccharides, carotenoids, and polyphenols, have been suggested to be involved in stimulating immune activities, and polysaccharides are one of the potential candidates. Because *G. amansii* gel was prepared by boiling in water, and because *G. amansii* contains up to 72–80% carbohydrates [17] and its hot-water extracts contain 67% sugar [18], it is plausible to propose that the macrophage-stimulating activity of PBS extracts in *G. amansii* is mainly due to the polysaccharides. Polysaccharides isolated from green algae have been shown to contribute to immune-enhancing activities in both animals and cultured macrophages [19,20]. Fu et al [18] reported that the phagocytic

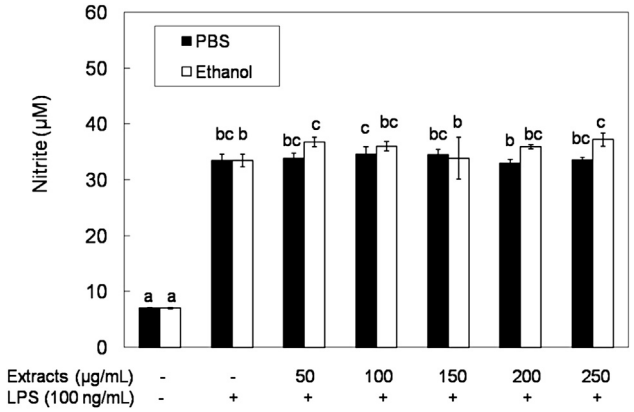

Fig. 5 – Effect of extracts of *Gelidium amansii* gel on NO release into the medium by LPS-activated RAW 264.7 macrophages. Cells were treated with 100 ng/mL LPS and various concentrations of PBS or ethanol extracts of *G. amansii* gel for 24 hours. The nitrite content in the medium was determined by a Griess reagent. Values represent the mean ± SD from three measurements. The experiment was repeated three times, and similar results were obtained. Data in the same treatment with different letters significantly differ ($p < 0.05$). LPS = lipopolysaccharide; NO = nitric oxide; PBS = phosphate buffered saline; SD = standard deviation.

activities and clearance efficiency against *Vibrio alginolyticus* were enhanced in shrimp treated with hot-water extract of *G. amansii* for 28 days. However, this possibility needs to be further investigated. Moreover, the different dose effects of the PBS extracts on cell proliferation and the production of immune mediators suggest that the increased cell proliferation is not the major reason for the increased production of these immune mediators.

Prolonged inflammation has been linked to various diseases, including cancers, cardiovascular diseases, rheumatoid arthritis, and obesity, thus plenty of dietary components that possess anti-inflammatory effects through modulating the production of immune mediators have been shown to have protective or health-promoting effects [6,7]. Our results indicated that the ethanol extracts possess anti-inflammatory activities by suppressing the LPS-stimulated production of TNF-α, IL-6, and even completely inhibited IL-1β, although it affects neither cell proliferation, nor NO production. By contrast, the roles of PBS extracts on inflammation are not definite because it further enhanced the cell proliferation as well as the TNF-α production, but reduced the IL-1β production in LPS-activated macrophages. Shu et al [21] reported that methanol extracts from red algae *Gracilaria changii* possessed anti-inflammatory activities by inhibiting the expression of TNF-α and IL-6 in phorbol 12-myristate 13-acetate (PMA)-induced differentiated U937 cells. Similarly, Yang et al [22] have shown that ethanol extracts from Korean seaweed suppress NO, PGE_2, TNF-α, and IL-6 production in LPS-stimulated RAW 264.7 cells. Different lipid-soluble compounds from algae may be extracted by ethanol, including carotenoids, vitamins, polyphenols, terpenoids, and phytosterols, etc, which

