

Blue-Green Algae as an Immuno-Enhancer and Biomodulator

Gitte S. Jensen, PhD,¹ Donald I. Ginsberg, MS,² Christian Drapeau, MS²

1. Holger N.I.S. Inc., Port Dover, Ontario, Canada
2. Medical Student, McGill University, Montreal, Quebec, Canada
3. Desert Lake Technologies LLT, Keno, Oregon

INTRODUCTION

In the evolving health management paradigm,¹⁻⁴ the general regulation of the immune system as well as the enhancement of specific immune functions have become a growing point of interest, and rightly so. Many health problems result from the inability of the immune system to stop a disease process in its initial stage. This paper will review the scientific evidence for the immunomodulatory effects of blue-green algae and some of the demonstrated effects of blue-green algae on health and disease.

The human body is constantly being exposed to foreign organisms such as bacteria, viruses, fungi, and parasites, all of which coexist to a certain degree in the skin, the mouth, the respiratory tract, the intestinal tract, and the genital tract. Some microorganisms are essential for optimal health, and the healthy human body is well-equipped to keep such organisms from becoming a problem. However, when the natural barriers are compromised, or when we are exposed to more highly infectious organisms, serious disease may result. This includes not only acute infectious diseases, but also chronic inflammatory and autoimmune diseases. Optimal support of the immune system is important for prevention of and intervention with diseases with microbiological involvement, whether acute illness or chronic degenerative disease. Inflammation sets the stage for chronic disease, and for the initiation and progression of cancer. Enormous research efforts are currently pursuing nutritional and botanical intervention of inflammatory processes.

*Correspondence:

Gitte S. Jensen, PhD
 Holger N.I.S. Inc.
 12 Denby Road
 Port Dover
 Ontario, Canada N0A 1N4
 e-mail: gitte@holgernis.com

BLUE-GREEN ALGAE AS FOOD

Blue-green algae (cyanobacteria) are among the most primitive life forms on Earth. Their cellular structure is a simple prokaryote. They share features with plants, as they have the ability to perform photosynthesis. They share features with primitive bacteria because they lack a plant cell wall. Interestingly, they also share characteristics of the animal kingdom as they contain on their cellular membrane complex sugars similar to glycogen. Among blue-green algae we find both edible and toxic species, adapted to almost any of the most extreme habitats on Earth, including deep-sea vents, hot springs, and Antarctica's ice. Edible blue-green algae, including *Nostoc*, *Spirulina*, and *Aphanizomenon* species have been used for food for thousands of years. Habitats with sufficient algae growth include the Pacific Ocean near Japan and Hawaii, and large freshwater lakes, including Lake Chad in Africa, Klamath Lake in North America, Lake Texcoco in Mexico, and Lake Titikaka in South America. African and American natives recognized the value of including blue-green algae in their diet and stored dried algae for year-round use and trade.

Still today, edible blue-green algae are a nutrient-dense food. As for any other crop, differences exist with regard to harvest procedures, quality control for contaminating species, adherence to proper processing to preserve nutrients from degradation, and storage conditions. The nutrient content depends on the location and environment in which the algae was grown as altitude, temperature, and sun exposure can greatly affect lipid and pigment composition. *Spirulina* is an algae species grown at sea or in man-made ponds, and the mineral profile will differ from fresh-water algae such as *Aphanizomenon*. Algae grown in a natural environment will differ from algae grown in canals or tanks due to differences in aeration, nutrient circulation and availability, and degree of competition with other algal species. As we learn more about the phytochemical components of different blue-green algae species, the optimal growth conditions for obtaining optimal yields can be determined.

The nutrient profile is subject to much variation between habitats and harvest procedures which influences the content of vitamins and antioxidants delivered in the final product. Certain features are common to all blue-green algae, including a high content of bioavailable amino acids and minerals, including zinc, selenium, and magnesium. Industrial standards still vary greatly in terms of documenting product composition to the consumer. However, blue-green algae have the appeal of being a raw, unprocessed food, rich in carotenoids, chlorophyll, phycocyanin, and many other bioactive components.

BEYOND NUTRITION

Among blue-green algae, many species have documented biomodulatory effects. This paper will review scientific evidence for immunomodulatory effects of blue-green algae and some of its demonstrated effects on health and disease. The research studies span the use of the whole algae of various species in both human and animal studies, as well as in vitro studies on algae extracts and purified compounds (Table 1).

