RAAD ALGEN

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AFA

THE MIRACLE FORMULA

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2013-2014

PREFACE

This work represents the net result of faithful efforts carried out by Raad Algen scientific office. We are hoping that it will provide the reader with scientifically proven data about the therapeutic effects and their possible mechanisms beside all the basic data about this amazing God made alga concerning its origin, composition, history of use, safety and so on. We would like to thank all the scientists who provided the humanity with these valuable scientific data specially Dr. Jeffrey I. Bruno the author of (Edible microalgae; A Review of the Health Research) and Dr. Christian Drapeau the author of (Primordial Food Aphanizomenon Flos-Aquae). This essay is different from previous works in paying some attention (in brief) to the normal physiology of different body systems and the underlying causes and pathogenesis of different diseases. The scientific data presented here are interacting and overlapping with each other making dividing this essay into distinct chapters was so difficult, so it is recommended to read the whole book to view the full image. Also we should clarify that this essay is a pure scientific work without any other purposes. Some of the therapeutic effects are shared between blue green algae, while others are unique to AFA. The diverse therapeutic effects of AFA still need years and years of extensive researches. We have mentioned only the scientifically and clinically proven data provided by many professors and ignored patients' reports of improvement in extremely wide disease entities waiting for their scientific explanations. We will be grateful for you for providing us with your suggestions and feed back to correct any unintended mistakes and avoid them in next editions.

The authors

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List of abbreviations

AA: Arachidonic Acid

AFA: Aphanizomenon flos-aqua

AFB1: Aflatoxin B1

AFA-W: AFA Water Extract

AD: Alzheimer's Disease

ADD: Attention Deficit Disorder

ADHD: Attention-Deficit Hyperactivity Disorder

Anti-DNP: Anti-Dinitrophenyl

ASC: Adult Stem Cells

BM: Bone Marrow

BMSCs: Bone Marrow Stem Cells

BUN: Blood Urea Nitrogen

CAM: Complementary and Alternative Medicine

c AMP : Cyclic adenosine mono-phosphate

CARET: Carotenoid and Retinol Efficacy Trial

Ca-SP : Calcium spirulan

CD: Cluster determinant

Chla: Chlorophyll

CHL: Chlorophyllin

CL: Chemiluminescence

CNS : Central Nervous System

Con-A: Concanavalin A

COX: Cyclo-Oxygenase Enzymes

CRP: C-Reactive Protein

CV-N: Cyanovirin-N

DHA: Docosahexaenoic Acid

DLT: Desert Lake technologies

DMSO: Dimethylsulphoxide

DSHEA: Dietary Supplement Health and Education Act

DTH: Delayed type hypersensitivity

EDTA: Ethylene-diamineteraacetic acid

EFA: Essential Fatty Acid

ELISA: Enzyme Linked Immunosorbent Assay

EPA: Eicosapentaenoic Acid

ESC: Embryonic Stem Cells

FDA: Food and Drug Administration

GL: Glycolipid

GO: Glucose Oxidase

GM-CSF: Granulocyte/macrophage colony stimulating factor

H2o2: Hydrogen Peroxide

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

HLA: Human leukocyte antigens

HVR: Hypervariable regions

IASCR: Induction of Adult Stem Cell Recruitment

IDU's: Injection drug users

IFN: Interferon

IFN/RBV: Interferon/Ribavirin

Ig : Immunoglobulin

IL: Interleukin

IPA: Insulin Potentiation Therapy

LA: linoleic Acid

LCL: luminol-enhanced chemiluminescence

LD: Lethal Dose

LDL: Low density Iipoprotein

LNA: linolenic acid

LPS: Lipopolysaccharide

LSL l: selectin ligands

MAAs: Mycosporine-like Amino Acids

MAC: Maximum Acceptable Concentration

MAO: monoamine oxydase

3MC: 3-Methylcholanthrene

MHC: Major histocompatibility complex

MS: Multiple sclerosis

NAIDS: Nutritionally acquired immune deficiency syndromes

NCI: National Cancer Institute

NIH: National Institutes of Health

NK: Natural killer

NOAEL: No Observable Adverse Effect Level

NPS: Non-prematurely senescent

NSAIDs: Non-steroidal anti-inflammatory drugs

$O2^{\cdot-}$: Superoxide Anion

OH-: Hydroxyl radical

OCl- : Hypochloride ions

ODA: Oregon Department of Agriculture

OSU: Oregon State University

PAA: Phenylacetic Acid

PBMC: Peripheral blood mononuclear cells

PC: phycocyanin

PCR: Polymerase chain reaction

PEA: Phenylethylamine

PHA: Phytohem agglutinin

Phe: Phenylalanine

PL: Phospholipid

PMN: Polymorphonuclear cell

PPIA: Protein phosphatase inhibition assay

PS: Prematurely senescent

PTDI: Provisionally Tolerable Daily Intake

PUFA: polyunsaturated fatty acids

PWM: Poke-weed mitogens

RDS: Reward Deficiency Syndrome

SAD: standard American diet

SCs: Stem Cells

SDF1: Stromal derived factor1

SGL: Sulfoglycolipids

SIV: Simian immunodeficiency virus

SVR: Sustained virological response

ROS: Reactive oxygen species

RPMC: Rat peritoneal mast cells

Th: Helper T cells

TNF-α: Tumor necrosis factor alpha

Tyr: Tyrosine

UV: Ultraviolet

VLDL: Very low density lipoprotein

Introduction to AFA

"When I was a child I often dreamed of becoming an oceanographer. Now as a psychologist I dream of how these tiny "living flowers of the water" offer keys to open a treasure chest of optimal health, longer life and a green sustainable future. Where some see an ugly primal slime, I see a beautiful reminder that if we but take the time to appreciate nature 's web, we will see simple patterns that can inform our hearts and minds and raise our humanity"

Jeffry J. Bruno, Ph.D.

Pacifica, California

"It is risky business to write a review of this sort aimed at a very diverse audizence. For the interested layperson, there may be too many references to scientific research, technical nomenclature, and complex biological mechanisms. For the serious scientist, there are likely to be too many questionable studies, conjectures, and hypotheses that require further research. For the medical doctor, the health benefits may appear too diverse to be credible, bringing to mind terms like "panacea" or "cure-all." In short, there is something in these pages to bother anybody!"

Jeffrey J. Bruno, Ph.D. 2001

Edible microalgae A Review of the Health Research

It was extremely difficult to cover all the aspects of the microalgae concerning the biochemistry, therapeutic uses, biologic uses and future's hopes. So we were satisfied to convey a sense of the enormity of the possibilities that microalgae can offer to humanity. The sheer number of studies cited—most published in peer-reviewed journals—ought to at

least pique anyone's interest in the benefits of microalgae. Even among the many scientists who have studied microalgae, few appear to have taken the time to view the big picture. It is important that the full range of this information reach a broader audience.

This essay provides the kinds of scientific evidence that are required for scientists and doctors to understand the potential therapeutic uses of microalgae and thereby encourages further research and clinical application; and spurs global thinking regarding microalgae's practical role in helping to solve a number of urgent problems facing humanity in the 21st century.

Diet or Drug, what do we need?

Medicine has never been so sophisticated. Although there are more medications available today for the treatment of diseases than ever before, health is declining at an alarming rate. Spending for health is now second only to defense. While antibiotics have been a good answer to numerous threatening diseases, experience has shown that they are far from the answer to all problems. The antibacterial model has skewed our vision of health by making us think in terms of eliminating disease rather than regaining and maintaining health [2].

Health is gained and maintained by proper nutrition, attitude, sleep, exercise and the judicious use of dietary supplements. We often think of drugs and pharmaceuticals as an effective way of regaining and maintaining health, and we may see it as the only real option. But what about dietary supplements? In the drug model, dietary supplements are

most often assumed to be ineffective or coincidental to recovery. Yet, the effectiveness of certain dietary supplements is undeniable [1].

Influenced by the pharmaceutical model, people seek dietary supplements that will rapidly eradicate symptoms; that is not how dietary supplements work. They work by helping the body regain normal healthy metabolism, they bring support to various organs so they can function well, they assist the body in the essential processes of elimination, nourishment and regeneration; and this is not easily demonstrated. Gaining greater health often means a myriad of small transformations -- greater energy, better mood, better sleep, elimination of little aches-, which altogether lead to a greater quality of life. It is not like measuring reduction in cholesterol level or decrease in blood pressure. It is much more complex, it goes much deeper into the meaning of health, and is by consequence often more difficult to quantify. It is nonetheless very real [2].

When dietary supplement are studied for what they are, building blocks of health and not more or less temporary remedy to eliminate discomforts or reverse a diagnosis, the approach is then completely different, though not less powerful in revealing the health promoting properties of specific herbs or plants. One such plant is the cyanophyta Aphanizomenon flos-aquae (AFA), which has clearly been shown in scientific studies to warrant a great deal of attention [1].

Perhaps one of the reasons microalgal nutrients appear to work in so many areas is that nature is conservative in its designs. Solutions that work are retained. For example, chlorophyll, an “invention” that allows organisms to capture sunlight and produce sugars, first appeared in blue-

green microalgae billions of years ago and is now used as a survival strategy by all higher plants. Animals in turn depend upon chlorophyll-containing plants, directly or indirectly, as a food source [2, 3].

These kinds of threads are repeated countless times throughout nature. Ancient organic molecules, such as amino acids, which were found in blue-green microalgae at the dawn of life, now act as basic building blocks for all of earth's creatures. Potent antioxidants (e.g., beta-carotene or glutathione) that originated in primitive microalgae are conserved and widely used across nature. Likewise, essential fatty acids (EFAs) are critical structural components of cell membranes and play a foundational role in our brain chemistry. Microalgae are the primary source of EFAs in the food chain! In short, microalgae at the bottom of the food chain provide an ancient biomolecular "pharmacopoeia" upon which most of cellular life now depends[1].

More than eleven areas of research are reviewed, ranging from algae's ability to enhance brain function to issues of safety A few common components found within microalgae, such as antioxidants, essential fatty acids, and amino acids, are significant across a range of topics.
i.e. Thousands of testimonials support the health benefits of AFA[1].

Why dietary supplements are not supported by pharmaceutical companies?

You can see it would be foolhardy for a pharmaceutical company to invest millions of dollars in scientific research to demonstrate the efficacy of a given plant and then millions of dollars to promote the

product if this investment cannot be protected by a patent. Consequently, a decision to research a substance is not based on its known effectiveness, but first and foremost on whether it can be protected by patent and will bring substantial financial gain. Dietary supplements are nonetheless -- and fortunately -- available on the market place, but without the protection of a patent few companies feel comfortable or able to invest funds into scientific research that will prove the health benefits of dietary supplements [4].

In other words, most dietary supplement companies do not have the money to perform the scientific studies that would provide the data needed to meet the demands shaped by the pharmaceutical model. Influenced by the pharmaceutical model, the market requests proof of efficacy, toxicity studies, studies on pharmacokinetics, determination of active components and dose studies. But the financial resources within the dietary supplement industry are not sufficient to carry such extensive research work. So dietary supplements cannot be proven to the extent that pharmaceutical compounds can, in spite of the obvious health benefits brought by numerous dietary supplements, without side effects [4].

Multivitamins or whole diet, does it matter?

"Vitamania," as some critics call it, is sweeping the country. Sales of supplements are soaring. In 2000 an estimated 100 million Americans spend over $7 billion a year on nutritional supplements. That is over twice as much as the $3 billion spent in 1990, according to the **Council** for **Responsible Nutrition** in **Washington, DC,** a trade group for the

vitamin industry. Although many people are convinced that vitamin supplements are effective, one large-scale, 13-year government study of 10,758 Americans found multivitamin supplements provided no long-term benefits in terms of increased lifespan or reduced cancer risk.[13]

In fact, taking large doses of synthetic vitamins might even be associated with an increased risk of some kinds of chronic disease.[15] On the other hand, the benefits of eating foods that contain a wide range of nutrients are well supported by scientific research.[15] Eating the right nutrients in the form of whole foods clearly increases life span and decreases stroke and cancer risks.[16] According to the **Surgeon General**, eight of the ten leading causes of death in the United States are diet related.[17]

Despite the widespread use of multivitamins to fortify foods, most consumers don't realize that synthetic forms of vitamins, which are chemically manufactured, may not provide the same benefits as vitamins derived from whole food sources. Giant pharmaceutical companies (e.g., **Merck and Hoffman-La Roche**) and chemical companies (e.g., **Eastman Kodak**) manufacture most of the vitamin isolates that are put into multivitamins and processed foods. Some of the same multinational corporations that sell synthetic vitamins later reap huge profits by selling drugs to treat diet-related diseases. This is not a small business.

Alarmingly, some of these same pharmaceutical-connected companies also sell genetically modified seeds, pesticides, and petrochemical fertilizers to farmers. Obviously, such corporations have little financial incentive to spend millions of dollars to conduct clinical trials on the health benefits of organic foods and herbs, which are not patentable and are therefore less profitable. These corporations also have little incentive

to make sure that the food on your table best supports and increases your health. [1]

For the sake of bigger profits, longer shelf life, and cosmetic appearance, whole foods are robbed of their nutritional value and are then "enriched" or "fortified" with synthetic vitamins and inorganic minerals to comply with the ***FDA****'s minimum recommended daily allowances* ***(RDAs)***. [15]

For example, **General Mills** uses a distillation process to extract vitamin E and other essential substances from soybean and cottonseed to produce vitamin E capsules. What remains is then used as margarine and cooking oil for commercial food products, so damaged by the heat and distillation process, it is no longer a health-building food. Unfortunately too, because of the distillation process the fractionated vitamin E capsule doesn't offer whole food benefits either.[15]

"Milling of whole grain to make refined flour results in loss of 85 percent of the magnesium, 86 percent of the manganese, 40 percent of the chromium, 78 percent of the zinc, 89 percent of the cobalt, 48 percent of the molybdenum, and 68 percent of the copper, in addition to comparable losses of selenium, vitamin E, and essential fatty acids''[20]. Moreover, heavy metals such as cadmium become more concentrated in refined flour, while the protective nutrients, such as biologically chelated zinc, which helps to eliminate that cadmium from our bodies are
mostly removed. To make matters worse, large amounts of sugar, saturated fats, artificial coloring, and preservatives are added to refined

foods. Since a host of nutrients are required to best utilize the calories we consume, the intake of refined foods typically nutritionally poor, but calorie rich, tends to create longer-term nutritional deficiencies that are not remedied by fortification with synthetic vitamins and minerals. [1]

"The real test of the value of refined (fortified) foods would be to put a group of lab animals on a diet of white bread and compare them to a group fed a diet of whole-grain bread. In one such experiment, two thirds of rats kept on a diet of enriched white bread died before the experiment was finished.'' [21]

What's now? Is there any solution?

Before reviewing the extensive research on the health benefits of microalgae, it is important to consider why microalgae are so seldom mentioned in the herbal, botanical, nutritional, and alternative therapies literature.

