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Effects of Sea Tangle and Its Constituents in Diet on Immune Functions of Diabetic Mice

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Abstract

Dietary effects of sea tangle on immune functions were investigated in diabetic mice. Four groups of ICR mice weighing 33.36±1.01 g were fed either an AIN-76 diet only (control), or with additional sea tangle powder, sea tangle water extract, and alginate at the level of 13.6%, 4%, and 1%, respectively by weight. Cellulose was omitted in sea tangle powder and alginate diets. After 10 days of feeding respective experimental diets, all mice were made diabetic by five consecutive intramuscular injections of streptozotocin (40 mg/kg body weight per day) and fed the diets for four more weeks. Plasma IgG concentrations but not those of IgM were significantly higher in mice fed sea tangle powder, extract or alginate than those on the control diet. Plasma TNF α levels were, however, lower in those fed sea tangle powder or water extract than control and alginate fed groups. TNFα releases from macrophages isolated from four groups and cultured with 5 μg/mL LPS for 24 hours showed a similar tendency to the results of plasma concentrations in the respective groups, but IL-1β releases were not different among four groups. Lymphocyte proliferation in response to LPS (10 μg/mL) measured using splenocytes cultured for 3 days was highest in the alginate fed group (594±38%) and those of sea tangle powder (536±47%) and extract (547±34%) fed groups tended to be higher than the control (523±30%). It is concluded that sea tangle contains immunomodulatory components besides alginate that could enhance humoral immunity of itself. The immunomodulatory effects of sea tangle constituents is regarded as beneficial for diabetic subjects.

Key words: sea tangle, antibody, TNFα, lymphocyte proliferation

INTRODUCTION

The autoimmune response against islet β-cells leading to insulin-dependent diabetes mellitus (IDDM) is believed to result from a disorder of immunoregulation. A current hypothesis is that Th1 cells and their cytokine products, interleukin (IL)-2, interferon γ (INFγ), tumor necrotic factorβ (TNFβ), active macrophage (Mϕ) and cytotoxic T cells destroy β cells, causing IDDM, whereas Th2 cells and their cytokine products, IL-4 and IL-10 suppress Th1 cells and cytokines and thereby prevent IDDM (1). On the other hand, diabetes, either IDDM or NIDDM, increases susceptibility to various infections, which implies reduced immune functions (2-4). Numerous investigators reported that the susceptibility of diabetics should be ascribed to malfunction of polymorphonuclear leukocytes, although Rayfield et al. (5) stressed the importance of glycemic control and McElhaney et al. (6) reported that IL-2 activity was not affected by NIDDM. In streptozotocin-treated mice, weights of lymphoid organs were lower along with reductions of delayed type hypersensitivity and antibody-forming activity (7).

Many nutrients and other food components have been recognized as playing roles as immunomodulators in the body. In addition to protein-energy malnutrition, deficiencies of micronutrients, vitamins, minerals and essential fatty acids impair immunity, among which Zn is a prime example (8). Among other food components, various types of complex polysaccharides have attracted attention for their immunomodulating effects (9-15) . Algal polysaccharide that comprises 30~70% of dry weight of seaweed is various in kind (16-19). Glycoprotein and fucan extracted from seaweed have been shown to affect immune functions such as increasing phagocytic activity (13), B lymphocyte proliferation, TNFα production (14) changes in activities of complement system (15), and antitumor activity (20,21). Most of these studies were done by the addition of whole or partially purified extracts from seaweed *in vitro*. Moreover, it has been rarely tested whether these components from seaweed could restore immune functions altered by diabetes. In our previous studies, we reported that whole sea tangle in the diet enhanced LPS-stimulated proliferation of splenocytes and macrophage production of IL-1β derived from normal mice but these changes were not found when diabetic mice were used (22,23).

In the present study, we tested effects of seaweed either as whole sea tangle (*Laminara supp.*) powder or as its water extract and its major component, alginic acid in diets for diabetic mice on serum levels of antibodies and TNFα as well as on the proliferation of splenocytes and IL-1β production

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from macrophages.