EFFECTS OF BLUE-GREEN ALGAE ON INNATE (NON-SPECIFIC) IMMUNITY

Several studies have examined the use of whole blue-green algae in the context of the normal functioning immune response. In our lab, one study using oral doses of 1.5 grams of the blue-green algae *Aphanizomenon flos-aquae* on healthy human volunteers revealed it slightly decreases the phagocytic activity of polymorph nucleated cells in vitro.⁵ This may indicate an anti-inflammatory, rather than anti-phagocytic effect on human neutrophils.

In a study looking at the phagocytic function of cat bronchoalveolar macrophages in vitro, the percentage of

cells that phagocytosed cells increased when they were exposed to a water-soluble extract of *Spirulina* for two hours.⁶ The number of particles ingested by the phagocytic macrophages did not change when compared to control cultures.

In another study, mice were fed a *Spirulina*-supplemented diet (10% of the dry weight of food) for ten weeks, and the ability of peritoneal macrophages to ingest latex particles was evaluated in vitro. The results of this study showed a slight increase in the percentage of phagocytic cells (4.6%; from 91.3 to 95.9%).⁷ A similar effect was observed in chickens.⁸

In addition, murine peritoneal macrophages exposed in vitro to a hot-water extract of *Spirulina* for 24 hours secreted a substance, (speculated to be IL-1), which induced thymocyte proliferation.⁷ In the same study, the ability of spleen cells extracted from algae-fed mice to proliferate in response to mitogens was examined in vitro. These experiments showed that splenic cells isolated from algae-fed mice proliferated more when exposed to certain mitogens compared to control mice.

The effect of blue-green algae on non-specific immunity has also been examined at the level of natural killer (NK) cell activity. Using a standard chromium release assay, splenic leukocytes from chickens fed blue-green algae were shown to exhibit greater anti-tumor cell activity when compared to those of control animals.⁸ The authors speculate that blue-green algae may increase NK cell activity via the production of cytokines such as interferon.

In a study designed to investigate the mechanism behind the immunostimulatory effect of blue-green algae on the human monocyte/macrophage cell line THP-1, a crude extract of the blue-green algae *Aphanizomenon flos-aquae* was used to stimulate the cell line. The extract was half as potent as LPS in activating NF-kB, and the purified molecule is ten times more potent than LPS (Pasco, manuscript in press). The molecule responsible for this activation has been identified as a novel polysaccharide.⁹

Thus, multiple studies on whole blue-green algae in humans, mice, rats, cats, and chickens have demonstrated an effect on phagocytosis, NK cell function, and inflammation. Some differences exist in the data, including the mild reduction of phagocytic activity in humans after algae consumption, in contrast to the increase of phagocytosis among bronchoalveolar macrophages. The cell types and experimental set-ups vary, and further studies are needed to establish the exact biochemical mechanisms involved.

EFFECTS OF BLUE-GREEN ALGAE ON SPECIFIC IMMUNITY

Hayashi et al⁷ examined the effect of an algae-supplemented diet on the ability to build a specific immune response

Table 1. Research On Blue-Green Algae As Biomodulators

Studies on	Route of administration	Compounds investigated
Human	Oral consumption*	Whole algae
Chicken	Oral consumption	Whole algae
Rodents	Oral consumption**	Whole algae
	Injection	Isolated fractions
	Injection	Purified compounds
In vitro	Added to media	Isolated fractions
	Added to media	Purified compounds

*) Humans: oral dose was 1.5 – 2.8 grams per day for adult subjects

**) Mice: oral dose varied up to 800 mg/kg

Table 2. Immuno-Modulatory and Anti-Inflammatory Effects of Whole Blue-Green Algae

