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Enhancement of Mucosal Immune Functions by Dietary *Spirulina platensis* in Human and Animals

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This paper reviews the effects of *Spirulina platensis* and its extracts and phycocyanin, a blue photosynthetic pigment protein in *Spirulina* on the mucosal immune functions in humans and animals as follows:

- **IgA antibody response and other classes in mucosal immunity of mice treated with *Spirulina platensis* and its extract.**
- **Effect of *Spirulina* phycocyanin ingestion on the mucosal antibody responses in mice. - Distinct effects of phycocyanin on secretory IgA and allergic IgE antibody responses in mice following oral immunization with antigen-entrapped biodegradable microparticles.**
- **Influence of dietary *Spirulina platensis* on IgA level in human saliva.**
- **A study on enhancement of bone-marrow cell-proliferation and differentiation by *Spirulina platensis* in mice: *in vivo* and *in vitro* study**

Key words : *Spirulina platensis,* Macrophage, IL-1, IgA, IgE, IgG1, Mucosal immunity

INTRODUCTION

Spirulina platensis, which is a helicoidal filamentous blue-green alga or cyanobacterium, had a history of being used as food for over a thousand years[1),2)] and has been commercially produced for the last thirty years or more as a food supplement. *Spirulina* is known to have nutritional advantages of high-quality protein contents and other components such as vitamins; minerals; essential fatty acids, including γ-linolenic acid, and β-carotene[3)]. Recently, more attention has been given to the study of the therapeutic effects of *Spirulina*. In addition to effectiveness in reducing hyperlipidemia, diabetes, and high blood pressure in man and animals, anti-viral and anti-cancer effects of orally administered *S. platensis* involving immune functions have also been reported[4)-6)]. Previously, we reported that *Spirulina* and its extracts enhanced immune responses in mice mainly through increased production of IL-1 in macrophages[7),8)].

IgA Antibody Response and Other Classes in Mucosal Immunity of Mice Treated with *Spirulina Platensis* and its Extract.

Primary immune response and macrophage function: As the beginning of the study we demonstrated that mice fed laboratory chow containing 10 or 20 % (w/w) dried *Spirulina platensis* for 7 weeks showed increased numbers of splenic antibody-producing cells in the primary immune responses to sheep red blood cells[8)]. The percentage of phagocytic cells in peritoneal macrophages as well as proliferation by mitogens of spleen cells from the mice fed *Spirulina* diet was also significantly enhanced. Adding a hot-water extract of *Spirulina platensis* (SpHW) itself as well as the supernatant of macrophages stimulated with SpHW to the culture medium resulted in enhancement of *in-vitro* antibody production of the spleen cells. Thymocyte-proliferation assay and ELISA showed that the supernatant of the macrophages stimulated with SpHW contained signifi-cantly increased interleukin-1 (IL-1) activity and TNFα level, respectively. These results suggested that *Spirulina* enhanced the immune responses in mice, particularly the primary response, by stimulating macrophage functions[8)]. It has been also reported that intraperitoneally injected polysaccharides of *Spirulina* increased the percentage of phagocytic cells in peritoneal macrophages in addition to the hemolysin content in the blood of mice[9)]. Bactericidal activity as well as NO production of macrophages in the broiler chickens was enhanced by feeding *Spirulina* containing 0.5 - 2.0% in the feed for 6 weeks[10)].

IgA antibody response in the intestinal mucosa and other antibody responses: Numerous studies in humans

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and animals have provided convincing evidence that protection against a variety of viral and bacterial mucosal pathogens can be obtained by oral or intranasal immunization. Secretory IgA antibody is known as the predominant isotype in most secretory tissues or mucosal surfaces and exhibits various biological properties such as agglutination of microorganisms; neutralization of bacterial enzymes, toxins, and viruses; immune exclusion; and inhibition of antigen or allergen absorption[11].

It was noted that IgA antibody level in the intestinal contents was significantly enhanced in the mice treated with a hot-water extract of *Spirulina* for 5 weeks concurrently ingested with antigen, in comparison to that of the mice treated with the shrimp antigen alone[12]. Enhancement of IgA antibody production was also observed in culture supernatant of lymphoid cells, especially in the spleen and mesenteric lymph node from the mice treated with the *Spirulina* extract for 4 weeks prior to antigen stimulation. IgG1 antibody response was also enhanced by the *Spirulina* extract while IgE antibody was not further enhanced. These results suggest that *Spirulina* ingested both concurrently with antigen and prior to antigen stimulation may significantly enhance the IgA antibody level to protectively affect on infectious or allergic reaction, and that *Spirulina* may at least neither induce nor enhance allergic reaction like food allergy which depends on IgE antibody.

Effect of *Spirulina* Phycocyanin Ingestion on the Mucosal Antibody Responses in Mice.

- Distinct effects of phycocyanin on secretory IgA and allergic IgE antibody responses in mice following oral immunization with antigen-entrapped biodegradable microparticles.