MATERIALS AND METHODS

Materials

Streptozotocin, α-cellulose, *dl*-methionine, choline bitartarate, lipopolysaccharide (LPS), Hank's balanced salts, phosphate-buffered saline (PBS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), goat anti-mouse IgG Ab, goat anti-mouse IgM Ab, bovine serum albumin (BSA), mouse IgG, mouse IgM, alkaline phosphatase-conjugated antiglobulins, and *p*-nitrophenyl phosphate (*p*NPP) tablets were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Casein, vitamin mix and mineral mix were purchased from Teklad Co. (Madison, WI, USA) and sodium alginate, from Bioserv Inc. (Frenchtown, NJ, USA). Corn starch was obtained from Samyang Genex (Seoul, Korea) and sucrose, corn oil, and lard, from a local market. Eagle's minimum essential medium (EMEM), penicillin-streptomycin and gentamycin were from GIBCO BRL Co. (Gaithersburg, MD, USA) and fetal calf serum (FCS) from Hyclon Co. (Logan, Utah, USA). ELISA kits for determination of IL-1β and TNFα were purchased from Genzyme Co. (Cambridge, MA, USA).

Animals and diets

Forty male ICR mice (Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea) with average weight of 33.86±1.01 g were divided into four groups and fed respective experimental diets for 35 days before sacrifice. The experimental diets were based on an AIN-76 diet (24). One control diet consisted of 15 g corn starch, 50 g sucrose, 20 g casein, 1.7 g corn oil, 3.3 g lard, 5 g cellulose, 1 g vitamin mix, 3.5 g mineral mix, 0.3 g DL-methionine, and 0.2 g choline bitartarate in 100 g diet. The sea tangle powder diet contained 15 g of dry powder of whole sea tangle instead of 5 g of cellulose to have same level of dietary fiber in the resultant total of 110 g diet. Sea tangle water extract and alginate diets were formulated simply by adding 4 g and 1 g of each material to the control diet. Dry sea tangle obtained from Taegu Marine Product Co. (Taegu, Korea) was washed to remove salt and dried at 40°C until its water content was 8% (w/w). The clean and dry sea tangle was ground to a 30 mesh powder. About 500 g of the dry sea tangle powder was extracted with 1.5 L of methanol by refluxing at 70°C for 5 hrs. To the resultant residue after methanol extraction, 5~7 L of water was added and it was soaked for 40 hrs at 25°C and concentrated under vaccum and finally freeze-dried to obtain the water extract of sea tangle for the respective diet. At 10th day after feeding the diets, diabetes in mice were induced by intramuscular injections of STZ at the level of 40 mg/kg for five consecutive days (25) and by detecting blood glucose from tail vein using One Touch^{R} from Johnson and Johnson Co. (Milpitas, CA, USA).

Lymphocyte proliferation

The spleen of each mouse was removed and the suspension of splenocytes was prepared at a concentration of 5×10^{6} cells/mL in EMEM containing 5% FCS, 100 U/mL penicillin-streptomycin and 10 μg/mL gentamycin. Triplicate cultures of splenocytes on a 96 well plate were treated with 10 μg/mL LPS for 72 hours. Lympocyte proliferation was then determined by the MTT assay (26).

Macrophages

Peritoneal macrophages were obtained from mice 4 days after injection with 3 mL sterile fluid of 3% thioglycolate (27). Peritoneal exudate cells (PEC) were collected after injection of 6~7 mL of Hank's buffer, centrifuged and resuspended in EMEM with 5% FCS containing 100 U/mL penicillin-streptomycin and 10 μg/mL gentamycin. Hundred microliters of PEC suspension were placed on 96 well plates at a concentration of 1×10^{6} cells/mL and incubated at 37°C for 2 hours. After removing non-adherent cells by rinsing with Hank's buffer, 200 μL of EMEM containing 5 μg/mL LPS was added to the remaining adherent cells. The macrophages were cultured for 24 hours and culture supernatant was used to determine TNFα and IL-1β.

Immunoglobulin and cytokine assays

A sandwich ELISA was used to determine total plasma IgG and IgM levels (28). Hundred microliters of goat anti-mouse IgG or IgM antibody was loaded in each well of 96 well plates, respectively. After one-hour incubation at 37°C, wells were washed five times with 0.05% Tween 20 in PBS (TPBS) and added 300 μL of blocking solution (0.3% BSA/0.05 M Tris, pH 9.5). After another hour of incubation and rinsing with TPBS, 100 μL of the appropriately diluted plasma was placed and incubated for one hour. After washing, 100 μL alkaline phosphatase-conjugated anti-mouse IgG or IgM was added. To this 50 μL of p-NPP (1 mg/mL diethanolamine buffer) was added and absorbance was measured at 405 nm. TNFα and IL-1β from plasma and culture media were measured using ELISA kits.