<u>Algae Species</u>	<u>Introduced as:</u>	<u>Test Species</u>	<u>Effects</u>	<u>Reference</u>
<i>Spirulina</i> sp.	Food	Human	Reversal of tobacco-induced oral cancer	Mathew et al, 1995
	Food	Mouse	Proportional reduction of IgE, increase of IgA	Hayashi et al, 1998
	Food	Mouse	Increased phagocytic activity Increased spleen cell proliferation Increased antibody production	Hayashi et al, 1994
	Food	Chicken	Increased phagocytic activity Increased NK cell-mediated anti-tumor activity Increased antibody production	Qureshi et al, 1996
	Extract	In vitro, cat	Increased phagocytic activity	Qureshi & Ali, 1996
	IP injection	Rat	Inhibition of mast cells Decrease in local allergic reaction Decrease in serum histamine levels Reduced allergy-induced mortality	Kim et al, 1998 Yang et al, 1997
<i>Aphanizomenon flos-aquae</i>	Food	Human	Increased transient recruitment of NK cells into tissue Increased mobilization of T and B cells into blood Mild modulation of PMN-mediated phagocytic response	Jensen et al, 2000
	Food	Rat	Decreased serum levels of arachidonic acid	Kushak et al, 2000
	Food	Rat	Source of linolenic acid (omega-3) Increased serum levels of EPA and DHA	Kushak et al, 2000
	Extract	In vitro, rat	Activation of macrophages (NF-kappaB, cytokines)	Pasco, in press

to sheep red blood cells. After immunizing mice (either once to measure the primary response or twice for the secondary response), they found that mice fed with the algae-supplemented diet showed increased numbers of splenic IgM antibody-producing cells when compared to control animals. Interestingly, this finding only held true for the primary immune response, as the IgG antibody production in the secondary immune response was hardly affected. In experiments involving chickens, there were no differences observed in anti-sheep red blood cell antibodies during primary responses, while antibody titers for the secondary response in algae-fed chickens were augmented compared to control animals.⁸ The differences may reflect the anatomical differences between the rodent and chicken immune systems.

Hayashi et al¹⁰ examined other antibody classes such as IgA and IgE in the context of mice orally immunized with a crude shrimp extract. They found that whereby both IgA (intestinal) and IgE (in serum) levels increased with antigen challenge, only IgA levels showed a greater enhancement in secretion with concurrent treatment with *Spirulina* extract (five-week feeding regimen).¹⁰ From this study they concluded that blue-green algae does not seem to induce or enhance food allergic IgE-dependent reactions. Furthermore, they suggest that when ingested along with or before a potential antigenic threat, blue-green algae may enhance IgA anti-

body levels to protect against food allergies.

Along the same lines, further studies have suggested that blue-green algae may inhibit mast cell-mediated type I allergic reactions and even the anaphylactic reaction in rats.^{11,12} By injecting a blue-green algae extract intraperitoneally (100-1000mg/g body weight) one hour prior to an allergic challenge, mortality induced by the anaphylactic compound 48/80 was decreased, local allergic reaction activated by anti-dinitrophenyl (anti-DNP) IgE was inhibited, and serum histamine levels were decreased. In vitro experiments from this group provided similar results.

The effects of blue-green algae on IgE-production and allergic reactions are encouraging, and warrant further studies in humans.

EFFECTS OF BLUE-GREEN ALGAE ON LEUKOCYTE TRAFFICKING

Much attention with regards to dietary modulation of the immune system has been given to stimulating activity of various immune cell types such as the phagocytic activity of macrophages, or the tumoricidal activity of natural killer cells. However, immune cell trafficking and the recruitment of immune cells from the systemic circulation are of equal importance. A recent study by Jensen et al⁵ involving

Table 3. Bio-modulatory Effects of Purified Compounds from Blue-Green Algae

<u>Species</u>	<u>Compound</u>	<u>Effects</u>	<u>References</u>
<i>All blue-green algae</i>	C-Phycocyanin	Anti-inflammatory (reduces leukotriene B4) Free radical scavenger Selective inhibition of COX-2 Reduced tissue damage in acetic acid-induced colitis Hepatoprotective effect	Romay 1999 Bhat & Madyastha 2000 Reddy et al, 2000 Gonzalez et al, 1999 Vadiraja et al, 1998
<i>Spirulina</i>	Calcium Spirulan (Ca-Sp)	Selectively inhibits penetration of virus into host cell (Herpes Simplex, human cytomegalovirus, measles, mumps, Influenza A, HIV-1)	Hayashi et al, 1996
		Reduces lung metastasis of melanoma cells by inhibition of tumor cell invasion of basal membrane	Mishima et al, 1998
	Cyanovirin-N	Irreversible inactivation of several strains of HIV (inhibited cell-to-cell and virus-to-cell fusion)	Boyd, 1997
	Extracellular products	Promotion of lactic acid bacteria growth in vitro	Parada et al, 1998
<i>Aphanizomenon flos aquae</i>	Unknown	Induces apoptosis in some human tumor cell lines	Jensen, msp in prep
	Polysaccharide	Stimulate the macrophage activity	Pasco et al, in press.
<i>Lyngbya lagerheimii</i> <i>Phormidium tenue</i>	<i>Sulfolipid</i>	<i>Inhibits syncytium formation upon HIV infection</i>	<i>Gustafson et al, 1989</i>
<i>Phormidium tenue</i>	Digalactosyl diacylglycerols	Inhibition of chemically induced skin tumors	Tokuda et al, 1996