First, in Western botanical traditions herbs are by definition land-based plants, which categorically exclude aquatic algae. For all practical purposes, Western herbal medicine seldom reaches below the water's surface. Significantly, the **Physician's Desk Reference for Herbal Medicine (1999)** makes no mention of microalgae.[22] Neither does **Dr. Mervyn Werback's (1993) Nutritional Influences on Illness**: A Sourcebook of Clinical Research—one of the most comprehensive nutritional reference books to date.[23]

Second, microalgae can potentially affect many different systems in the body. This multiplicity of effects runs against the Western

mechanistic search for a "magic bullet" one treatment for a specific illness and thus falls into the order of "panacea." Panaceas, or "cure-alls," are not widely embraced by Western medicine; such therapies might include diet, exercise, prayer, or laughter as part of the treatment. Only lately have we seen the emergence of holistic health models that better explain, for example, why the same treatments that improve intestinal function might also benefit our brain and immune systems.[1]

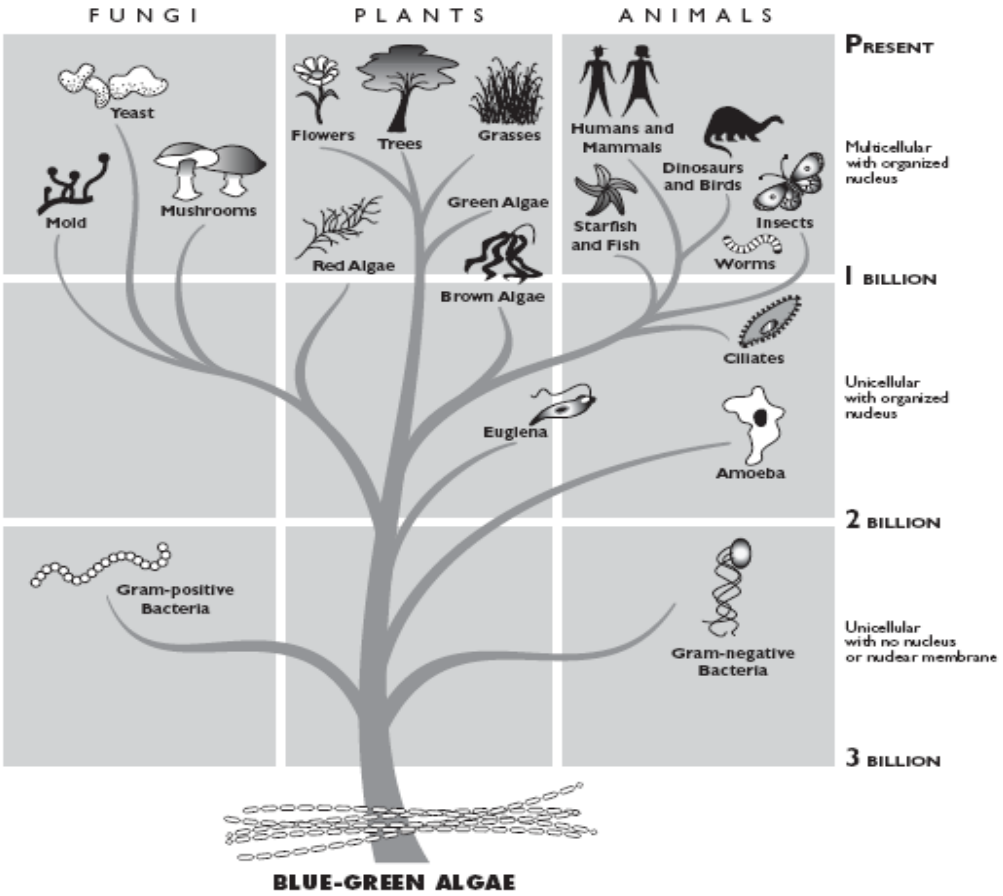


Fig. 1; The tree of life (From Algae to the Rescue!, by Karl Abrams, Logan House Publications, 1996)

Review Of Microalgae

Oriental medicine experts, such as macrobiotic counselors or Chinese doctors, though, do utilize algae and recognize their health benefits. However, these Eastern approaches tend to rely more upon empirical observations and tradition rather than experimental research methods. [1]

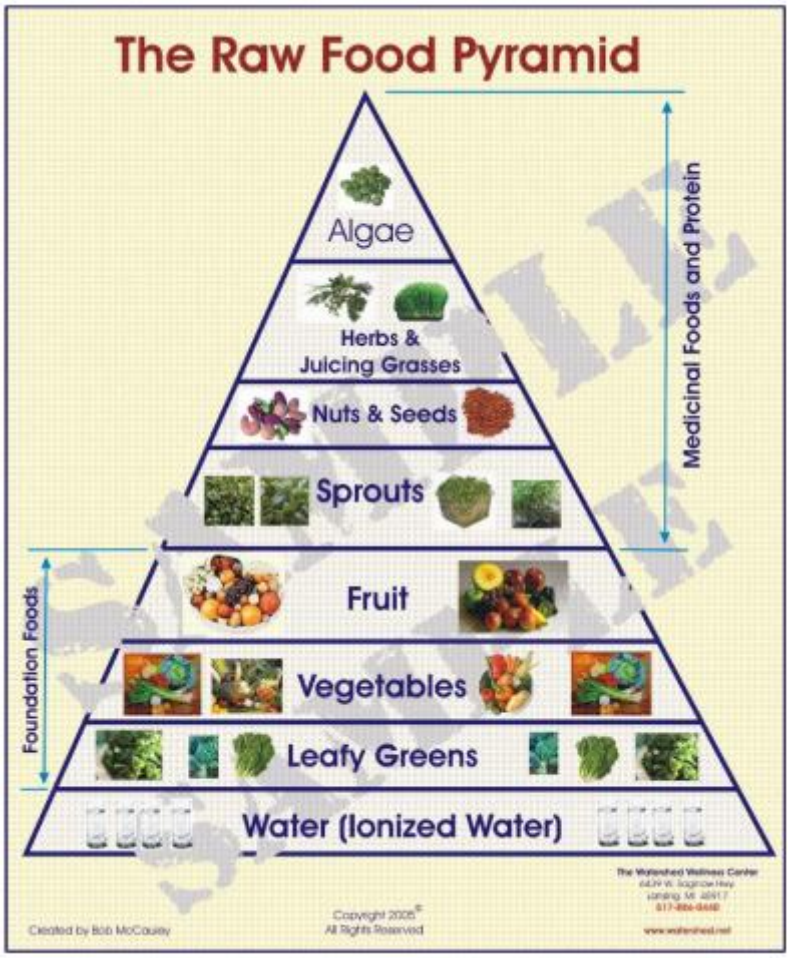


Fig. 2 ; The raw food pyramid.

Another area where algae are medically established is in the European health spas. Around **1867, Bonnardiere**, a French physician, coined the word "thalassotherapy" (from the Greek thalassa or "sea"). He adapted sea therapies that had been used for centuries into a health spa regime,

which included seafood and sea-vegetable diets, seawater drinks, hot seawater baths (38°C), bathing in brown kelp seawater solutions, kelp meal and seawater massages, skin fomentation with ocean bottom mud, and sunbathing.[1]

Numerous health spas continue to use forms of thalassotherapy and algotherapy in France, West Germany, Belgium, Spain, Italy, Yugoslavia, and along the Black Sea coast. Throughout these countries and the Orient these sea- and algae-based therapies have long been used for:

"...treatment of such problems as chronic rheumatism, gout, neuralgia, asthma, wounds, eczema, hemorrhoids, scrofulosis, neuroses, stress-related diseases, and aging, as well as rehabilitation as performed by qualified specialists. [Also] In Japan, Eisenia and Ecklonia added to hot bath water are supposed to prevent or cure palsy and hypertension."[1, 24]

In Western Europe, powdered sea vegetables (Fucus, Ascophyllum, or Laminaria) are kneaded into a paste and sometimes combined with other fomentation agents for use as plasters on arthritic joints or used in combination with massage. In some instances, powdered sea vegetables and effervescent salts are added to the bath water to beautify the skin"[24] For a more comprehensive list of medical benefits of marine algae read: **Hoppe H. Marine Algae in Pharmaceutical Science. De Gruyter and Co, 1975.**[25]

By some estimates there are more than 30,000 different species of microalgae. Microalgae make up half of the plant kingdom—chiefly a separate and unexplored kingdom, as unknown and potentially valuable

as the rainforests. Microalgae can be separated into two large categories, based on their cellular organization. [1]

The blue-green microalgae are closely related to bacteria, and are, in fact, known scientifically as cyanobacteria. All other algae, which are considered more advanced in their cellular organization, are separated into ten different phyla, which are designated by their color (e.g., brown, golden-brown, green, red). Precious few algal species have been researched for medical or nutritional usage. Many species are probably still unidentified— waiting to be discovered. The most popular edible species of algae in North America are large seaweeds, like kelp and nori, microalgae like Chlorella and Dunaliella (green), and Spirulina and phanizomenon flos-aquae (AFA), both blue-green. [1]

While microalgae share some similarities, they also have important and unique differences from species to species. For example, Chlorella (a green microalgal species) contains more chlorophyll, less protein, and has an indigestible outer cell wall, which needs to be mechanically broken down before the cell contents can be digested. Spirulina (a blue-green microalgal species) has widely studied sulfolipids and readily grows in man- made ponds, especially in warm climates. [1]

Aphanizomenon flos-aquae (AFA) is a blue-green microalgal species, like Spirulina, but most AFA is harvested from the wild in volcanic regions, leading to high levels of trace minerals. It thrives in cold climates, resulting in higher levels of essential fatty acids. Dunaliella (a reddish-colored, green microalgal species) is mass cultivated and has the highest levels of beta-carotene, but also the lowest protein and chlorophyll content of the commonly eaten microalgal species. The

unique health advantages of the various species are only beginning to be understood with the vast majority of microalgae still to be studied; yet many common characteristics and benefits are shared by these primitive organisms. [1]

The environment in which algae grows, what nutrients are available, the pH, light, and temperature levels and the manner in which it is processed, further contribute to important differences. For example, the more expensive, low-temperature freeze-dried forms of some AFA algae result in a higher net protein utilization.

*According to chemistry professor **Karl Abrams**, AFA's net protein utilization is "75%, while that of Spirulina and Chlorella are only 37% and 20%, respectively." Toxins in the environment, such as heavy metals or pesticides, may contaminate microalgae; this possibility has posed a concern with some imported Chlorella or Spirulina.* [26]

Blue-green algae is one such product marketed as a health promoting supplement. It is often sold in the form of capsules and drops meant to be ingested daily. As a food source, it has been researched extensively in terms of its nutritional content and application for cultivation. More recently however, there has been particular interest in the potential use of blue-green algae as a supplement with activity as a biological response modifier. That is, the potential for use of blue-green algae as a therapeutic tool in preventing and combating certain disease States. Although anecdotal claims for the effects of blue-green algae supplementation range from complete recovery from chronic illness to improved learning

in children, there are unfortunately not enough well structured animal or clinical studies to fully back up such claims.

Although the consumption of microalgae is not commonplace on a global scale, there are several reasons why microalgae have become increasingly attractive as a commercial food crop:

1. Biomass: The amount of biomass microalgae produce is extraordinary. They can produce more food per acre and per unit time than any other of the common crops can.[9, 10, 11] Microalgae culture systems can produce up to 15,000 kg of protein per acre per year. In comparison, soybeans, a crop relatively high in protein content, typically produce less than 750 kg of protein per acre per year. [12]

2. Nutrients: They are the most nutrient-dense food currently known. [9, 28, 29, 30] For example, AFA is 60- 70% protein by weight and contains a rich source of vitamins, especially vitamin B12 and β-carotene, minerals, and is one of the few dietary sources of γ-linolenic acid.

3. Environmental aspects: Cultivation of microalgae does not contribute to soil erosion, requires little or no pesticides or herbicides, and requires a minimum of energy to cultivate and process. Microalgae are indeed a very energy, and land-efficient protein source.[12]

Nonetheless, the current price per kilogram of protein derived from microalgal sources remains high in developed countries.

Microalgae: A Historical Perspective

"In blue-green algae we find three and one-half billion years of life on this planet encoded in their nucleic acids (RNA/DNA). At the same time, all microalgae supply that fresh burst of primal essence that manifested when life was in its birthing stages. At a moment in history when the survival of the human species is in jeopardy, many people have begun instinctively to turn to these original life forms for nutritional support." [1, 7]

For thousands of years, algae have been used worldwide as a food source or as a remedy for a wide variety of physical ailments and diseases. In coastal regions of the Far East, notably in Japan, there is evidence that algae were used as a food source around 6000 BC, and there are records of many species of seaweed used as food around 900 AD. Many reports during the time of the Spanish conquest reveal that the natives of Lake Texcoco, near the city of Tenochtitlan (Mexico City today), collected blue-green algae from the waters of the lake to make sun-dried cakes called tecuitlat. [8]

Prescott reported in his book Conquest of Mexico that a "slime" was gathered from the lake by the inhabitants of Tenochtitlan and eaten as a nutritious cake. They collected this green substance floating at the surface of the water and dried it in the sun. They gave it the name tecuitlatl, or excrement of stone, as they believed it came from the stones. They consumed the algae as cheese, which it resembled in aroma and taste, and they sold it in the marketplace. In addition, **Dangeard,** a French phycologist, documented that natives of the Lake Chad area also used

microalgae as food.[9] It is used to make hard cakes called Dihé. Like the inhabitants of Tenochtitlan, these natives collect patches of floating micro-algae and sun-dry them in shallow holes dug in the sand along the shores of the lake. Once dried, the sand is cleaned from the algal cakes. They are broken into pieces and are then ready to be eaten or sold at local markets.

Reports by the United Nations have documented the superior overall health condition of people living around lake Chad who eat these Dihé cakes.[1, 2, 7, 8]

In a brief presented to the Food and Drug Administration (FDA) Select Committee on the Worldwide Use and Safety of Algae as a Food Source, the authors reported that some cultures have relied on algae for up to 25% of their diets.[31]

Yet, while a number of ancient cultures understood and used algae as a food, it wasn't until the early 1950s that the use of microalgae as a food source for humans began to gain momentum in the West. Microbiologists began to speculate that since algae have such high nutritive value (as much as 65-70% protein); large-scale production methods could lead to a revolution in agriculture.

In the 1960s, this speculation fueled a sort of "algae space race" between the United States and the USSR. ***Dr. William Oswald*** *of the University of California at Berkeley demonstrated a means by which algae could be used to support the entire metabolism of an adult man.* [32] *He called his life support system an "****algatron****": waste was recycled, oxygen created, and food grown on board a spaceship.* [33] *His results were*

soon supported by research in Japan and the Soviet Union that pointed to algae as an ideal food for long-term space missions.