Statistical analysis

Data were analyzed by analyses of variance and group differences were considered statistically significant at $p<0.05$ by Tukey's test.

RESULTS

Body weights and blood glucose

Average body weights of mouse groups fed sea tangle powder and water extract were slightly higher (34.9±1.9 g and 34.4±2.0 g, respectively) than those of the control and alginate groups (32.8±1.4 and 33.4±1.5 g, respectively) but it was not statistically significant. This trend was maintained all throughout the diet period. All groups increased their body weights up to 39.5 to 42.1 g until STZ injection, which stopped further body weight gains (Fig. 1A). Two weeks after the final STZ injection body weights of mice decreased by 1.2 to 2.7 g to give a final average body weight of all mice of 39.0±1.6 g.

Blood glucose levels were significantly elevated to 366±

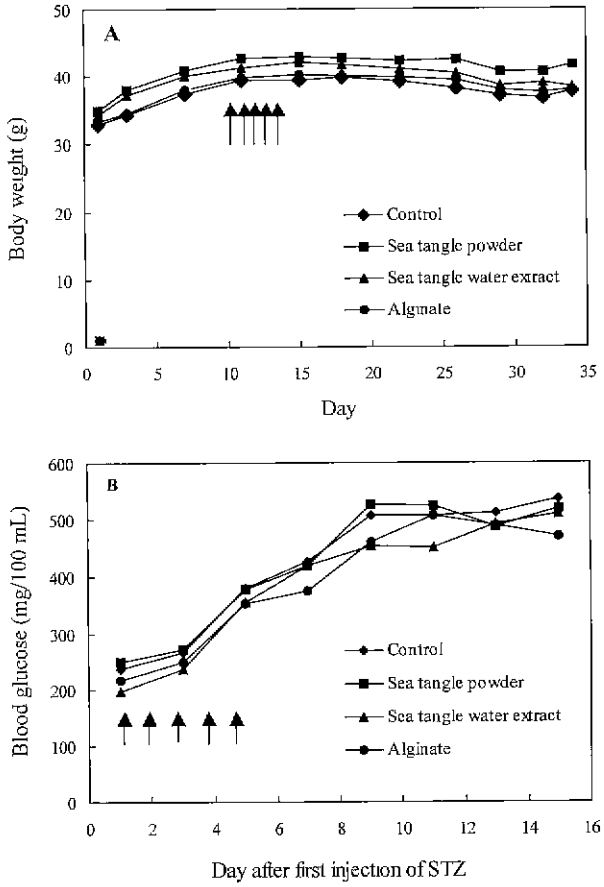

Fig. 1. Changes in body weights (A) and levels of blood glucose (B) of mouse groups during experimental diets. Arrows in panels A and B represent injections of STZ (40 mg/kg bw) for five consecutive days.

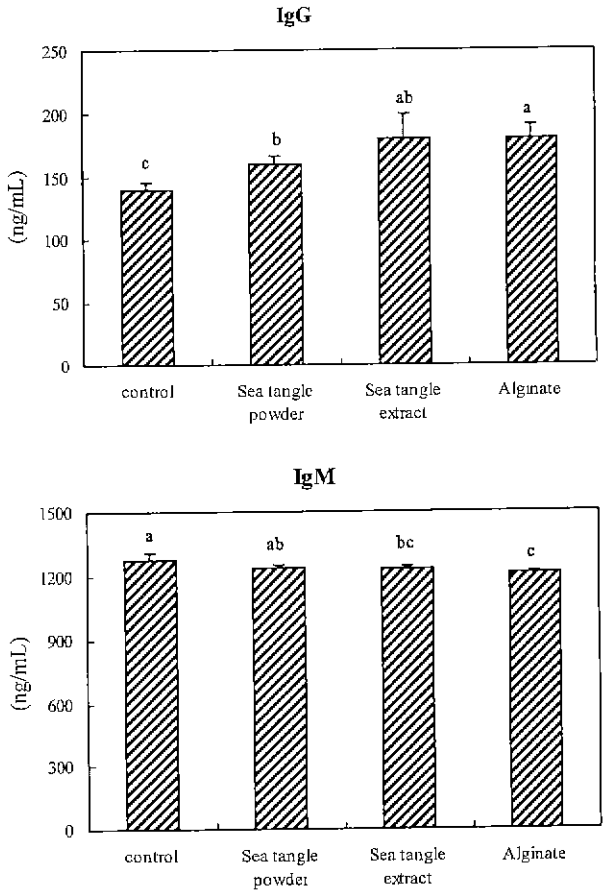

Fig. 2. Concentrations of plasma IgG and IgM of STZ-induced diabetic mice fed four experimental diets. Values are the mean± SEM and those with different alphabet letters are significantly different each other at $p<0.05$.