We investigated then effect of *Spirulina* phycocyanin ingestion on immune responses of the intestinal mucosa as one of the body's defense mechanisms against allergies and infectious diseases. The study showed that ingestion of phycocyanin was found to promote IgA antibody production in the intestinal mucosa particularly in Peyer's patches of the mice immunized with ovalbumin (OVA) as an antigen[13]. The antigen-specific IgA antibody levels, however, in both Peyer's patches and mesenteric lymph nodes were too low to be detected probably due to using aqueous solution of OVA as an antigen, that is, aqueous solution of OVA might have not enough retention time in the digestive tract to demonstrate sufficient antigenicity and might be easily metabolized. In order to solve this problem, we prepared OVA antigen-entrapped biodegradable microparticles made of poly (DL-lactide-co-glycolide), and used as a stimulating antigen[14,15] for local antibody response in the mucous.

In the study, we measured IgA antibody responses in the intestinal mucosa of the mice ingested phycocyanin by using OVA-entrapped biodegradable microparticles as an orally stimulating antigen. We also examined the effects of phycocyanin on type I allergies by measuring serum IgE antibody levels and on intestinal vascular permeability with respect to inflammation. In the mice ingested phycocyanin for 6 weeks, a marked increase in the antigen-specific IgA antibody level as well as the total IgA antibody level was observed in the intestinal mucosa, the Peyer's patches and mesenteric lymph nodes, all of which comprise the intestinal mucosal immune system, and also in the spleen cells[16]. Phycocyanin ingestion for 8 weeks, on the other hand, suppressed the production of antigen-specific IgG and IgE antibody in the serum. Inflammation of the small intestine, determined as vascular permeability by Evans blue-leaking method, was also reduced by 6-week ingestion of phycocyanin at least. These results suggest that phycocyanin also enhances biological defense activity against infectious diseases and reduces allergic inflammation through sustaining functions of the mucosal immune system.

Increase of IgA Antibody Levels in Human Saliva by Ingesting *Spirulina Platensis* as Health Food

We investigated, in the study, the effect of *Spirulina* on salivary IgA antibody level of the subjects who ingested the commercial *Spirulina* tablets as health food in various period of usage in their daily life, and detected correlation between the salivary IgA level and the amount of *Spirulina* ingested. Salivary glands have been recognized as part of the common mucosal immune system, and saliva has been used by researchers for studies of the influence of various parameters on the human mucosal immune system[17].

One hundred and thirty four employees of a manufacturing company, average age; 43.2±12.1 years old (from 20 to 62 years old), were enrolled in the study as subjects and asked to offer saliva specimens and to answer some questionnaires about duration and experience of *Spirulina* (SP) ingestion. One hundred and twenty seven saliva specimens from 91 men and 36 women were collected after having obtained informed consent from each subject. Each saliva specimen was collected in dental roller cotton being kept in the mouth for three minutes. Saliva specimens were obtained as supernatant after centrifugation and secretory-IgA (S-IgA) antibody level in saliva was measured by ELISA. Total S-IgA level of the group ingesting SP continuously (Continuous group) in men was significantly higher ($p < 0.05$) than that of the group ingesting SP discontinuously (Discontinuous group). Total S-IgA level of the group ingesting SP for more than one year (>1 year group) was significantly increased ($p < 0.01$) in comparison to the group ingesting SP for less than half a year (<0.5 year group). Further, statistically significant correlation between S-IgA levels in the saliva and total amount of *Spirulina* ingested by the subjects was observed (correlation coefficient $R = 0.288$, $n = 72$, $p < 0.05$)[18].

A Study on Enhancement of Bone-Marrow Cell-Proliferation and Differentiation by *Spirulina platensis* in Mice (in vivo and in vitro study)

Phycocyanin, a characteristic photosynthesis pigment protein in *Spirulina*, is known to show antioxidant activity and to enhance differentiation of a human monocytoid leukemic cells, U937, to monocytes or neutrophils[19]. These findings suggest that *Spirulina* including its components such as phycocyanin affects enhancing or sustaining immune functions normally as a consequence of promoting immune competent-cell proliferation or differentiation in lymphoid organs. Multi potent colony-stimulating factors or IL-3 produced by a variety of cells, such as lymphocytes, monocytes, and fibroblasts, can proliferate and differentiate immature hematopoietic cells to be matured. *Valtieri et. al*[20] reported that G-CSF stimulated terminal differentiation of the IL-3-dependent granulocytic lineage, 32D C13 cells, derived from normal murine bone marrow into neutrophilic granulocytes.

In this study, we focused on the investigation of effects of *Spirulina* and its extracts such as a hot-water extract and phycocyanin on induction of colony stimulating factor(s) and on their proliferation and differentiation activity for hematopoietic-cell in mice[21]. Culture supernatants of the spleen cells, which were stimulated with the hot-water extract of *Spirulina* (SpHW), phycocyanin, and cell wall components (SpCW) in culture, induced colony-stimulating factor(s) (CSFs) that were detected by colony forming assay by using bone marrow cells in soft agar culture assay, and granulocyte-macrophage-colony stimulating factor (GM-CSF) was detected in the culture supernatants of the spleen cells by ELISA method. The colony forming activity was also detected in the spleen cells and peritoneal macrophages of the mice which ingested SpHW and phycocyanin for 6 weeks, and ingestion of SpHW enhanced proliferation of bone marrow cells into lymphocyte or granulocyte.

These results suggested that *Spirulina* and its extract or phycocyanin enhanced immunological functions in man and animals as a consequence of summation of each resultant activity, such as induction of IL-1, antibody-producing and cell-proliferating activities, and promoting cell-differentiation. Details should be investigated further.

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