During the 1970s, international research on the mass production of microalgae led to the early conclusion that microalgae were not cost competitive when compared to less expensive protein sources, such as soybeans. An understanding of the health benefits of microalgae would come later. The emerging "Green Revolution" turned in a different direction to feed the world's hungry, relying increasingly on the use of chemical fertilizers, pesticides, and genetic engineering to provide food for the masses. While this so-called Green Revolution did significantly increase food supplies worldwide, it has also resulted in serious problems associated with decreased seed stock, genetically engineered foods, and over-reliance on petrochemical fertilizers and pesticides. [1, 34]

While microalgae are generally too expensive to be considered a staple food for mass consumption, in wealthier countries, such as Japan and the United States, the use of microalgae as a nutritional health supplement is part of a multi-billion dollar industry that is contributing to a consumer-led health revolution in modern medicine.[1, 34]

A hundred years ago, when medicinal herbs and natural remedies were the most common means of regaining and maintaining health, the blue-green alga Aphanizomenon flos-aquae (AFA) growing abundantly in Klamath Lake might have been a household name. It would have had a reputation for being a food which gave a person added energy and mental clarity, boosted the immune system, and had a remarkable regenerative effect, even when consumed in small quantities.

Today, AFA enjoys a growing popularity among hundreds of thousands of people who report a wide range of benefits. Nearly three decades ago, it was discovered growing in wild abundance in a pristine lake in Southern Oregon. It has recently come to the attention of medical researchers, who are now conducting studies on this unique food to understand and uncover its secrets. Some call it nature's most complete food.[1, 2]

Ecology of Klamath Lake [35]

Klamath Lake is the largest freshwater lake in Oregon (125 miles^2; 325 km^2) with a watershed drainage exceeding 3,800 miles^2 (9850 km^2). This shallow lake with an average depth of eight feet is flanked by the Cascade Mountains to the west and the Winema National Forest to the east. It is 4,139 feet above sea level and has two major tributaries, the Williamson and Wood Rivers, as well as many smaller springs and stream inflows, providing Klamath Lake with waters of exceptional purity.

Klamath Lake, along with Tule Lake, are the shrunken remnants of ancient Modoc Lake. There have been many opinions regarding the age of the Klamath Basin, but the most recent work estimates the formation of the Klamath bed sediments at the Pliocene epoch, more than two million years ago. At that time, Modoc Lake is estimated to have covered over a thousand square miles. At the end of the Pleistocene epoch (about 12,000 years ago), Klamath River began to form, slowly draining Modoc Lake and lowering its surface altitude. Sites of higher elevation began to show and to divide Modoc Lake into several smaller bodies of water, leading to today's Klamath Lake and Tule lake.

Fig. 3; Eruption of Mt. Mazama—Painting by Paul Rockwood

Fig. 4; At 7,100 feet altitude, Crater Lake is located in the caldera of former Mount Mazama. Crater Lake is 2,000 feet deep, and for decades its water has been used as a standard for water purity.

North of Klamath Lake is located the remnant of Mount Mazama, originally estimated to stand at 12,500 feet. Nearly 7,000 years ago, Mount Mazama erupted, pulverizing the top 5,000 feet of the mountain and throwing millions of tons of ashes into the atmosphere. The magnitude of Mount Mazama's eruption is estimated at 300 times that of Mount St. Helens. The ashes covered most of the state of Oregon and reached as far as seven other states. After the explosion, Mount Mazama collapsed, forming a caldera that is today's Crater Lake.

At a depth of more than 2,000 feet, Crater Lake is the deepest lake in the United States. Its extremely low temperature and purity create physical characteristics that reflect light in a manner that gives the water a unique and vibrant deep-blue color. While standing at the rim of Crater Lake, it is easy to understand the spiritual fascination that the lake has held for the native people and the early settlers.

Crater Lake to Klamath Lake [35]

Nearly 90 percent of the water flowing into Klamath Lake comes from springs and rivers of exceptional beauty, bringing nearly 5,400acre-feet of water every day, 650 billion gallons annually. Although the question remains officially unanswered, most estimates indicate that the spring waters flowing into Klamath Lake come from Crater Lake, after a journey of approximately 15 miles through mineral-rich underground aquifers.

Generally, algae are found in bodies of water that are stagnant or deteriorating. Klamath Lake is an exception; it has always been and is still extremely robust and supports not only a tremendous biomass of AFA but also fish, waterfowl, and predatory bird species. When ice was first collected from the lake in 1906, it was reported to be green with algae. Lake sucker fish were so common that people used pitchforks to harvest them. Ospreys were reported in densities of up to 10 nests per square mile.

Today, the Klamath Basin is still home to the largest wintering congregation of bald eagles in the lower 48 states and is the largest stopover for waterfowl in the Pacific flyway. Klamath Lake is located in a relatively undeveloped area, surrounded by publicly owned land such as the Crater Lake National Park, the Winema National Forest, the Lower Klamath National Wildlife Refuge and the Tule Lake National Wildlife Bird Refuge. With the Cascade Mountains to the west, thousands of square miles of National Park to the north and east, and the city of Klamath Falls located downstream at the southern end of the lake, Klamath Lake is virtually untouched by industrial activity and pollution.

Stories that Klamath Lake is polluted come from the fact that at certain seasons, fish die from oxygen deprivation due to the changes brought on by massive algal growth. During AFA blooms, the water of Klamath Lake can reach a pH of 11, and dissolved oxygen can go under 3 ppm. This can be deadly for fish. Klamath Lake is actually rather pristine. It is devoid of industrial activities and surrounded by national parks: Crater Lake National Park to the north, Winema National Forest to the

east, and the Cascade Mountains to the west. The city of Klamath Falls is downstream to the south.

Fig. 5; A map demonstrating the position of Klamath lake.

Why AFA Flourishes [36]

AFA is unique in its ability to fix atmospheric nitrogen. This very characteristic gives it a rare advantage over other types of algae existing in Klamath Lake. In the harvest season, it grows by consuming atmospheric nitrogen and the lake's available nutrition, creating an enormous bloom that is virtually 100 percent pure AFA. Many of the lake's characteristics are responsible for this unusual ecosystem, allowing for such abundant bloom of AFA:

FIRST, the lake is so old that it contains 30 feet of mineral-rich sediment at its bottom, much of it donated by the explosion of Mount Mazama. In their 1967 study of the lake, Miller and Tash estimated that the top one inch of the lake's sediment alone contains enough nutrients to sustain a full algal bloom.

SECOND, the average depth is less than ten feet with a median depth of about five feet. In more than 50 percent of the lake, you can stand on the bottom.

THIRD, the lake is nearly 25 miles long and five miles wide, providing a longitudinal shape that fosters strong winds and turbulence. When the wind blows, it applies pressure to the shallow lake's surface, forcing the water to turn over. The turbulence grabs the mineral-rich sediment, bringing up into suspension a wealth of nutrients that further promote algae blooms. This cycle explains the exceptionally abundant growth of AFA in Klamath Lake.

The Water of Klamath Lake [37]

The most fascinating and unique characteristics of the water flowing into Klamath Lake is its color and temperature. Crater Lake is known for its emerald blue water. This condition is due to its clarity, temperature, and chemical matrix, allowing light to reflect the spectrum of blue to the viewer. It is generally known that mineral concentrations are the predominant factor creating this anomaly.

Fig. 6; View of Mount McLoughlin from the east side of Klamath Lake, where harvested AFA is brought to shore. Klamath Lake is a wonderful natural ecosystem filled with abundant wildlife.

Water testing has confirmed that high mineral content creates this "blue color" condition and is most often seen in unpolluted high mountain lakes and streams. Certain minerals have specific color spectrums due to the electrical or ionic activity created by increasing amounts of ions present in the water. In most cases, minerals are the prime energy conductors in water.

It was assumed that the spring sources supplying Klamath Lake were naturally high in mineral concentrations due to the blue color of the springs. Oddly enough, advanced testing has revealed the opposite. Several of the main springs have very little entrained minerals, yet they still have the blue color. There is no general consensus of how the "blue

water" condition exists without the required mineral matrix. One obvious explanation is that the effect is not solely derived from minerals alone.

Conductivity, pH, and specific gravity typically reveal information assuming the presence of minerals. We must look at the importance of electrical potential as a possible contributing factor. Looking at millivolt values (mV) of the source water, we can determine what the potential for electrical activity is. The mV values are scaled from positive to negative. The higher the negative number, the more electron activity is possible. A value of +75 mV has very little potential for electron activity, whereas a value of -75 mV has a tremendous amount of electron potential. Testing the springs at their source reveals values of -60to -80 mV without the presence of minerals, indicating the source water is highly electrical.

It is widely known that natural electrical charge does create a color phenomenon, known as the "piezo effect." This is caused by the compression of crystalline structures like quartz releasing a static or electrical charge. The resulting silica-quartz piezo spark is blue-white in color. The surrounding watershed happens to be rich in quartz sands, allowing the source water to percolate through it, possibly creating the piezo effect. This could be a major contributing factor to the electric-blue color of the spring water.

In addition, water with temperatures of 4ºC (or 39ºF) creates conditions for maximum density and energetics. Cold water is more vital and able to distribute minerals, electrical ions, and nutrients than warm

water. The spring sources supplying Klamath Lake range from 5 to 5.7ºC and are some of the coldest ground water temperatures in the United States.

In summary, chemical testing confirms the spring source water to be very low in mineral concentrations but extremely high in electrical potential. This allows the water coming into the lake to have greater capacity to collect nutrients and minerals, and an increased ability to respond to sunlight. These unique characteristics help to create the unusual water environment of Klamath Lake.

Harvesting Today [38]

For more than two decades, the naturally occurring AFA growing in Klamath Lake, Oregon, has also been harvested and sold as a unique dietary supplement filled with health-promoting compounds. Although AFA grows in many other areas of the world, the biomass that accumulates every year in Klamath Lake is unique in its abundance as well as its purity. High-quality AFA is currently being harvested in Klamath Lake, at the site of rich algal blooms. Small harvesting platforms pass through the algal blooms that gather in large, thick patches at the surface.

These harvesters are equipped with rotating screens that lift the algae from the water surface or a pumping system that pumps lake water onto screens, and the concentrated AFA is then transferred on a screened conveyor system, where the initial de-watering step is performed. The

AFA is then slowly pumped through a refrigeration system that brings the concentrated AFA down to a temperature of 5ºC (38ºF).

The AFA is then filtered through a centrifugal sieve to remove debris and undesirable species of algae. Purified and concentrated AFA is finally transported to the drying facility to be dried or stored deeply frozen.

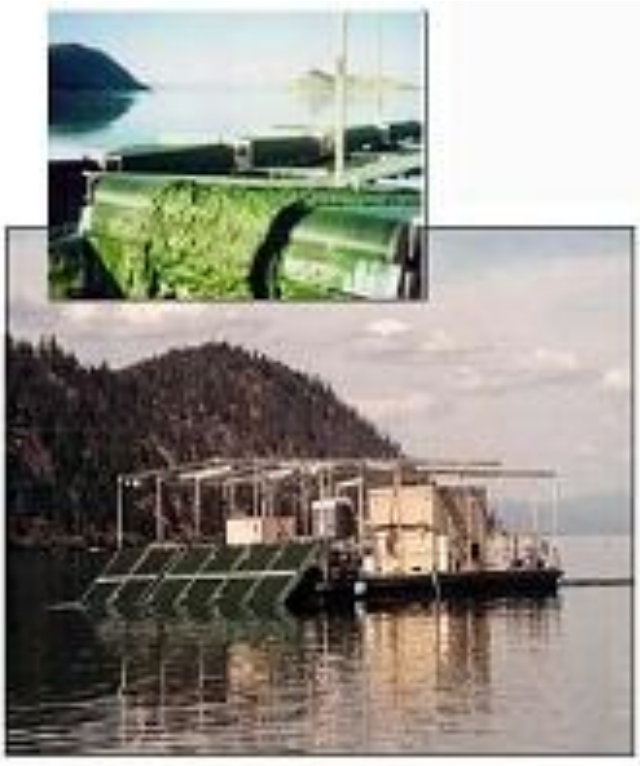


Fig. 7; Harvesting AFA.

A Superior Drying Method [39]

Various drying methods have been utilized to dry AFA. A three-year study comparing the performance of these different drying methods established the superiority of Refractance Window™ technology over other methods, including freeze-drying and spray-drying.

In brief, when water is placed over a heating source, infrared energy is transferred throughout the water by convection. The heat energy then radiates from the water, primarily through evaporation. If the water is covered by a transparent membrane, evaporation and its associated heat loss is blocked or "refracted." The membrane acts like a mirror reflecting the infrared energy back into the water.

When a moist, raw material such as algae is placed on the membrane's surface, the water in the material creates a "window" that allows for the passage of infrared energy through the membrane and also through the material. Heat is directly transferred to the water present in the material.

In a matter of a few moments, the water in the material on the membrane's surface evaporates, and the "window" of infrared energy closes and "refracts" back into the heated water source, no longer exposing the material to heat.

When comparing various drying technologies, the degree of preservation of a material's original color and flavor indicates the quality of the drying process utilized. Studies performed at Washington State University's Department of Biological Systems Engineering and Department of Food Science and Human Nutrition established the

preservation superiority of the Refractance Window™ drying technology over all other methods of drying, including spray drying and freeze-drying.

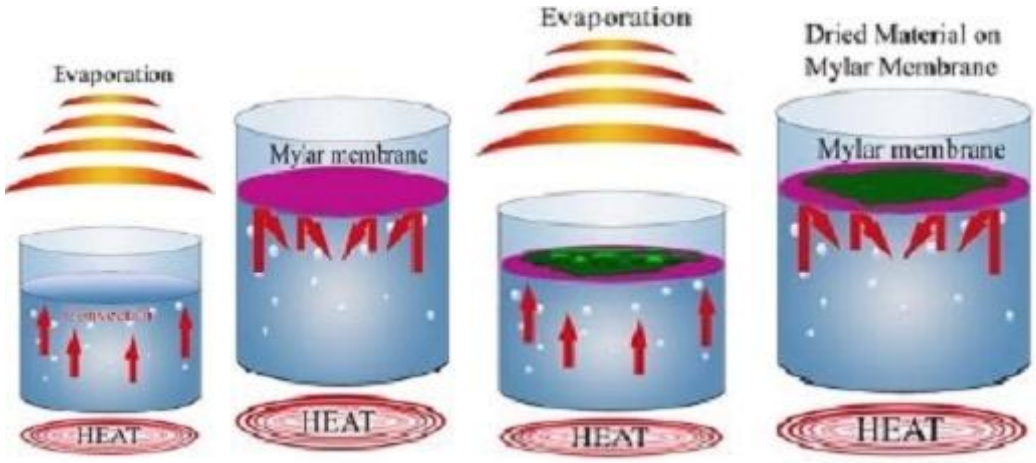


Fig. 8; AFA drying method.