12 mg/ 100 mL on 5th day of STZ injection and continued to rise up to 497±28 mg/100 mL on 11th day and the blood glucose level was maintained until sacrifice (Fig. 1B). Changes of blood glucose during and after STZ injections were almost the same as reported by Like and Rossini (25). There was no significant difference in the blood glucose levels among experimental groups after induction of diabetes.

Concentrations of IgM, IgG and TNFα in plasma

Plasma IgG concentrations were significantly higher in mice fed with sea tangle powder, extract or alginate than control diet, but IgM concentrations showed the reverse trend between control and three other experimental groups (Fig. 2).

Plasma TNFα concentrations were changed in a different manner compared to immunoglobulin concentrations as shown in Fig. 3. Mice groups fed with sea tangle powder or water extract had lower TNFα levels than control group but those fed alginate maintained a similar or slightly higher plasma TNFα levels compared with control group.

In vitro cytokine productions from macrophage and splenocyte proliferation

Peritoneal macrophages were obtained from four experimental mice groups and cultured in the presence of 5 μg/mL

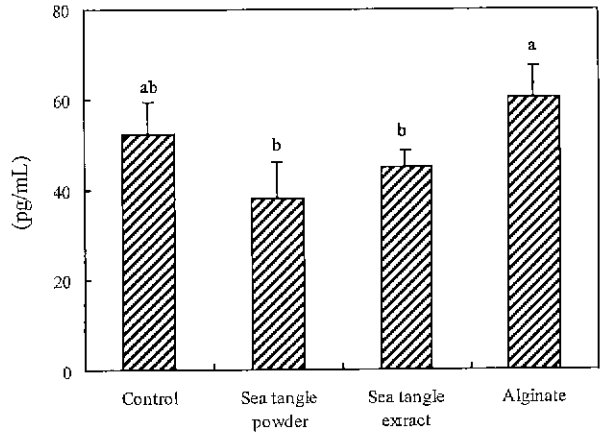

Fig. 3. Concentrations of plasma TNF-alpha of STZ-induced diabetic mice fed four experimental diets. Values are the mean± SEM and those with different alphabet letters are significantly different each other at $p<0.05$.

LPS for 24 hours. The productions of TNFα and IL-1β of the cultured macrophages are shown in Fig. 4. TNFα releases

from macrophages from four groups showed a similar tendency, although only those of group fed with sea tangle extract were significantly lower than control group (Fig. 3). On the other hahd, IL-1β releases from macrophages were not much different among four groups, even though those from three other experimental groups showed increasing tendency compared with the control group.

Effects of dietary sea tangle and its constituents on lympocyte proliferation were determined using splenocytes and LPS (10 μg/mL) as a mitogen. As shown in Fig. 5, stimulation of splenocyte proliferation in response to LPS was higher in alginate fed group (594±38%) than the other three groups. Those of sea tangle powder (536±47%) and extract (547±34%) fed groups tended to be higher than the control (523±30%).

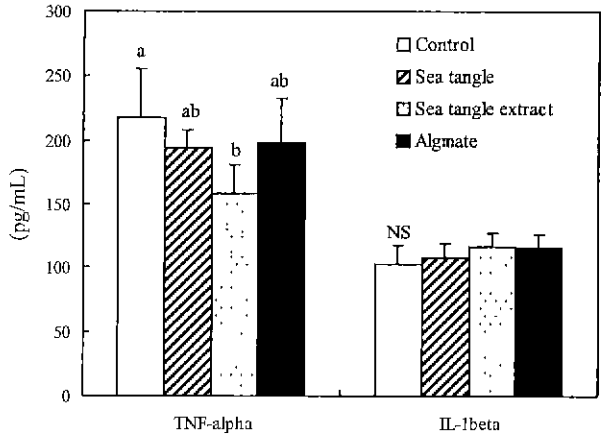

Fig. 4. Effects of dietary sea tangle and its constituents on TNF-alpha and IL-1beta production by macrophages. Adherent peritoneal macrophages isolated from each group of diabetic mice were stimulated with 5 μg/mL LPS for 24 hrs. Values were means±SEM and those with different alphabet letters are significantly different each other at $p<0.05$. NS represents not significant..