humans demonstrated that the blue-green alga *Aphanizomenon flos-aquae* was able to trigger within two hours the migration of nearly 40% of the circulating natural killer cells. This effect was significantly more pronounced in long-term consumers than in naïve subjects. In the same study, *Aphanizomenon flos-aquae* was also shown to stimulate the mobilization of T and B lymphocytes. This effect appeared cell-type specific since no changes were observed on polymorph nucleated cells.

ANTI-INFLAMMATORY PROPERTIES OF BLUE-GREEN ALGAE

Blue-green algae in general contain a significant amount of carotenoids, namely beta carotene, lycopene, and lutein, providing it with good antioxidant properties. By their quenching action on reactive oxygen species, antioxidants carry intrinsic anti-inflammatory properties. However, blue-green algae also contains specific anti-inflammatory properties as a result of their high phycocyanin content. Phycocyanin is a photoharvesting pigment that provides the intense blue color in blue-green algae. It can constitute up to 15% of the dry weight of a blue-green algae harvest. C-phycocyanin is a free radical scavenger,²⁶ and has significant hepatoprotective effects.²⁷ Phycocyanin was shown to inhibit inflammation in mouse ears²⁸ and prevent acetic acid induced colitis in rats.²⁹ The anti-inflamma-

tory effect seemed to be a result of phycocyanin to inhibit the formation of leukotriene B4, an inflammatory metabolite of arachidonic acid.²⁸

In a study performed in rats, the blue-green algae *Aphanizomenon flos-aquae* was also shown to decrease the plasma level of arachidonic acid.³⁰ *Aphanizomenon flos-aquae* contains significant amounts of the omega-3 alpha-linolenic acid. Omega-3 fatty acids have been shown to inhibit the formation of inflammatory prostaglandins and arachidonate metabolites. Since *Spirulina* contains significant amounts of omega-6 gamma-linolenic acid, the anti-inflammatory properties of *Spirulina* must be due to different biochemical pathways.

ANTI-VIRAL EFFECTS

As part of its program aimed at discovering new anti-tumor and anti-viral agents in natural sources, the National Cancer Institute isolated extracts of blue-green algae (*Lyngbya lagerheimii* and *Phormidium tenue*) that were found to protect human lymphoblastoid T cells from the cytopathic effect of HIV infection. Upon further investigation, a new class of HIV inhibitory compounds called the sulfonic acid-containing glycolipids were isolated; the pure compounds were found to be strikingly active against the HIV virus in the p24 viral protein and syncytium formation assays.¹³ Since this discovery, there has been further investigation into other

species of blue-green algae for compounds with anti-viral properties. Some compounds worthy of mention include a protein called cyanovirin-N which appears to irreversibly inactivate diverse strains of the HIV virus and to inhibit cell-to-cell and virus-to-cell fusion.¹⁴ Other studies using a water-soluble extract of blue-green algae have found a novel sulfated polysaccharide, calcium spirulan (Ca-SP), to be an antiviral agent. This compound appears to selectively inhibit the penetration of enveloped viruses (Herpes simplex, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1) into host cells, thereby preventing replication.¹⁵⁻¹⁷ A review of anti-HIV activity of extracts from blue-green algae has been recently published.¹⁸

ANTI-CANCER EFFECTS

An early study on blue-green algae's cancer-preventive properties in humans was performed on tobacco-induced oral leukoplakia.¹⁹ Mathew et al found that oral supplementation with *Spirulina fusiformis* resulted in complete regression of 57% of subjects with homogenous leukoplakia. After discontinuation of *Spirulina* supplementation, almost half of the complete responders developed recurrent lesions.