- **DEFINITIONS**:

  - Cyanophyta—The current scientific classification for the blue-green algae family
  - Aphanizomenon flos-aquae—the scientific name of the freshwater cyanophyta found in Upper Klamath Lake
  - AFA—The abbreviation for Aphanizomenon flos-aquae
  - Microalgae—A common term to describe single-cell blue-green algae (Cyanophyta)
  - Blue-green algae— A class of microorganisms containing the blue pigment phycocyanin. This term is used frequently in the health care field to describe edible microalgae like AFA
  - Phycocyanin—The blue pigment in AFA

UNIQUE COMPPOSITION OF AFA

Remarkable Nutritive Qualities of Microalgae

Gram-for-gram microalgae may be the most nutrient dense food on Earth.[40]

The primitive character of microalgae's cellular organization gives it a number of advantages over higher plants and animals as a food source. For starters, practically the entire organism can be nutritious, with minimal indigestible structures. By contrast, typically less than half of the dry weight of plants and animals has nutritional value. Primitive blue green algae are composed almost entirely of nutritionally useful and uniform cells. Furthermore, microalgae exhibit superior photosynthetic efficiency, using light approximately three times more efficiently than higher plants. [41]

Microalgae are among the most productive organisms on the planet. [41]

AFA contain more micronutrients than any other known food. AFA cells are about 20 to 30 times smaller than the cells within
the food we usually eat. Because of this, AFA contains 20 to 30 times the membrane surface area'.'' AFA's smaller cell size means a larger ratio of cell membrane surface compared to the rest of the cell. In the case of blue-green algae, the cell membrane is where some of the most important nutrients are concentrated. AFA algae produce more cell membrane material without getting larger by creating a vast system of membrane inpouchings similar to the brain's infoldings. In other words, if the cell

membrane were ironed flat, it would be many times the apparent size of the cell. [42]

One of the most remarkable nutritional aspects of microalgae is its high content of usable protein ranging from 50% to 70%! This is a far higher percentage than the choicest edible parts of any higher plant or animal.

Algal protein has shorter and less complex polypeptide chains—making it easier to digest than plant or animal protein. Red meat has a surprisingly low net protein utilization index of 18%, compared to AFA's 75% [26] *.The net protein utilization index is a measure of how completely amino acids are assimilated by humans. In fact, some microalgae, such as AFA, contain all ten essential amino acids that humans require from their diets—in a profile similar to that recommended by the National Academy of Sciences.* [43]

Not least, "microalgae are considered to be the primary source of unsaturated fatty acids in the food chain." [44] *Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two relatively rare and valuable fatty acids found in microalgae.* [45] *The reason that fish oils are so rich in polyunsaturated fatty acids (PUFAs) is that microalgae are abundant in their food chain. Unlike seafood, microalgal oils are cholesterol free. The nutritional value and therapeutic merits of PUFAs have been widely documented.* [46]

AFA is almost 100% usable by the body compared to synthesized vitamins which are only 5-25% usable. In Amounts Perfectly Balanced by ALLAH.

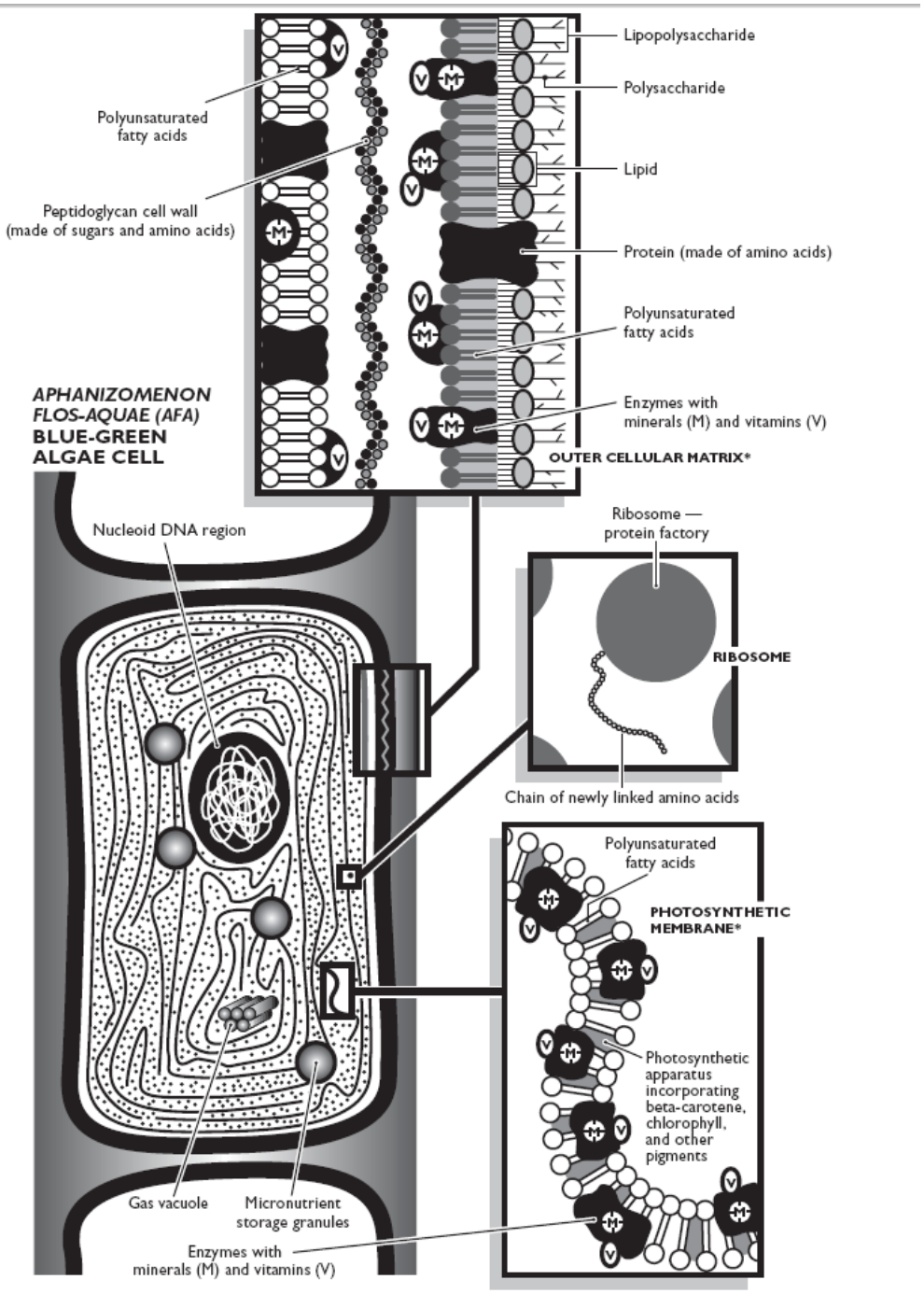


Fig. 9; Schematic of AFA cell. These simple illustrations leave out most of the complexity and mystery of the cell (From Algae to the Rescue!. by Karl Abrams, Logan House Publications, 1996)

Typical Nutrient Data Klamath Lake AFA

Typical Nutrient Composition (per gram) [47-55]

Protein: 60 – 70%

Carbohydrate: 20-30%

Calories: 260kcal/100g

Minerals: 3-9%

Lipids: 2-8%

Pigments: 1-4%

Moisture: 3-7%

Chlorophyll .55%

ESSENTIAL FATTY ACIDS

Alpha-Linolenic Acid (Omega 3) 29.50 mg

Gamma-Linolenic Acid (Omega 6) 6.00 mg

VITAMINS

Provitamin A Beta Carotene	2000 IU
Thiamin (B1)	4.70 mcg
Riboflavin (B2)	57.30 mcg
Pyridoxine (B6)	11.10 mcg
Niacin (B3)	.16 mg
Pantothenic Acid (B5)	6.80 mcg
Inositol	160 mcg
Vitamin E	1.70 IU

Ascorbic Acid (Vitamin C)	6.70 mg
Biotin	0.30 mcg
Folic Acid	1.00 mcg
Choline	2.30 mcg
Cobalamin (B12)	8.00 mcg
Vitamin K	45.52 mcg

Table, 1; **Vitamins present in AFA.**

MINERALS

Boron	.15mg	Molybdenum	3.30 mcg
Calcium	14.00 mg	Nickel	5.30 mcg
Chloride	0.47mg	Potassium	12.00 mcg
Chromium	0.53 mcg	Phosphorus	5.20 mcg
Cobalt	2.00 mcg	Selenium	0.67 mcg
Copper	4.30 mcg	Silicon	186.50 mcg
Fluoride	38.00 mcg	Sodium	2.70 mg
Germanium	.27 mcg	Tin	.47 mcg
Iodine	.53 mcg	Titanium	46.60 mcg
Iron	350.70 mcg	Vanadium	2.70 mcg
Magnesium	2.20 mg	Zinc	18.70 mcg
Manganese	32.00 mcg		

Table, 2; **Minerals present in AFA.**

TYPICAL AMINO ACID CONTENT (per gram)

Essential Amino Acids

Arginine	38 mg	Methionine	7 mg

Histidine	9 mg	Phenylalanine	25 mg
soleucine	29 mg	Threonine	33 mg
Leucine	52 mg	Tryptophan	7 mg
Lysine	35 mg	Valine	32 mg

Table, 3; **Essential amino acids present in AFA.**

Non-Essential Amino Acids

Alanine	47 mg	Glutamine	78 mg
Asparagine	47 mg	Glycine	29 mg
Aspartic Acid	7 mg	Proline	29 mg
Cystine	2 mg	Serine	29 mg
Glutamic Acid	4 mg	Tyrosine	19 mg

Table, 4; **Non essential amino acids present in AFA.**

AFA and spirulina compared: [47-55]

For the past several decades, people have been enthralled by a "green foods" revolution. During this time, several foods have been championed as the revolution's leader. Foods such as barley grass, chlorella, wheat grass juice, and sprouts lag far behind the two most popular blue green algae, aphanizomenon flos-aquae (AFA) and spirulina. Both are considered green superfoods; they have similarities but several important differences.

AFA is a wild food harvested from a wild environment. Spirulina is a wild species grown in a controlled environment. Generally this

indicates that AFA will contain more minerals than spirulina. Analytical research supports this conclusion.

Fig. 9; AFA (above) and spirulina (below)

One major difference is simply that AFA is the "greenest" superfood known, because it has the most of that wondrous green

photosynthesizing pigment chlorophyll. A ten-gram portion of AFA algae contains 300 mg. of chlorophyll, whereas a ten-gram portion of Spirulina has only 115 mg.

Both forms of blue-green algae have a similar overall concentration of proteins, carbohydrates, lipids, and minerals. However, the quality of their micronutrients is noticeably different because of specific growing and harvesting techniques associated with them. Spirulina, for example, is grown in concrete or plastic ponds with a "salinity factor" (sodium chloride salt content) often greater than 100 times that of AFA algae. Spirulina's nutrient composition is just a reflection of the substances that have been artificially added to it in the form of mineral (and other) supplements.

AFA, by comparison, is harvested from its own mineral-rich and natural Upper Klamath Lake, Oregon, habitat. Its micronutrients mirror what has existed naturally in the lake for thousands of years due to past volcanic activity and the interactions of rivers, streams, and unpolluted mountain rain, as well as a vast subterranean water supply originating from the nearby and pristine Crater Lake.

The richness of AFA's micronutrients are even more evident from the fact that 30 – 40 feet of organic nutrient sediment make up a treasure trove of minerals for AFA to feed upon. The vast richness of this sediment is reflected in the fact that AFA has about 40 percent more calcium and 100 percent more chromium than does spirulina with approximately five to ten times the vitamin C content of spirulina.

One interesting difference between these two cyanobacteria can be traced to the fact that whereas spirulina is a tropical algae, AFA is a heartier cold-climate species. In the warmer climate of the tropics, the cell membrane of a spirulina cell can easily maintain its flexibility by producing a rather high percent of saturated fatty acids. AFA algae, on the other hand, does not lead this life of "tropical luxury". The colder climate of Upper Klamath Lake forces AFA'' cell membrane enzymes to compensate by ingeniously manufacturing specific poly-unsaturated fatty acids (PUFAs) that enhance its life-sustaining membrane flexibility.

To be sure, both forms of algae are blessed with a rich array of phytochemical antioxidants such as the carotenes. Although spirulina contains slightly more betacarotene than AFA algae, one must be careful to take a more educated look. The presence of more PUFAs allows for wider variety of other carotenoids such as alpha and gamma carotene- to be spread out within the cell membrane itself. The true healing power of beta carotene cannot be fully realized unless a variety of other structurally related carotenoid compounds is present.

Carotenoid compounds in all forms of blue-green algae are also particularly sensitive to the type of harvesting techniques employed. The sun-drying and spray-drying techniques often used in processing spirulina invariably cause a marked decrease in beta carotene as well as the concentration of methioninea sulfur containing essential amino acid.

The assimilation of algae protein is also dependent upon how it is processed. When spirulina is sun-dried or spray-dried, its "net-protein utilization" (usually expressed as percent assimilation) is typically half that of AFA algae, which has been more carefully freeze-dried and flash frozen.

. In general, freeze-drying techniques are essential to maintain the viability of AFA enzymes and its delicately chelated minerals and vitamins. Spray-or sun-dried spirulina products tend to readily lose much of such heat-sensitive components.

AFA contains more vitamin C. AFA blue-green algae contains more than five times the vitamin C content of spirulina. AFA contains more essential fatty acids. AFA blue-green algae is a cold-water algae that insulates itself with essential fatty acids. In warmer, tropical, saltwater pools (where spirulina grows) less of the insulating, essential fatty acids are required.