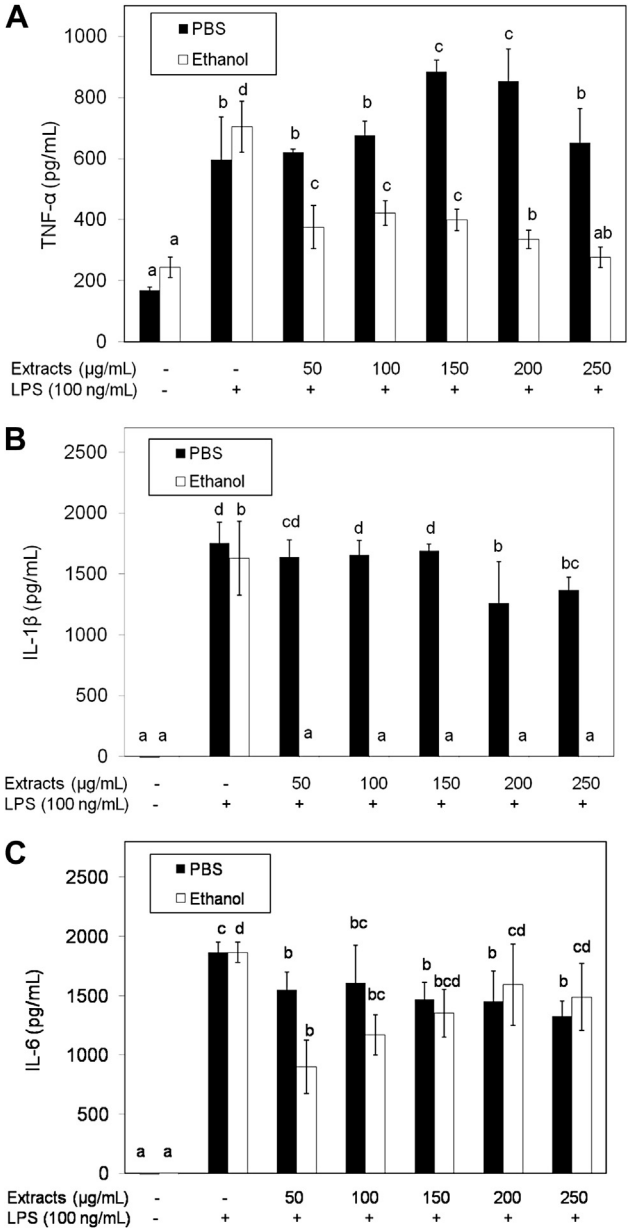

Fig. 6 – Effect of extracts of *Gelidium amansii* gel on LPS-induced TNF-α (A), IL-1β (B), and IL-6 (C) released into the medium by RAW 264.7 murine macrophages. Cells were treated with LPS (100 ng/mL) and various concentrations of PBS or ethanol extracts from *G. amansii* gel for 24 hours. The concentrations of cytokines in the medium were then examined by commercial ELISA kits. Values represent the mean ± SD from three measurements. The experiment was repeated three times, and similar results were obtained. Data in the same treatment with different letters significantly differ ($p < 0.05$). ELISA = enzyme linked immunosorbent assay; IL-1β = interleukin-1β; IL-6 = interleukin-6; LPS = lipopolysaccharide; PBS = phosphate buffered saline; SD = standard deviation; TNF-α = tumor necrosis factor-α.

all have been shown to have anti-inflammatory activities [7,23–26]. Some of them may act as cyclooxygenase inhibitors and some may act through inhibiting nuclear factor-kappa-B (NF-κB) activities [7]. Nonetheless, bioactive pigments, such as carotenoids and fucoxanthin, may not be candidates for the anti-inflammation due to depigmented *G. amansii* used in this study.

The PBS extracts of *G. amansii* gel not only activate macrophage activity by increasing cell proliferation and NO and cytokine production, but also modulate cytokine production, that is, they enhance TNF-α and reduce IL-1β and IL-6, in LPS-activated macrophages. Polysaccharides are composed of various structural compounds, which may play different roles in immune function. For example, sulfated polysaccharide extract from hot-water extracts of brown seaweed *Sargassum hemiphyllum* have been shown to inhibit LPS-activated release of NO, TNF-α, IL-1β, and IL-6 by blocking NF-κB translocation and down-regulating expression of inducible nitric oxide synthase (iNOS) protein [27]. By contrast, the LPS-induced macrophage activation requires LPS receptor complex, which plays vital roles in binding and in mediating the response to LPS [28], and the binding of LPS to the receptor complex initiates a cascade of signal transduction pathways. Because LPS and the extracts are simultaneously added to the culture medium, the possibilities of the bioactive components in PBS or ethanol extracts interacting with the LPS or interfering with the LPS binding to the LPS receptor complex cannot be ruled out. Verification on these possibilities needs further study. Finally, polysaccharides, phenolic compounds, and bioactive components in *G. amansii*, to our knowledge, have not been identified or characterized, so these possibilities remain to be explored.

In conclusion, we confirmed that the extracts from *G. amansii* gel possess immune-modulation activities, in which the PBS extracts may initiate innate immunity by increasing macrophage proliferation, NO production, and cytokine secretion, while ethanol extracts have anti-inflammatory activities by suppressing LPS-stimulated TNF-α, IL-1β, and IL-6 secretion. The mechanisms by which the different extracts affect immune modulation need to be explored further.