In other studies, extracts of blue-green algae have been used to treat cancer in animal models. In one model, ingestion of an extract of *Spirulina* and *Dunaliella* was shown to inhibit chemically-induced carcinogenesis in hamster buccal pouches.^{20,21} Earlier studies often attributed the anti-cancer effect of algae to its content in carotenoids since beta-carotene has been shown to have an effect similar to that of algae extract. A more recent study, however, showed that the sulfated polysaccharide mentioned above, Ca-SP, appears to inhibit tumor invasion and metastasis.²² Both the in vitro and in vivo effects of Ca-SP suggest that the intravenous administration of Ca-SP reduces the lung metastasis of melanoma cells by inhibiting the tumor invasion of the basement membrane. A water-based extract of *Aphanizomenon flos aquae* containing high concentrations of phycocyanin inhibited the in vitro growth of one out of four tumor cell lines tested, indicating that at least some tumor cell types may be directly sensitive to killing by phycocyanin (Jensen et al, manuscript in preparation). Another fresh-water blue-green algae, *Phormidium tenue*, contains several diacylglycerol compounds which effectively inhibited chemically-induced skin tumors in mice.²³ In addition, *Spirulina* was shown to have a modulatory effect on hepatic carcinogen metabolizing enzymes.²⁴

Of major interest to ongoing research in inflammation as well as breast cancer is the finding that C-phycocyanin selectively inhibits COX-2, but has no effect on COX-1.²⁵ The COX enzymes are involved in prostaglandin synthesis. Since COX-2 is over-expressed in many breast cancer cells, and inhibition of COX-2 leads to a markedly reduced tumor growth and blocks angiogenesis, the finding that phyco-

cyanin specifically interferes with this pathway holds promise.

BLUE-GREEN ALGAE AS A BIOMODULATOR

Besides their effects on the immune system, blue-green algae have also been reported to modulate other systems and improve metabolism. In the past few years increasing attention has been given to the study of the therapeutic effects of blue-green algae. The anecdotal claims for such effects are numerous. Although there is limited data from controlled animal or clinical studies, such claims include improvement in condition of Alzheimer's patients, overall enhancement of immune response, improvement in fibromyalgia, control of hypertension, alleviation of depression and chronic fatigue, increased stamina, healing of internal and external lesions, increased mental acuity, and general improvement in overall well-being. This last section will review the scientific evidence supporting the therapeutic effects of blue-green algae.

EFFECTS ON METABOLISM

Several reports from different labs have shown that certain species of blue-green algae have cholesterol-lowering effects in animal and human models. In feeding experiments in rats, two studies have reported that the elevation in total cholesterol, LDL, and VLDL cholesterol in serum caused by cholesterol feeding was reduced when the high cholesterol diet was supplemented with 16% and 5% blue-green algae, respectively.^{31,32} In addition, Kato found that adipohepatosis induced by a high fat and high cholesterol diet was cured rapidly when the diet was supplemented with algae.³¹ Investigations into the mechanism of this phenomenon led to the finding that the algae-fed group showed a statistically significant increase in the activity of lipoprotein lipase, a key enzyme in the metabolism of triglyceride-rich lipoproteins.³³

The hypocholesterolemic effect of blue-green algae was also observed in humans in a study conducted on 30 patients with mild hyperlipidemia and mild hypertension.³⁴ Patients took 4.2 grams of algae or placebo per day, and were observed for two months. At the end of the study, patients taking the algae showed a significant reduction of LDL-cholesterol ($p < 0.05$) compared to the control group. LDL cholesterol increased back to baseline levels after administration of the algae was discontinued. In addition to lowering LDL cholesterol levels, the atherogenic index (a measure of fat deposition in arteries) declined significantly after four weeks of algae consumption.