These differences and more are summarized in the next tables:

	AFA	**Spirulina**
Moisture	6%	5%
Protein	63%	65%
Total lipid	3%	5%
Fatty acids	n/a	n/a
Carbohydrate	27%	18%
Ash	7%	7%
Calcium	140.0 mg	100.0 mg
Phosphoms	51.0 mg	90.0 mg
Iron	6.4 mg	15.0 mg

Sodium	38.0 mg	60.0 mg
Chlorine	46.0 mg	44.0 mg
Chromium	40.0 mg	28.0 µg
Copper	60.0 µg	120.0 µg
Magnesium	16.0 mg	40.0 mg
Manganese	0.3 mg	0.5 mg
zinc	0.3 mg	0.3 mg
Potassium	100.0 mg	120.0 mg
Vitamins		
Ascorbic acid	5.0 mg	0.5 mg
Carotene	2000 RE	2300 RE
Biotin	3.6 µg	0.5 µg
Cobalarnin	8.0 µg	3.2 µg
Choline	2.6 mg	n/a
Folic acid	1.0 mg	1.0 µg
Pyridoxine	67.0 µg	80.0 µg
Panthothenic acid	130.0 µg	10.0 µg
Niacin	0.65 mg	1.46 mg
Thiamin	0.03 mg	0.31 mg
Inositol	n/a	6.4 mg
Vitatnin E	1.2 IU	1.0 µg
Pigments & Other Compounds		
Carotenoids	N/a	37.0 mg
Phycocyanin	1500.0 mg	1500.0 mg
Chlorophyll	300 mg	115.0 mg
y-linolenic acid	n/a	135.0 mg
Glycolipids	n/a	200.0 mg
Sulfolipids	n/a	4-10.0 mg
Nucleic acids	n/a	4.5%

Table 4a; Summary of comparison between AFA and spirulina composition (figures are given by 10 gram of dry weight)[48]

STEMTech
The Stem Cell Nutrition Company

COMPARISON CHART
Aphanizomenon flos-aquae - Spirulina - Chlorella

Health Benefits	*Aphanizomenon flos-aquae* (AFA)	Spirulina	Chlorella
Stimulation of NK cell migration	AFA was shown (1.5 g daily) to stimulate the migration of NK cells from the blood to the tissues. NK cells are known to be scavengers and killers of cancer and virally infected cells.	When tested, Spirulina did not show any effect on NK cell migration.	When tested, Chlorella did not show any effect on NK cell migration.
Stimulation of macrophage activity	AFA contains a unique polysaccharide that was shown to stimulate macrophage activity	Spirulina contains a unique polysaccharide that was shown to stimulate macrophage activity, though the potency is roughly ¼ that of AFA	Chlorella contains a unique polysaccharide that was shown to stimulate macrophage activity, though the potency is roughly ½ that of AFA
Mobilization of immune cells	AFA was shown (1.5 g daily) to stimulate the mobilization of lymphocyte B and T from lymphoid tissues and increase the number of circulating lymphocytes.	Spirulina was not show to have any effect on lymphocyte mobilization.	Chlorella was not show to have any effect on lymphocyte mobilization.
Production of free radicals by immune cells.	The immune system is one of the main contributors to oxidation in the body. AFA was shown to reduce the background production of free radicals by polymorph nucleated cells.	No data available.	No data available.
Anti-inflammatory properties of phycocyanin	AFA contains a significant amount of phycocyanin, which was shown to be a potent specific COX-2 inhibitor. COX-2 inhibitors are the novel generation of anti-inflammatory remedies.	Spirulina contains a significant amount of phycocyanin.	Chlorella contains no phycocyanin and has no specific effect on inflammation.

Table 4b; Summary of comparison between AFA and spirulina

Biochemistry of AFA

In this section, we will mention in brief the role of unique nutrients present in AFA on human health. We will limit this discussion to nutrients that are specific to AFA or present in exceptional quantities.

Chlorophyll

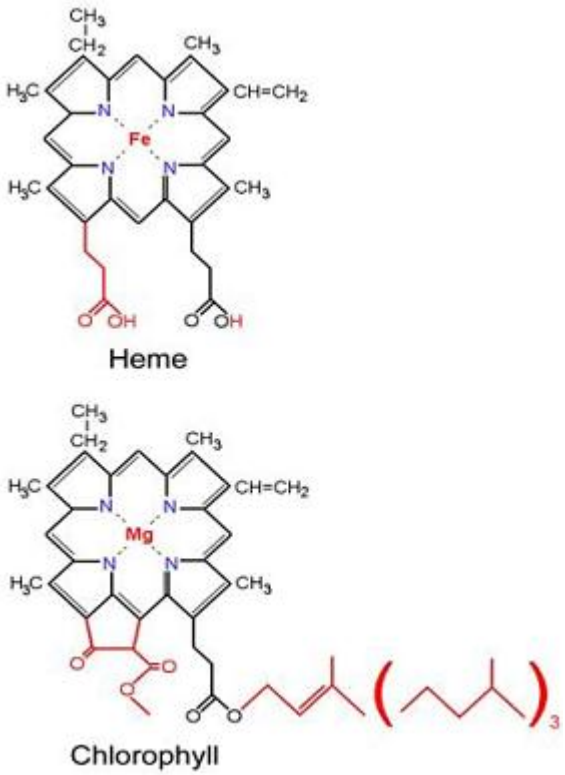


Fig. 10; Structure of heme and chlorophyll.

It is the green pigment found in plants, chlorophyll is responsible for the transformation of light energy into chemical energy. Although chlorophyll is present in all green vegetables, it is exceptionally abundant in AFA (about one percent of dry weight). Interestingly, the molecular structure between chlorophyll and our hemoglobin (red blood cells) is remarkably similar. The main difference is that the central atom in chlorophyll is magnesium, whereas in hemoglobin it's iron. Thus,

chlorophyll is considered a major blood purifier, and is very beneficial to overall health.[2]

Although the biochemical pathway that would allow the transformation of chlorophyll into hemoglobin has never been investigated, data suggests that eating foods containing high chlorophyll content could stimulate the synthesis of hemoglobin in the body. In **1936, Hughes and Latner** [179] carried an experiment in which they triggered severe hemorrhage in dogs. The dog is often used in cardiovascular studies because of the similarity of its cardiovascular system with that of man. The control group was allowed to recover without any treatment whereas the experimental group received daily dose of chlorophyll. The group that consumed chlorophyll daily recovered much faster and showed much higher blood hemoglobin content.

Scientific research has revealed the anti-cancer properties of chlorophyll. (Refer to chapter of cancer) [180-183]

*According to **Paul Pitchford**, author of "Healing with Whole Foods," chlorophyll is attributed to numerous health benefits: it stops bacterial growth in wounds, eliminates bad breath and body odor, removes drug deposits, counteracts all toxins, builds blood, renews tissue, counteracts radiation, promotes healthful intestinal flora, activates enzymes to produce vitamins A, D, and K, reverses anemic conditions, reduces high blood pressure, strengthens the immune system, relieves nervousness and serves as a mild diuretic.*[184] *Finally, popular medicine has also produced evidence of the healing properties of chlorophyll. Topical application as well as oral intake of chlorophyll was*

shown to prevent and help eliminate infections.[185-186]*Topical application of chlorophyll was noted to promote healing of the skin as well as stomach ulcers.* [187]

Beta-Carotene and Other Carotenoids [188-201]

AFA is an exceptional source of carotenoids (more than 240 retinol equivalents per gram). Beta-carotene, as well as other carotenoids, has been shown to be a powerful antioxidant, helpful in the prevention of cardiovascular diseases and cancer (refer to specific chapters for details)

Polyunsaturated Fatty Acids

Dietary essential fatty acids, especially Omega-3 essential fatty acids, have been shown to be beneficial to the immune, cardiovascular, and nervous systems. Nearly 50 percent of the lipid content of dried AFA is composed of Omega-3 essential fatty acids (mostly alpha-linolenic acid).The average North American diet is known to be lacking in Omega-3 fatty acids. Such deficiency is increasingly linked to cardiovascular diseases, [46,202-206] *immunosuppression,* [207] *arthritis,* [208] *mental problems,* [210-213] *and skin problems*.[214]

In addition, Omega-3 fatty acids were shown to prevent platelet aggregation [215-216] *and to lower cholesterol.*[216,230] *Consumption of essential fatty acids, mostly Omega-3, was also shown to inhibit many forms of cancer, namely breast, prostate, pancreatic and colon.*[219,220]

There is also evidence that Omega-3 fatty acids may help in neuropathic conditions associated with diabetes.[221,222]

Significant interest has been raised by the relationship between essential fatty acids and nervous system functions. Epidemiological studies in various countries and in the United States suggest that decreased Omega-3 fatty acid consumption correlates with increasing rates of depression.[210] *Consumption of foods containing Omega- 3 fatty acids may constitute an alternative treatment for depression. Furthermore, decreased concentrations of certain essential fatty acids in the plasma have been found in children diagnosed with Attention-Deficit Hyperactivity Disorder (ADHD).*[211]

Chemoprotection and Polysaccharides

A substance is "chemoprotective" when it protects against the toxic effects of chemicals or compounds present in our food or environment. Heavy metals and pesticides are examples of such compounds extremely deleterious to health. Various species of microalgae have been demonstrated to absorb heavy metals, and their consumption may promote the elimination of heavy metals.[223-225] *Scientific studies have shown that cyanophyta offer significant protection against heavy metal toxicity to the kidneys.*[226] *A sugar present on the cell membrane of microalgae has also been confirmed to bind and eliminate pesticides in the intestine.*[227] *Phycocyanin, the blue pigment present in AFA, has also been shown to have chemo-protective properties.*

Anti-diabetes and anti-obesity effects

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.[665] In addition, in a double-blind crossover study versus placebo involving human patients, supplementing the diets of obese outpatients with 2.8 gram of blue- green algae three times daily over a four week period resulted in a statistically significant reduction of body weight.[666]

Other medical effects include:

Improvement in condition of Alzheimer's patients, overall enhancement of the immune response, increased fertility, reduction in size and occurrence of tumors, improved digestion and elimination, healing of internal and external lesions, increased stamina, increased mental acuity, and general improvement in overall well-being. Nonetheless, there have been pre-clinical and clinical studies supporting several of these therapeutic effects such as the reduction of cholesterol and cancer, enhancement of the immune system, increasing intestinal lactobacilli, mild diuretic, reducing nephrotoxicity by heavy metals and drugs, and radiation protection. [667, 48]

How best to take it?

(By Dr. Kate James MBBS, Integrative Medical Doctor)

It is best to take the algae in the morning, shortly before or with your breakfast, and depending on how much you are taking again midmorning and/or early afternoon. Our digestive system is at its strongest earlier in the day and so the earlier the better!

For most people it is best to take a cup of herbal tea on waking to warm the body and help our digestive system to wake up. This is wonderful preparation for taking algae.

The 2 best ways to take the algae to help your body to use it optimally are either about 20-30 minutes before meals, or as part of a simple meal including fruit, and or vegetables in some form. (This is ideal however not essential.)

I give our children their algae by opening a capsule and mixing it with about an egg cup full of water before they tuck into their breakfast. In fact as I now edit this article I realise the elder two just swallow it whole and Theo likes his in Cherry juice.

I have taken my algae in many different ways at different times. What feels right to us now often changes over time. A big factor here in the North of England is often the weather and seasons. Sometimes I've taken algae in green vegetable juices or sometimes in fruit based smoothies and sometimes sprinkled on berries or other seasonal fruit together with my porridge!.

The key points are to take it before or early in the meal, ideally as part of a simple meal, and with some fruit or vegetables in some form as algae has what's called an enormous synergy with those types of foods.

In other words it is able to work to its best effect in our bodies, being absorbed an assimilated to its maximal potential, and thus it can work even better than if we took it on its own. It is interesting to note that AFA is understood to be 97% assimilable as opposed to vitamin and mineral supplements which at best tend to be only 30-50% assimilable ie you can only absorb 30% of their content.

It is also very important to avoid taking it with black tea or coffee, and ideally best taken at a different time to eating meat and fish.

Further readings;

1-BMJ | 10 SEPTEMBER 2011 | VOLUME 343 pp519; Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis Evan Mayo-Wilson et. al

2-Vitamin K Supplement Along with Vitamin D and Calcium ; Reduced Serum Concentration of Undercarboxylated Osteocalcin While Increasing Bone Mineral Density in Korean Postmenopausal Women over Sixty-Years-Old, Sang Hyeon Je et. al, J Korean Med Sci 2011; 26: 1093-1098

3-Zinc-Altered Immune Function1Klaus-Helge Ibs and Lothar Rink2; Institute of Immunology, University Hospital, Technical University of Aachen, D-52074 Aachen, Germany, J.Nutr.133:1452S–1456S,2003.

4-Importance of Zinc in the Central Nervous System: The Zinc-Containing Neuron1 Christopher J. Frederickson, et. al

5- Effect of a Klamath algae product (“AFA-B12”) on blood levels of vitamin B12 and homocysteine in vegan subjects: a pilot study. Institute of Biomathematics, University of Urbino “Carlo Bo”, Italy.

QUALITY AND SAFETY OF AFA FROM KLAMATH LAKE

Many foods contain a wide variety of very potent toxins such as aflatoxins, a mycotoxin present in corn, peanuts, and other crops that is such a potent liver carcinogen that it is regulated at 20 µg/kg, 50 times lower than the limit established for microcystins.[57] *Cabbage and related vegetables contain glucosinolates, which have goitrogenic activity.* [58] *Sweet potatoes or legumes may produce hepatotoxins as well as compounds able to produce neuropathy and mental confusion.* [59] *Solanine may be found in potatoes, especially when improperly stored, which may result in headaches, incoherence, hallucination, and dizziness.* [60,61] *Many sources of wild-harvested fish contain significant levels of mercury, and shell fish may contain potent toxins responsible for several deaths every year.* [62]

So the concern is not the existence or even the presence of toxins in food but rather their concentration. When a compound is found below levels that have been established as safe, then a food is considered safe.

Christian Drapeau MSc ; TOWNSEND LETTER-DECEMBER 2010

Microcystin Toxicity;

The blue – green algae harvested from Klamath lake and currently sold on the market is more than 99% aphanizomenon flos– aquae. This

blue – green alga from Klamath Lake is absolutely non – toxic, as demonstrated by many years of extensive testing. During a few weeks in the summer, microsystis, a co – occurring blue – green alga capable of producing the toxin microcystin, is found as a minor constituent of the Klamath Lake phytoplankton community. The phenomenon is not recent and microsystis has always been present in very small amounts in Klamath Lake. Despite its presence, microsystis is not a problem, since Desert Lake technologies **(DLT)** has developed a method to separate this alga from aphanizomenon flos – aquae.[20, 13]

In **1996, John McPartland** wrote an article that was published in Townsend Letter ("Why Blue-Green Algae Makes Me Tired ?"), expressing his exasperation at being approached by distributors from what was then called Cell Tech, a multilevel marketing company selling the blue-green algae Aphanizomenon flos-aquae (AFA). Although his exasperation was understandable, given the aggressive business practice of some distributors at times, the information in his article was extremely misleading: a very negative report about AFA, attributing to this blue-green algae a slew of problems and suggesting that consumers were taking significant risks by simply consuming this species of algae as a dietary supplement – which was completely untrue. [56,71-81]

McPartland listed several references reporting the neurotoxicity of AFA strains collected in various parts of the world, leading the reader to believe that AFA from Klamath Lake was producing a series of neurotoxins such as neosaxitoxin and anatoxin. He also claimed that AFA was producing microcystin with an LD 50 of 50µg/kg. He further

suggested that AFA could be a vector for Legionella pneumophila, the cause of Legionnaires' disease, and Vibrio cholerae, which causes cholera, even though the quoted literature pertained to other species of blue-green algae growing in entirely other parts of the world. [56, 71-81]

While microcystins are indeed potent toxins when consumed in signifcant levels, the LD 50 reported is that of intravenous injection of the toxins, which cannot in any way be compared to oral intake. Just think; the LD 50 for intravenous table salt is less than a tablespoon. Or imagine injecting a tablespoon of peanut butter in your vein! Intravenous and oral intakes are two completely different things when talking about exposure to a substance.[63,82-88]

And it's not that proper information was lacking; studies already published at the time had reported a no observable adverse effect level (NOAEL) – that is, not a toxic but a safe level – ranging between 40 and 280 µg/kg. *It is also important to mention that the potential presence of microcystin does not come from AFA, but rather from co-occurring Microcystis spp. at times present in Klamath Lake.* Based on all this information, a safe level has been established by **Oregon Department of Agriculture** as 1 µg/g. [63,82-88]