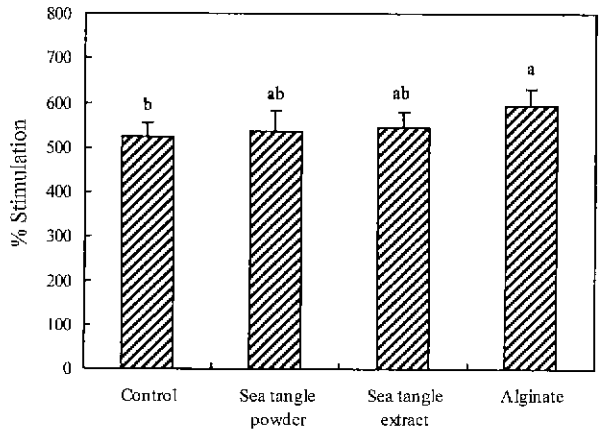

Fig. 5. Effects of dietary sea tangle and its constituents on LPS-induced lymphocyte proliferation. Splenocytes isolated from each group of diabetic mice were cultured in EMEM with FCS and 10 μg/mL LPS for 72 hrs. Lymphocyte proliferation was determined by the MTT assay. Values are mean±SEM and those with different alphabet letters are significantly different each other at $p<0.05$.

DISCUSSION

The present study demonstrates that whole sea tangle, its water extract and its major component, alginic acid change immune activities of diabetic mice *in vivo* but the effects of the three different materials were different. Elevation of plasma IgG under three different conditions is comparable to a report from Lim et al. (9) that soluble dietary fiber such as pectin and Konjak mannan increased serum IgG and IgA concentrations, although IgA was not measured in the present study. Therefore, alginate, a soluble dietary fiber of sea tangle appears to be a major factor for increasing plasma IgG levels. Alginic acid content was, however, higher in the whole sea tangle powder diet (4.3%) than in the pure alginic acid added diet (1%). This is due to the fact that the sea tangle powder was analyzed to contain 28.7% (w/w) alginate. A smaller increase in the immunoglobulin in whole sea tangle powder fed mice may be due either to a low release of alginate from sea tangle tissue matrices in the gut or to other components in the powder. Effects of dietary fiber on production of immunoglobulins have been shown to be more pronounced in cecum contents or mesenteric lymph node lymphocytes than in the serum (9,10). This seems plausible since the intestine is the very site where dietary fibers exert their actions on. To elucidate the effect of alginate on serum IgG in detail, it is required to examine intestinal production of immunoglobulins including secretory IgA. Splenocyte proliferation in response to LPS was increased only by alginate although it tended to be increased also by whole sea tangle or its water extract. The effect of alginate is regarded to contribute IgG production possibly from B cells which have been reported to be respond well by seaweed extracts (14).

Components other than indigestible polysaccharides including fucan in whole sea tangle cannot be excluded for their roles in stimulation both in humoral and cellular immunity. Dietary sesaminol and sesamin (29), curcumin (30) and lutein (31) have been reported to increase plasma IgG in rats and cats, respectivley. Tocopherol and tocotrienol in the diet have been also shown to enhance the production of IgA and IgG from spleen and mesenteric lymph node lymphocytes (32). Seaweeds contain considerable amounts of carotenoid, vitamins C, calcium (33), trace minerals (34) and vitamin B_{12} (35). It needs to be evaluated whether these compounds are related to the production of immunoglobulins. Whatever the mechanisms may be, enhancement of humoral immunity by sea tangle will alleviate susceptibility to infection in diabetes.

In contrast to increases in TNFα production from mesenteric lymph node lymphocytes by soluble dietary fiber *in vivo* (9) and from macrophages by seaweed glycoprotein *in vitro* (10), we did not find any increase in plasma conccentrations of TNFα or its production by macrophages but some decreasing tendency by dietary sea tangle or its components. The dif-

ferent results in the present study may be due to the lack of direct contact between algal polysaccharides and macrophages. Komatsu et al. (36) reported that some effective component(s) absorbed from oral administration of cabbage juice stimulated TNFα and IL-1β production from resident peritoneal macrophages, implying that low molecular weight compound(s) could affect cytokine production. On the other hand, extract of Evodia rutaecarpa had biphasic effects on production of the cytokines depending on concentrations. At present, it is not known how sea tangle component(s) are involved in cytokine production. But the tendency to reduce TNFα production by sea tangle is of significance since it could be beneficial to diabetic patients whose high TNFα level is not desirable.

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