In a recent study by Kushak et al, rats were fed the blue-green alga *Aphanizomenon flos-aquae* and total cholesterol level was monitored. After 43 days, cholesterol levels were significantly decreased when compared to the control group.³⁰ Although *Aphanizomenon flos-aquae* contains a significant amount of the omega-3 polyunsaturated linolenic

Table 4. Biomodulatory Effects of Whole Blue-Green Algae on Metabolism

<u>Algae Species</u>	<u>Introduced as</u>	<u>Test Species</u>	<u>Effects</u>	<u>Reference</u>
<i>Aphanizomenon</i>	Food	Rat	Reduction of cholesterol	Kushak et al, 2000
	Food	Rat	Reduction of blood glucose levels	Drapeau et al, 2001
	Food	Human	Modulation of brain activity (EEG)	Walker & Valencia, 1999
<i>Spirulina</i>	Food	Human	Reduced body weight	Becker et al, 1986
	Food	Rat	Reduction of cholesterol	Kato et al, 1984
	Food	Rat	Increased activity of lipase	Iwata et al, 1990
	Food	Rat	Reduced glucose levels	Takai et al, 1991
	Food	Rat	Inhibition of maltase and sucrose	Kushak et al, 1999
	Food	Mouse	Modulation of carcinogen metabolic enzymes	Mittal et al, 1999
	Food	Mouse	Modulation of lead toxicity	Shastri et al, 1999
	Food	Rat	Increased iron status during pregnancy and lactation	Kapoor & Mehta, 1998
<i>Nostoc</i>	Food	Rat	Reduction of cholesterol	Hori et al, 1994

acid, the effect on cholesterol levels seemed unrelated to the lipid content of the diets. Kushak et al³⁰ proposed that the hypocholesterolemic effect of *Aphanizomenon flos-aquae* may be due to its chlorophyll content which was shown to stimulate liver function and decrease blood cholesterol.³⁵

In a double-blind crossover study involving human patients, supplementing the diets of obese outpatients with 2.8 grams of blue-green algae three times daily over a four week period resulted in a statistically significant reduction of body weight.³⁶ In a study measuring the effect of blue-green algae on glucose levels in diabetic rats, the water-soluble fraction was found to be effective in lowering the serum glucose level at fasting, while the water insoluble fraction suppressed glucose levels at glucose loading.³⁷ In another study investigating the effect of the blue-green alga *Aphanizomenon flos-aquae* on rat intestinal mucosal digestive enzymes, it was observed that this alga specifically inhibited the activity of maltase and sucrose in a dose-dependent manner.³⁸ Furthermore, this decrease in enzymatic activity was accompanied by a dose-dependent decrease in blood glucose.

The overall conclusion is that blue-green algae may have benefits on lipid and sugar metabolism, as well as liver function. Further human studies are needed to address the feasibility of using blue-green algae in conjunction with cholesterol-lowering medication.

OTHER EFFECTS OF BLUE-GREEN ALGAE

Other research studies on blue-green algae consumption deserve mention. Many reports exist in the literature on its antimicrobial effects. The secretion of anti-microbial substances is an important part of the competition for ecological niches in the natural environment. However, an interesting caveat exists. In one study, *Spirulina* was cultured in vitro, and the extracellular medium was shown to stimulate the growth of lactic acid bacteria.³⁹ If the growth-promoting substance(s) exist in sufficient amounts intracellularly, blue-green algae may play a role in vivo by supporting friendly gut

bacteria. This leads to other facets of health including gut health and nutrient absorption. On that note, consumption of *Spirulina* was shown to support the iron status and hemoglobin of rats during pregnancy and lactation.⁴⁰ *Spirulina fusiformis* had a significant protective effect against lead-induced toxicity in rats.⁴¹ Finally, a report by Valencia et al has presented evidence that *Aphanizomenon flos-aquae* accelerates recovery from mild traumatic brain injury.⁴²

CONCLUSION AND SUMMARY

Research results based on the numerous isolated compounds from blue-green algae warrant the exploration of using whole algae as conjunctive therapy due to the possible synergistic effects of many phytochemicals within the whole algae. The emergence of composite algae supplements in contrast to single algae supplements may also yield further anti-inflammatory, immune-boosting, and metabolic benefits. A significant body of data suggests that blue-green algae immunoenhancing properties could be useful in the adjunct treatment of various diseases involving 1) suppressed or exhausted immune system, and 2) inappropriate immune response including allergies, autoimmune diseases, and chronic inflammatory conditions. The data presented also suggests that blue-green algae could be useful as an adjunct in the treatment of cancer and AIDS, and calls for the design of controlled human clinical studies.

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