University of Illinois *carried out reviewing more than 300 scientific articles, aimed at accurately evaluating the risk associated with microcystin as a possible contaminant of blue – green algae products. This risk assessment determined that 10 ug/g was considered a safe level.*

A similar safe level (5 µg/g) was later confirmed by a risk assessment performed by ***Dr. Gary Flamm****, former head toxicologist at the FDA in Washington, DC. This safe level of 5 ug/g was also supported by* ***Dr. Wayne Carmichael*** *in a written testimonial.* [63, 64]

Despite the written opinions of many experts and the significant amount of data indicating that levels of 5 µg/g and even 10 ug/g were safe for human consumption, even children, the ***Oregon Department of Agriculture (ODA)*** *decided to pass a regulation establishing 1 µg/g as the maximum acceptable concentration (MAC). The actual safe level determined by animal studies was between 2,500 and 6,000 ug of microcystin per day. To add a margin of safety, this safe level was further divided by a factor of 1,000. The adopted safe level of 1 µg/g is therefore 1,000 times lower than level established as safe in animal studies, ensuring complete safety for children.*

Microcystin is indeed a liver toxin; however, it is completely safe at the levels currently found in blue – green algae products. Liver damage only occurs at levels that exceeds10, 000 times the adopted safe level of 1 ug/g. one would have to eat more than 5,000 capsules per day to reach such levels !!. [63,65] However, collaboration between **ODA** and **FDA** in Washington state, as well as with independent universities and institutions, has failed to produce a validated test for the precise measurement of microcystin at low levels. The tests currently utilized that have been developed and refined over the past 5 years, an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) , are precise enough to monitor compliance, even though levels

found in a same sample analyzed on different occasions, different laboratories, can at times show significant variations. [66-68]

In conclusion, the blue – green algae industry has been extremely pro – active with the problem of the presence of microcystis in Klamath lake. Members of the Klamath lake algae industry have worked with the Oregon Department of Agriculture to raise the regulatory level to 5 µg /g. however; **DLT**'s position has been to fully integrate the regulatory level of 1 µg/g, and to develop ways to reduce microcystin content. As stated before, **DLT** has developed and implemented a method to separate Microcystis for aphanizomenon flos-aquae. Lots of AFA harvested since 2000 all tested at less than 1 µg/g. [68]

Upper Klamath Lake has sometimes been referred to as polluted because of the lake's incredible bounty of Aph. Flos-aquae. The most observable influence of this blue green algae is the change in the chemical properties of the water around the blooming algal masses, namely dissolved oxygen, pH and ammonia[64]. Fish will congregate near inflow areas of better water quality, yet their density and stressed condition renders them susceptible to outbreaks of disease and die-offs. [69]

Klamath Lake such fish kills **(1971, 1986, 1995)** are generally attributed to outbreaks of "Columnaris" disease. These outbreaks have been common in fish hatcheries under crowded, high temperature conditions. various testing for pesticides, petro-chemicals and other contaminants over the past 10 years failed to reveal the presence of any such contaminants. [70]

A few reports of neurotoxicity in the scientific literature have raised unwarranted concern. Aside from these reports, nearly ten years of regular testing (more than 300 samples tested) has failed to reveal the presence of any neurotoxins. In the late 1990's two lawsuits were filed against companies harvesting from Klamath Lake for neurotoxins. Both cases were dismissed after considerable effort to detect neurotoxins proved unsuccessful. [67]

Finally, a study recently published used genetic technologies to determine that the previous reports of neurotoxins associated with Aph. Flos-Aquae had misidentified the algal species and the toxic algal samples were not AFA but a species of Anabaena. [66]

Below is a brief and more detailed account of evolution of the scientific data regarding the neurotoxicity of Aph. Flos-aquae:

Gorham determined that the sample was not pure Aph. Flos-aquae, but actually consisted of equal parts of Aph. Flos-aquae and Microcystis – an algae known to produce microcystins. **Gorham** concluded that the toxicity came not from the Aph. Flos-aquae, but from the Microcystis . [71]

It could not be concluded that Aph. Flos-aquae from upper Klamah Lake produced a neurotoxin. As quoted by **Gentile** (personal communication to W.W.C., **March 27, 1996**), "this anecdotal toxicity test

on Upper Klamath Lake Aph. Flos-Aquae should be rigorously restudied before it can be concluded that the alga produces a toxin". Periodic toxicity tests in the **1980**'s plus frequent regular testing since **1991** have failed to reveal any neurotoxins in Upper Klamath Lake Aph. Flos-aquae. **72,73**

Sawyer et al. (1968) and Gentile and Maloney (1969) reported toxicity of an atypical non-colony forming Aph. Flos-aquae that killed fish and laboratory mice. This Aph. Flos-aquae came from Kezer Lake in New Hampshire. **72-74**

More recently, **Rapala et al (1993)** reported toxicity of Aph. Flos-Aquae isolated from water blooms in Finland. These studies establish that Aph. Flos-aquae is toxic only in some geographical locations. This study also demonstrated that it was not possible, under the experimental conditions, to manipulate a non-toxic strain of Aph. Flos-aquae to become toxic. **75**

At this point in time, the general consensus among scientists was that some strains of Aph. Flos-aquae were capable of producing neurotoxins but most strains, include the Klamath Lake strain, were non-toxic.**68**

Recent development in genetics have provided the tools to determine, using genetic similarities, whether the toxics strains of Aph. Flos-aquae are the same species as the strain showed to be non-toxic. **68**

Li et al. (2000) have shown that all the toxic strains of Aph. Flos-aquae are genetically dissimilar to the non-toxic strains and most likely belong to the Anabaena genera. [69] The Wright State University study confirms that AFA growing in Klamath Lake is nontoxic.

Court Cases

It is interesting to briefly discuss two instances in which lawsuits were filed around the issue of neurotoxicity of Klamath Lake Aph. Flos-aquae.

In the first one[63,69] a man, **Mr. Fineman**, claimed that consumption of Aph. Flosaquae triggered neuropathy. The case revealed that **Mr. Fineman** had been suffering from diabetes since early childhood and had had many episodes of developing neuropathy.

After two years of contracting with various laboratories throughout the world to detect and identify a neurotoxin in Aph. Flos-aquae, Mr. Fineman had to withdraw the suit because of Lack of evidence. The court obliged **Mr. Fineman** to published the following statement :

*"I, **Samuel Fineman**, brought a lawsuit against Cell Tech and the Kollmans because I thought I had been harmed by some substance in Cell Tech's products. Testing and investigation (including testing for neurotoxins) did not confirm the presence of any such substance. Accordingly, I have withdrawn my lawsuit in its entirety."*

In a second case, [63,69] the aforementioned company Cell Tech filed a lawsuit against an individual, **Mark Thorson**, who had relentlessly published over the Internet that Aph. Flos-aquae from Klamath Lake contained a neurotoxins similar to cocaine and dangerous to consumers. Once again, after considerable effort to prove his allegations, **Mr. Thorson** lost his case. He was also asked to published the following statement over the Internet:

"During the last several years, I have from time to time posted this and other newsgroup a file of information called "An Anatoxin-a Primer." I now retract the statements made in the Anatoxin-a Primer. The Anatoxin-a Primer implied that Super Blue Green Algae from Klamath Lake, produced by Cell Tech, contains anatoxins-a (a neurotoxin I characterized as addictive) ,and that Cell Tech deliberately avoids testing for this toxin because anatoxin-a is responsible for the effects reported by SBGA users. I have since been advised that Cell Tech conducts regular tests that would disclose anatoxin-a, and that this toxin has never been found in Super Blue Green Algae. I had no basis for the suggestions I made in the Anatoxin-a Primer, and I hereby it in full."

These two cases are interesting as they both relied on the explicit demonstration that Aph. Flosaquae from Klamath Lake contained a neurotoxin. In both cases, many laboratories throughout the world with the capability and the expertise to detect and quantify neurotoxins were contracted to find neurotoxins in Aph. Flos-aquae from Klamath Lake, with no success.

In summary, the few instances of reports of neurotoxins of Aph. Flos-aquae pertained not to Aph. Flosaquae bur to species believed to be Anabaena spp. All samples shown to be Aph. Flos-aquae by PCR technology (genetics) were all reported to be non-toxic. In addition, two significant legal suits filed to detect the presence of any neurotoxin in Aph. Flos-aquae from Upper Klamath Lake. [68]

Dr. Gitte Jensen, an immunologist affiliated with McGill University, sought to establish a level at which uncontaminated AFA algae might exert a toxic effect on human blood cells. She created various dilutions of live blood mixed with AFA algae and observed the reactions. Even at extreme concentrations—the equivalent of a human ingesting 15.000 capsules of AFA per day—Dr. Jensen found no toxic effects on human blood cells.[668]

Taken altogether, the available data demonstrate the non-toxicity of Aph. Flos-aquae from Upper Klamath Lake.

Anti-inflammatory and Antioxidant Effects of AFA

"Many of the elderly in the United States—and quite a few of the not-so-elderly—experience terrible pain in their Joints Their fingers may become twisted and swollen, and they may be unable to button a coat without large doses of anti-inflammatory drugs. .Many come to feel crippled and useless. By the age of 35, 35% of Americans have diagnosable arthritis in their knees At least 85% of those over the age of 70 have it. and many have it severely."

—J. Robbins.Diet for A New America. 1957

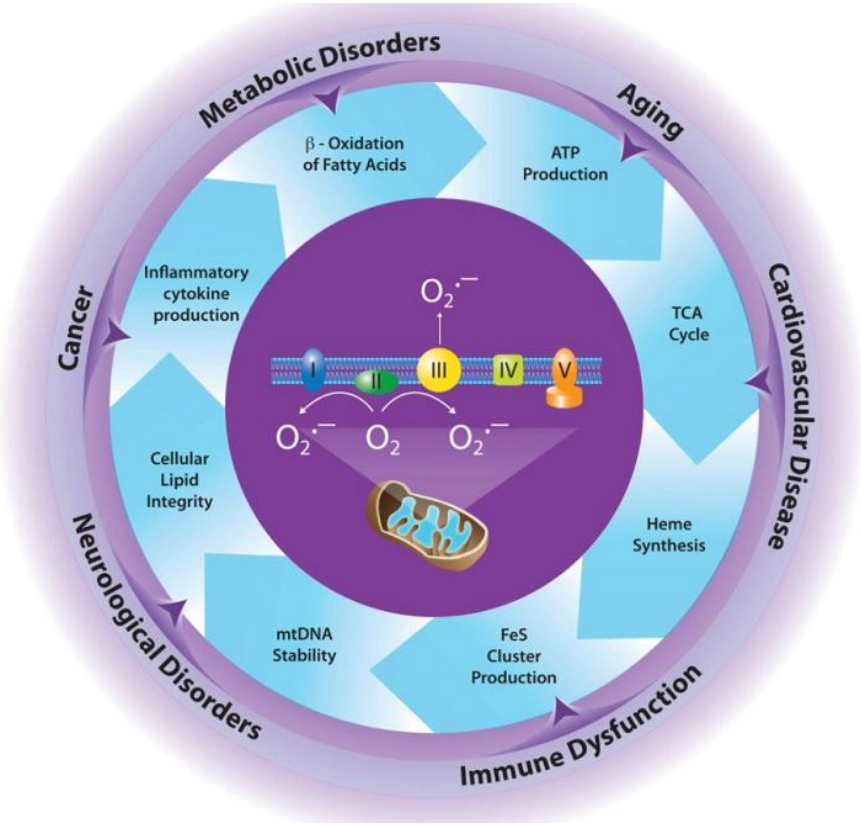


Fig. 55; Mitochondria are the major source of ROS in the cell through superoxide production at complexes I and III of the electron transport chain (center). Excessive ROS production can damage different components of mitochondrial metabolic pathways, resulting in altered mitochondrial function and an imbalance in cellular homeostasis (inner

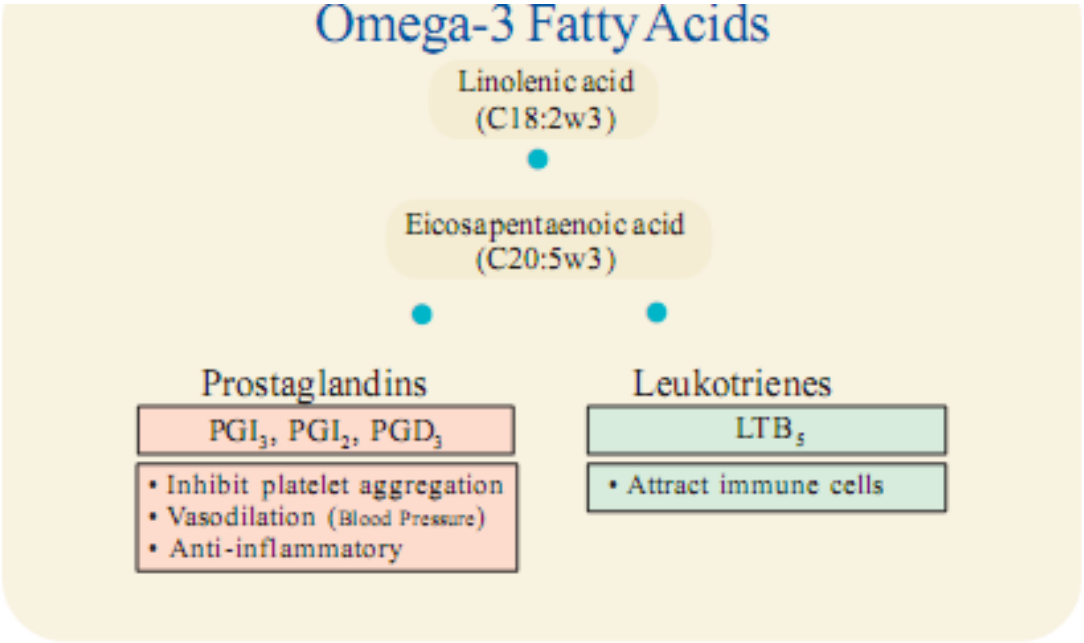
ring). Diminished mitochondrial function leads to the development of numerous diseases (outer ring).