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REFERENCES

[1] Yan ZQ, Hansson GK. Innate immunity, macrophage activation, and atherosclerosis. Immunol Rev 2007;219:187–203.
[2] Nagabhushanam V, Solache A, Ting LM, et al. Innate inhibition of adaptive immunity: *Mycobacterium tuberculosis*-induced IL-6 inhibits macrophage responses to IFN-gamma. J Immunol 2003;171:4750–7.
[3] Sladkova T, Kostolansky F. The role of cytokines in the immune response to influenza A virus infection. Acta Virol 2006;50:151–62.
[4] Yun KJ, Koh DJ, Kim SH, et al. Anti-inflammatory effects of sinapic acid through the suppression of inducible nitric oxide synthase, cyclooxygase-2, and proinflammatory cytokines expressions via nuclear factor-kappaB inactivation. J Agric Food Chem 2008;56:10265–72.
[5] Prasad S, Sung B, Aggarwal BB. Age-associated chronic diseases require age-old medicine: role of chronic inflammation. Prev Med 2012;54(Suppl.):S29–37.
[6] Gupta SC, Kim JH, Prasad S, et al. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev 2010;29:405–34.
[7] D'Orazio N, Gammone MA, Gemello E, et al. Marine bioactives: pharmacological properties and potential applications against inflammatory diseases. Mar Drugs 2012;10:812–33.
[8] Castro R, Lamas J, Morais P, et al. Resveratrol modulates innate and inflammatory responses in fish leucocytes. Vet Immunol Immunopathol 2008;126:9–19.
[9] Ji Z, Tang Q, Zhang J, et al. Immunomodulation of RAW264.7 macrophages by GLIS, a proteopolysaccharide from *Ganoderma lucidum*. J Ethnopharmacol 2007;112:445–50.
[10] Sohn DW, Han CH, Jung YS, et al. Anti-inflammatory and antimicrobial effects of garlic and synergistic effect between garlic and ciprofloxacin in a chronic bacterial prostatitis rat model. Int J Antimicrob Agents 2009;34:215–9.
[11] Liu JN, Yoshida Y, Wang MQ, et al. B-cell stimulating activity of seaweed extracts. Int J Immunopharmacol 1997;19:135–42.
[12] Katayama M, Fukuda T, Okamura T, et al. Effect of dietary addition of seaweed and licorice on the immune performance of pigs. Anim Sci J 2011;82:274–81.
[13] Baidu Encyclopedia, Introduction to *Gelidium amansii*. http://baike.baidu.com/view/48249.htm [accessed 2013/01/04].
[14] Yan X, Nagata T, Fan X. Antioxidative activities in some common seaweeds. Plant Foods Hum Nutr 1998;52:253–62.
[15] Chen YH, Tu CJ, Wu HT. Growth-inhibitory effects of the red alga *Gelidium amansii* on cultured cells. Biol Pharm Bull 2004;27:180–4.
[16] Yoshizawa Y, Ametani A, Tsunehiro J, et al. Macrophage stimulation activity of the polysaccharide fraction from a marine alga (*Porphyra yezoensis*): structure-function relationships and improved solubility. Biosci Biotechnol Biochem 1995;59:1933–7.
[17] Jeong GT, Park DH. Production of sugars and levulinic acid from marine biomass *Gelidium amansii*. Appl Biochem Biotechnol 2010;161:41–52.
[18] Fu YW, Hou WY, Yeh ST, et al. The immunostimulatory effects of hot-water extract of *Gelidium amansii* via immersion, injection and dietary administrations on white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish Shellfish Immunol 2007;22:673–85.
[19] Karnjanapratum S, You S. Molecular characteristics of sulfated polysaccharides from *Monostroma nitidum* and their in vitro anticancer and immunomodulatory activities. Int J Biol Macromol 2011;48:311–8.
[20] Lee JY, Kim YJ, Kim HJ, et al. Immunostimulatory effect of *Laminarin* on RAW 264.7 mouse macrophages. Molecules 2012;17:5404–11.
[21] Shu M-H, Appleton D, Zandi K, et al. Anti-inflammatory, gastroprotective and antiulcerogenic effects of red algae *Gracilaria changii (Gracilariales, Rhodophyta)* extract. BMC Complement Altern Med 2013;13:61.
[22] Yang EJ, Moon JY, Kim MJ, et al. Inhibitory effect of Jeju endemic seaweeds on the production of pro-inflammatory mediators in mouse macrophage cell line RAW 264.7. J Zhejiang Univ Sci B 2010;11:315–22.

[23] Kim KN, Heo SJ, Yoon WJ, et al. Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-kappaB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. Eur J Pharmacol 2010;649:369–675.
[24] Yoo MS, Shin JS, Choi HE, et al. Fucosterol isolated from *Undaria pinnatifida* inhibits lipopolysaccharide-induced production of nitric oxide and pro-inflammatory cytokines via the inactivation of nuclear factor-kappaB and p38 mitogen-activated protein kinase in RAW 264.7 macrophages. Food Chem 2012;135:967–75.
[25] Dutot M, Fagon R, Hemon M, et al. Antioxidant, anti-inflammatory, and anti-senescence activities of a phlorotannin-rich natural extract from brown seaweed *Ascophyllum nodosum*. Appl Biochem Biotechnol 2012;167:2234–40.
[26] Khan MN, Yoon SJ, Choi JS, et al. Anti-edema effects of brown seaweed (*Undaria pinnatifida*) extract on phorbol 12-myristate 13-acetate-induced mouse ear inflammation. Am J Chin Med 2009;37:373–81.
[27] Hwang PA, Chien SY, Chan YL, et al. Inhibition of lipopolysaccharide (LPS)-induced inflammatory responses by *Sargassum hemiphyllum* sulfated polysaccharide extract in RAW 264.7 macrophage cells. J Agric Food Chem 2011;59:2062–8.
[28] Fujihara M, Muroi M, Tanamoto K, et al. Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. Pharmacol Therap 2003;100:171–94.