The Eicosanoids Pathway

Eicosanoids are a group of oxygenated fatty acids containing 20 (eicosa) carbon atoms, produced by the body to support many body functions. Eicosanoids are ubiquitous substances considered local hormones because their activity is limited to the site where they are released, they are short lived and they are synthesized on demand and not stored in tissues. Their role in the body is such that their two main precursors, linoleic acid (LA; 18:2w6) and a-linolenic acid (LNA; 18:2w3), are called essential fatty acids and were even considered vitamins a few deoades ago (vitamin F). The action of specific enzymes leads to the transformation of both LA and LNA into whole families of eicosanoids having various roles in the support of immune and cellular functions.[432]

COX-2Inhibitors

There are two primary cyclo-oxygenase enzymes: COX-I andCOX-2. COX-I helps maintain platelet and kidney function and is integral to homeostasis maintenance. COX-2 leads to the production of substances that cause acute or chronic discomfort in joints. The adverse effects of the non-steroidal anti-inflammatory drugs (NSAIDs) come from the fact that they inhibit both COX-1 and COX-2. Specific COX-? inhibitors prevent the production of inflammatory compounds without affecting the activity of COX-1.[432]



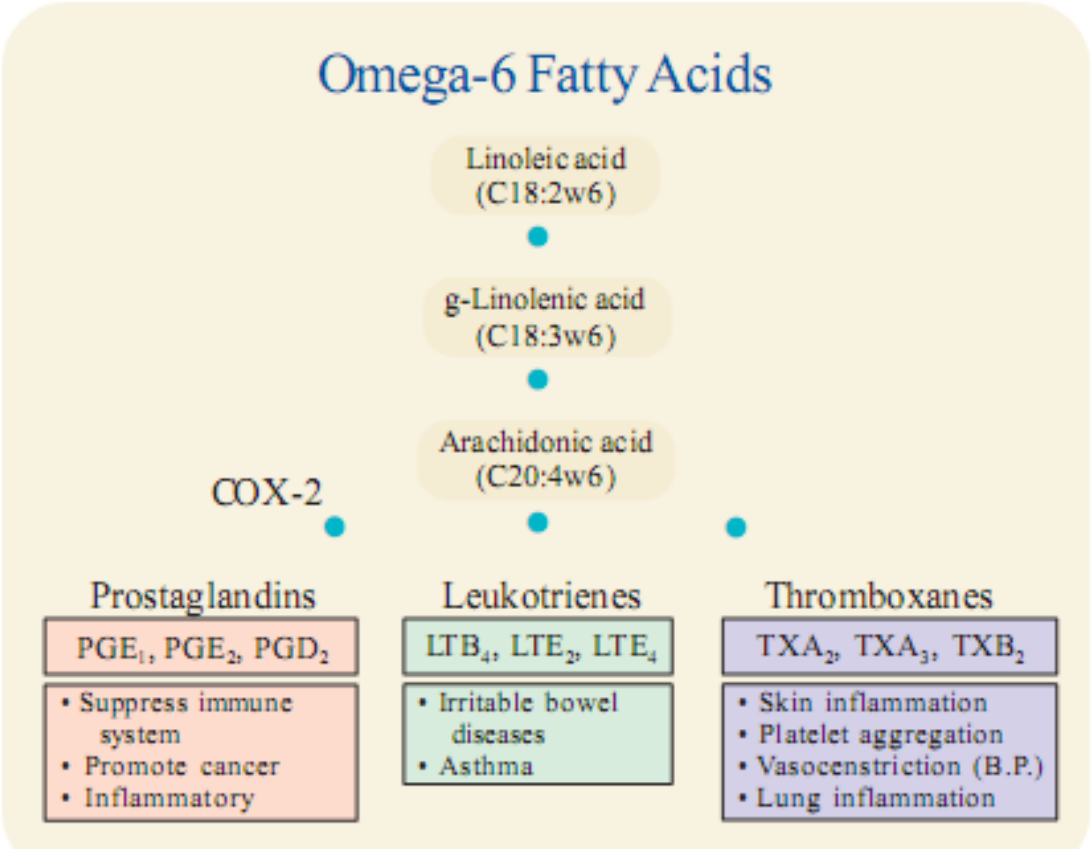


Fig. 56; Although this is an oversimplification, Omega-3 fatty acids are generally metabolized into health-promoting anti-inflammatory compounds, whereas Omega-6 fatty acids are precursors to inflammatory compounds in the body. COX-2 is involved in the transformation of arachidonic acid into inflammatory prostaglandins.

Human clinical trials with specific COX-2 inhibitors have shown anti-inflammatory and analgesic efficacy that compare with traditional treatments, without the secondary effects normally associated with NSAID. Phycocyanin was shown to be more effective than Celecoxib^{TM} and Rofecoxib^{TM} at specifically inhibiting COX-2. Phycocyanin was also shown to inhibit the enzyme lipoxygenase responsible for the transformation of AA into leukotriene B4, a compound involved in the pathophysiology of asthma. Recent drugs developed for the treatment of asthma are inhibitors of leukotriene B4 action.[177,178,208, 432-452]

Phycocyanin

Phycocyanin is the blue pigment present in all blue-green algae. In the living algal cells phycocyanin serves as a protein storage unit and as an antioxidant protecting the cell from the intense high-altitude sunlight. When taken orally, phycocyanin was shown to have strong antioxidant and anti-inflammatory properties. In various animal models of inflammation, phycocyanin was shown to reduce or prevent inflammation such as acetic acid-induced colitis in the rat. Recently the mechanism of action was identified when phycocyanin was shown to be a strong and specific inhibitor of the enzyme cyclo-oxygenase-2 (COX-2), blocking the production of the inflammatory eicosanoids in the body. [432-452]

Phycocyanin is a water-soluble, highly fluorescent protein derived from cyanobacteria[453] (blue-green algae) used in food coloring, cosmetics and biomedical research.[454] In this contest, we focused our attention on the edible microalga Aphanizomenon flos-aquae, in which PC represents around 15% of algal dry wet. In particular, we tested the efficacy of a novel natural extract from AFA enriched with PC in protecting human

erythrocytes and plasma samples against the oxidative damage induced by AAPH or CuCl2. The chromophore, named phycocyanobilin, is similar in chemical structure to bilirubin, and like the latter acts as a powerful scavenger of reactive oxygen species. C-phycocyanin is a free radical scavenger[455, 456] and has significant hepatoprotective effects

Anti-oxidant properties of AFA

Fossilized samples of algae show that these organisms have remained virtually unchanged for several billion years. Scientists describe blue-green algae as having reached a sort of "evolutionary perfection." as some species show little evidence of mutation or genetic damage. The success of ancient microalgae depended upon their developing powerful antioxidant pigment shields. Because there was little atmospheric protection from the harmful rays of a much brighter sun, antioxidant shields were vitally important to protect sensitive molecules— especially exposed nucleic acids and lipids—from the damaging effects of solar radiation. *By producing oxygen and converting inorganic carbon and nitrogen into organic forms, ancient microalgae helped to transform our planet from an inhospitable world into a life-sustaining one over billions of years. So effectively did microalgae develop antioxidant and genetic survival codes that they provided a foundational blueprint copied by many higher species that arrived later.*[375]

Prokaryotes, organisms without a nuclear membrane (e.g.. blue-green algae), display a more diverse array of antioxidant pigments and a broader selection of carotenoids than terrestrial plants and most green algae.

Scientists at the University of Wisconsin. Department of Food Microbiology, report that because of the remarkable health benefits of algal and microbial carotenes, there will likely be a substantial increase in the world-wide demand for a full range of these important antioxidants.[438]

Carotenoids represent one of the most widely distributed and structurally diverse classes of natural pigments, with important functions in photosynthesis, nutrition, and protection against photo-oxidative damage.

Rats and chickens fed a natural algal form of beta-carotene showed at least a tenfold higher accumulation of overall beta-carotene in their livers than those control animals fed equivalent amounts of synthetic all-trans beta-carotene supplement. The higher accumulation of the natural algal carotenoids. over the synthetic isolated beta-carotene likely indicates a greater therapeutic value, according to the researchers. [434]

Researchers have reported that natural algal beta-carotene is superior to a synthetic beta-carotene supplement in terms of raising lipophilic antioxidants (protecting PUFAs) in human serum [435] Also, natural algal extracts of 9-ds beta-carotene are shown to have a higher antioxidant potency compared to synthetic all-trans beta-carotene with in vitro experiments. [436]

Pigments, phytochemicals, vitamins, and trace elements from algae and higher plants can help boost the human body's antioxidant

defenses.[437,438] AFA has an unusually wide variety of antioxidants, such as tocopherols. beta-carotene. flavonoids. superoxide dismutase. glutathione, taurine, tryptophan, phenolic acid, and vitamins C.
E, B5. and B2. Antioxidants are biomolecules that protect organisms from the damaging effects of reactive oxygen species (free radicals) that are constantly formed in biological systems.

Oxidative stress is an important factor in the genesis of many pathologies, from cancer to cardiovascular and degenerative diseases.[457-459] In order to protect the body against the consequences of oxidative stress, an efficacious approach consists in improving the antioxidant nutrition. In this regard ,scientific studies have shown that the synergistic action of a wide spectrum of antioxidants is better than the activity of a single antioxidant, and that antioxidants from natural sources (primarily foods) have a higher bioavailability and therefore higher protective efficacy than synthetic antioxidants.[460]

Free radicals are molecules that lack one electron—a highly energetic particle—in what is usually a pair. To stabilize, free radicals randomly grab electrons from normal (i.e.. healthy) molecules. This creates new
free radicals, and like a row of falling dominoes, the damaging free radical cascade continues to spread. Free radicals are by-products of normal metabolism and form as a result of exposure to radiation and some environmental pollutants. Because they are highly reactive, they can damage cellular components, a particular concern with brain, vascular, and connective tissues and nucleic acids. They have been implicated in a variety of diseases and even in the acceleration of the aging process itself.[432-452]

"The more complicated the life form, the more sophisticated its handling of the oxygen molecule. In higher life forms, oxygen can be converted into several toxic oxygen species (free radicals) that are lethal to most potential pathogens. Then the host must also be capable of protecting itself from these toxic oxygen species; hence, antioxidant defenses were born... The pathogens, on the other hand possess inadequate antioxidant defenses against this sophisticated handling of oxygen." [439] For example, leukocytes can generate and launch toxic free radical attacks against pathogens, but they must also have their own effective anti- oxidant defense system.

Researchers at Oregon State University in Corvallis are starting to recognize that pigment particles in blue-green algae may have an antioxidant effect in humans, preventing unstable compounds from damaging healthy cells. Balz Frei. director of OSU's Linus Pauling Institute, speculates that perhaps algae might one day be commonly eaten to help lower the risk of cancer, perhaps becoming as popular as taking vitamin C. [440]

Blue-green algae contain a wide range of antioxidants in the form of specific trace minerals. amino acids, vitamins, and especially pigments - an impressive variety of carotenes along with potent green and blue pigments. Depending on the source of blue-green algae, the amount of phycocyanin can range up to 15% of its dry weight.

The first report about the antioxidant and anti-inflammatory properties of C-phycocyanin of blue-green algae (AFA) came from in

Pharmacology Department, National Center for Scientific Research, CNIC, Havana, Cuba by Romay C et al.[443] In this study, they have applied established invitro and invivo assays in order to evaluate the antioxidant action of c-phycocyanin. This natural product was able to scavenge alkoxy and hydroxyl radicals. Two methods were used to evaluate hydroxyl radical scavenging by c-phycocyanin because hydroxyl radical is one of the most potent oxidizing species and its extreme reactivity naturally poses problems with regard to its detection.[463] In both methods, inhibition was observed at relatively high concentrations of the product.

Phycocyanin also quenched the CL signal generated in the HX-XO system, but this effect cannot be ascribed to $O2^-$ scavenging, as demonstrated by double-quenching and NBT reduction assays. One possible explanation for this behavior is that phycocyanin quenches CL by binding to an intermediate or co-oxidising species that may be involved in the CL reaction. [464]

The inhibitory effect observed on microsomal lipid peroxidation most probably is due to a metal-binding capacity of c-phycocyanin, since chain-breaking antioxidants often introduce a lag period into the peroxidation process, corresponding to the time taken for the antioxidant to be consumed, where as metal-binding antioxidants will give a constant inhibition throughout the reaction.[465] Another indication of such an action, is the ability of c-phycocyanin to inhibit deoxy ribose damage in a site-specific manner (in the absence of EDTA).[465] In the deoxy ribose assay, a second-order rate constant calculated for phycocyanin was

similar to that obtained, by the same method, for some non-steroidal anti-inflammatory drugs, such as indomethacin and ibuprofen ($1{:}8 \times 10^{10}$ $M^{-1}S^{-1}$) [466]

Chemiluminescence of PMNLs is the final result of luminal oxidation by strong oxidants, such as oxygen radicals and peroxides, emanating from enzymatic reactions. For addition to the myeloperoxidase-H2O2-halide system, the release of arachidonic acid by phospholipaseA2 and of diacyl glycerol and inositol trisphosphate by phospholipaseC, the metabolism of arachidonic acid by the cyclooxygenase and lipooxygenase pathways, the activation of membrane NADPH oxidase by diacylglycerol and calcium mobilization by inositol trisphosphate are all able to induce the CL reaction. Inhibition of any of these mechanisms suppresses the CL response. [467]

Phycocyanin was able to inhibit the LCL in a dose-dependent fashion, most probably through its capacity to scavenge free radicals(OH•,H2O2,RO•) and peroxides arising during the respiratory burst of phagocytic cells. However, it is also possible that phycocyanin could diminish CL signals in otherways, e.g. by affecting enzymes involved in the production of reactive oxygen species by activated phagocytes, NADPH oxidase and myeloperoxidase, or by interfering either with the binding of the stimulant or the arachidonic acid metabolism pathway. In this regard, they have recent evidence for inhibition by phycocyanin of LTB4 release in an animal model of inflammation (manuscript in preparation).

The peroxide-induced inflammatory response is a valuable invivo model in order to test agents with potential scavenging effect against H2O2 and OH• .GO injected into the mouse paw reacts with endogenous glucose and generates H2O2 which subsequently produces OH• radicals; both together are responsible for tissue damage and for the accompanying inflammatory changes . Phycocyanin reduced the edema produced by glucose oxidase in the mouse paw. This anti-inflammatory effect must be due, at least in part, to the scavenging of hydroxyl radicals, taking into account the fact that DMSO, a well known scavenger of OH• radicals, also inhibited the inflammatory response induced by GO.

Currently, there is a consensus that much of the damage induced by H2O2 invivo is due to its conversion to highly reactive oxidants, mainly OH•.[465] Therefore, the scavenging action of phycocyanin against OH is probably relevant to its anti-inflammatory effects.

Very recently, research carried out in their laboratory has confirmed the anti-inflammatory effects of phycocyanin in other experimental models of inflammation such as cotton pellet granuloma in the rat and TPA-induced inflammatory response in the mouse ear (manuscript in preparation).

Taking into account that AFA is used as dietary supplement in many countries and that PC represents around 15% of algal dry wet, in our opinion it was of prime importance to investigate the biochemical properties of the protein in vitro. In this report, we evaluated for the first time the antioxidant activity of a novel natural extract from AFA enriched with PC. The extract was used for the inhibition of aqueous peroxyl radicals [2,2'-Azobis (2-amidinopropane) dihydrochloride, AAPH]-

induced oxidative hemolysis and lipid peroxidation of normal human erythrocytes. They also tested the protective role of the AFA extract in human plasma samples treated with the pro-oxidant agent cupric chloride (CuCl2).

In RBC treated with 50 mM AAPH, free radicals attack erythrocyte membrane components, such as proteins and lipids, and cause changes in the structure and function of membranes; as a result, a time-dependent RBC hemolysis was observed during cell incubation with AAPH. At the same time, a significant accumulation of TBARS, used as indicators of lipid peroxidation, was found during the incubation period with AAPH. Moreover, the treatment of RBC with AAPH resulted in up to 75% loss of intracellular GSH after 5h at 37^{o}C, indicating that GSH is very susceptible to AAPH mediated oxidative insult.

In RBC suspensions pre-incubated with the natural AFA extract, our findings clearly indicated that the extracts significantly reduced in a time-and dose-dependent manner the extent of lipid peroxidation and hemolysis of RBC treated with AAPH, thus protecting the cell against the oxidative damage. Contemporarily, the depletion of intracellular GSH induced by AAPH was delayed.

The AFA extract showed a similar antioxidant activity in human plasma samples treated with the pro-oxidant agent CuCl2 (100 µM): by evaluating diene formation at 245 nm, we found that the extract increased the plasma resistance to oxidation in adose-dependent manner; in the

same way, MDA accumulation induced by CuCl2 was significantly inhibited.

The involvement of the photosynthetic pigment PC in the antioxidant protection of the AFA extract against the oxidative damage was demonstrated by recording the spectral changes of PC induced by AAPH or CuCl2. In fact, the incubation of the extract with the oxidizing agents led to a significant decrease in the absorption of PC at 620 nm accompanied with disappearance of its blue color, thus indicating a rapid oxidation of the protein. Similar spectral changes under line a direct involvement of the chromophore phycocyanobilinin the radical scavenging activity of PC; infact, the spectroscopic properties and the brilliant blue color of the protein depend in large measure on the chemical nature of its chromophore.[462]

In the light of these invitro results, and taking into account that PC also has anti-inflammatory properties, we think that the oral supplementation with the natural AFA extract could be a helpful co-factor in the treatment of clinical conditions related to oxidative stress and inflammation.

Replicated studies with a range of experimental animal models have established the potent antioxidant and anti-inflammatory effects of phycocyanin. In rodents, experimentally-induced colitis as well as edemas of the paw and ear all responded positively to C-phycocyanin.[443-475]

"Bi-indoles isolated from a number of blue-green algae have anti-inflammatory and anti-allergenic properties.[444] Additionally, a chlorophyll-related compound, pheophytin, derived from an edible green alga, has potent anti-inflammatory effects in both in vitro
and in vivo experiments Both human and mouse immune cells showed an inhibited response to experimentally induced inflammation. Pheophytin has exhibited a significant suppression against edema formation in a mouse ear that was inflamed by toxic means[445]

Gitte Jensen, an immunologist from McGill University, and her team at the Royal Victoria Hospital in Montreal report that AFA algae may help to inhibit and to reverse inflammatory conditions. The researchers observed that small dilutions of AFA algae tend to dampen the release of reactive oxygen species from certain phagocytic cells in human blood. [446]

Scientists at the University of Padova. Italy, found that diatoms, golden brown unicellular algae, produce anti-inflammatory chemicals that are the main active ingredients in mud-pack treatments. In European health spas the use of mudpacks for the treatment of rheumatic and osteoarthritic patients has a long and relatively successful history. The maturation of thermal mud is dependent upon the full colonization of the mud by thermophilic microorganisms, with diatoms producing anti-inflammatory sulfoglycolipids (SGL). similar to those in
blue-green algae A typical cycle of treatments requires 12 packs of thermal mud.[1]

"On this basis we calculated the amount of SGL taken up by each patient in a cycle of treatments, and found a figure not far from the

recommended dose of non-steroid anti-inflammatory drugs utilized for the same pathology. However, unlike pharmaceutical preparations, the amount of SGL taken up by the patients after the mud-packs does not exert any adverse gastrointestinal effect on these patients" reported the scientists. [447] Additionally, the anti- inflammatory action of SGL is consistent with the decrease of serum interleukin-1 observed in arthrosic patients treated with mudpacks[448]

Biologists at Israel's National Institute of Oceanography fed blue-green algae to animals and discovered that the high levels of the important fatty acids EPA and DHA in blue-green algae reduced the levels of arachidonic acid in the blood and liver. [449] Arachidonic acid increases brain cell oxidative damage and impairs the flexibility of cell membranes. It is a precursor of the inflammatory prostaglandins—the molecules people are trying to suppress when they take anti-inflammatory drugs.

Essential fatty acids, especially the omega-3s found in coldwater microalgae and fish oils, can be helpful in a variety of inflammatory conditions, such as rheumatoid arthritis[208] Fish oils are essentially derived from the consumption of aquatic algae-derived PUFAs. either directly or indirectly. Cold-water fatty fish are good sources of these health-promoting fatty acids because of the cold-water algae in their food chain. In a retrospective study of 208 documented cases, medical researchers found positive evidence suggesting that AFA (a coldwater blue green algae) may be helpful in the treatment of fibromyalgia, a painful inflammatory condition with multiple etiologies[450]

Overall, evidence suggests that microalgae demonstrate at least four antioxidant properties:

1. Scavenging of reactive oxygen species (free radicals).
2. Regeneration of endogenous antioxidants, such as SOD and glutathione reductase.
3. Chelation of heavy metals.
4. Repair of oxidation-damaged proteins.

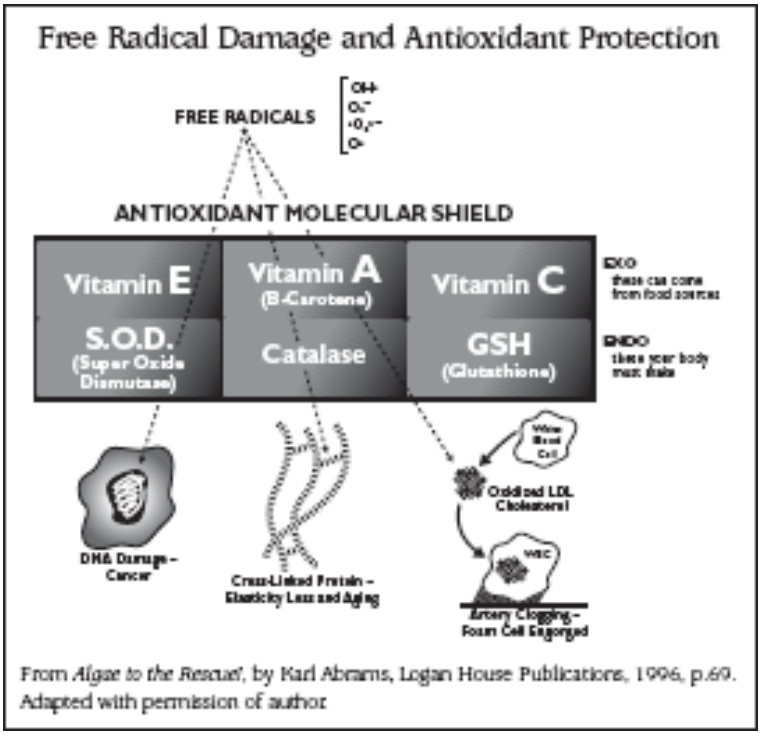


Fig. 57; **Free Radical Damage and Antioxidant Protection**[1]

DETOXIFYING EFFECT OF AFA

"Since 1950 at least 70.000 new chemical compounds have been invented and dispersed into our environment through new consumer commodities, industrial products, arid food. We are by default conducting a massive clinical toxicological trial .And our children and their children are the experimental animals."

—H. Needleman and P. Landrigan.Raising Children Toxic Free, 1994

"Toxic substances are everywhere - in the air we breathe, the food we eat. and the water we drink. Even our bodies and the bacteria in the intestines produce toxic substances, It can be strongly said that the health of an individual is largely determined by the ability of the body to detoxify.'

—Murray and Pizzomo.Encyclopedia of Natural Medicine. 1991.

"Methionine was probably one of the first amino acids available in Earth's ancient primordial seas billions of years ago. This amino acid was (and is still) used by primitive bacteria and blue-green algae to biosynthesize glutathione, possibly Earth's first antioxidant (protection) tripeptide molecule. Methionine in this form has been shown to help humans detoxify lead and copper contamination in the blood [391]

Methionine's most useful metabolite, glutathione, is a peptide made of three amino acids—glycine, cystine, and glutamic acid—all present in microalgae. Also, methionine can be restored from homocysteine—its alterego, sister form—with the help of B vitamins, especially folic acid in

conjunction with B-6 and B-12. Elevated blood homocysteine levels are a risk factor for heart attack, the number one killer of adults in America.[392]

Blue-green algae are one of the richest food sources of detoxifying polypeptides, including methionine and glutathione, along with B-vitamin precursors Once ingested, these molecules are modified as needed. Such polypeptides are essential in the protection of DNA. the family jewels, and are essential in the chemistry of detoxification.[393]Also glutathione, along with ascorbate. may help to protect against polyunsaturated fatty acid (PUFA) oxidation.[394]

Glutathione is the most important worker in the body's detoxification department. In fact, detoxification is the most important activity in the body's entire biochemistry and the biggest consumer of energy for making new molecules. Biochemically, the glutathione reductase enzyme present in blue-green algae shows amino acid sequence similarities to human reductases.[395] Among prokaryotes, only two groups, the purple bacteria and the cyanobacteria—also known as blue-green algae—produce glutathione.[396]

Detoxification of harmful substances from the body is a life-long process. Our ability to effectively detoxify and eliminate toxins (eg., heavy metals, solvents, pesticides, microbial toxins) shapes and determines our state of health. Two major organ systems, the kidneys and liver, are primarily involved in detoxification. Additionally,
healthy intestinal bacteria and a healthy mucosal lining are essential to reduce the absorption of toxins into the blood through the intestines. Microalgae can support, directly and indirectly, these primary detoxification pathways to better eliminate toxins.

The liver acts as a major organ for detoxification. All blood returning from the stomach, intestines, spleen, and pancreas is “detoxified” by the liver, reducing and helping to eliminate many toxins. Blue-green algae have multiple liver-protective factors, including amino acids (e.g.. methionine, arginine, and isoleucine), chelating trace minerals, and potent antioxidants, such as phycocyanins and superoxide dismutase (SOD). [397-399]

When microalgal supplementation was given to rats consuming a high fructose (60%) diet, a preventive effect on the liver triglyceride level was observed, along with lowered plasma cholesterol. The researchers reported that the microalgae helped reduce liver fats that were elevated by the excessively fructose-rich diet. [400] Chlorophyll, which microalgae contain in abundance, can help to stimulate liver function, increase bile secretion, and protect cellular functions. [401]Also, "chlorophyll appears to promote regeneration of damaged liver cells. [402]

Researchers from the National Institute of Oceanography in Haifa. Israel, reported that the increased absorption of essential fatty acids (EFAs) found with consumption of certain microalgae can support healthier liver function.[403] Additionally, experimental animals fed oxidized (i.e.. rancid) oil combined with a Dunaliella algal extract were better able to maintain their hepatic stores of beta-carotene and vitamin A than control animals given a synthetic all-trans beta-carotene. The control group showed significant liver (provitamin losses, likely due to a greater utilization of their antioxidant vitamin reserves Beta-carotene helps to reduce hepatic and erythrocyte peroxidation associated with the consumption of oxidized oils. [404] Exposure to air naturally oxidizes the oils in our food; light and heat both speed up the process.

In the Orient algae has a long tradition as a master cleanser. Macrobiotic counselor Steve Gagne reports ”When ingested, algae begin to go to work like a janitor, cleaning, purifying and strengthening the internal environment. [405] Japan has been subject to several tragic incidents of severe chemical poisoning. The use of algae has stimulated the excretion of some contaminants, notably cadmium, at an accelerated rate in test patients. Lead and mercury are also excreted, without the detrimental effects associated with conventional chelation therapy[406]

Japanese researchers from Chiba University found that blue-green algae effectively reduces kidney toxicity from high doses of mercury and pharmaceutical drugs administered to laboratory rats. [407] These scientists used two indicators for kidney toxicity, blood urea nitrogen (BUN) and serum creatinine. They checked levels of both indicators before and after administering various toxic doses of mercury, para-aminophenol (a common pain-killer), gentamicin (an antibiotic), and cis-dichloro-diamino-platinum (an anticancer drug). In all cases, rats fed blue-green algae (up to 30% of their diet) exhibited significantly reduced BUN and serum creatinine levels that returned back to near normal levels after the experimental poisoning. This was not the case with the poisoned, untreated, control animals.

Medical researchers have demonstrated that green microalgae increase the detoxification of harmful chemicals like chlordecone. dioxin. and PCBs.[408] In a study of chlordecone poisoned rats, ingested algae decreased the half-life of the chemical toxins from 40 to 19 days[227]

Several grams of AFA blue-green algae eliminated excessive aluminum from children in a three-month study Also, parents reported

significant decreases in negative health symptoms, suggestive of improved detoxification pathways. [151] Aluminum exposure in humans is unavoidable. Some aluminum absorption occurs with the ingestion of food and medicines. Greater amounts of aluminum are present in antacids.[409] Blue-green algae may be helpful for dialysis patients, who have a greater risk for aluminum accumulation and an increased risk of neurotoxicity.

Blue-green algae have been shown to reduce lead toxicity, as well [410] The beneficial effects of blue-green algae may be due to the presence of the abundance of antioxidants, including beta-carotene and SOD enzymes. Numerous studies have demonstrated a strong relationship between childhood learning disabilities and body stores of heavy metals, particularly lead. [411-412]

Andrew Valencia reported that AFA significantly reduced "leaky gut syndrome" in patients, based on functional test measures[413] Compromised intestinal permeability increases the uptake of toxic compounds and macro-molecules that can exhaust the detoxification capability of the liver A combination of leaky gut and dysfunctional
liver detoxification may lead to increased tissue stores of toxic compounds and depressed immune status.

Treatment with both reduced glutathione and selenium-enriched Spirulina algae helped to normalize the intestinal permeability of rats exposed to an experimental anaphylaxis reaction. Reduced glutathione administered by itself did not help normalize the intestinal permeablility of these sensitized rats [414]

Chlorophyll, the green pigment in algae, was found to be helpful in controlling body and fecal odors in 62 geriatric patients.[415] Chlorophyll may help to restore intestinal balance by reducing toxic bacteria and fungi in the walls of the intestines. In natural therapies, chlorophyll is considered a "detoxifying agent.”

RADIATION PROTECTION EFFECTS OF AFA

"The transfer of energy that is produced by radiation is similar to that caused by other forms of acute injury such as an automobile crash or a bullet wound... The difference between a bullet and an X ray lies principally in the size of the particle. While a bullet destroys tissues and entire organs, a particle of radiation collides with single atoms or molecules deep within the cells."[416]

—H. Needleman and P. Landrigan.Raising Children Toxic Free. 1994

High doses of radiation destroy cells directly, while lower doses of radiation can damage cellular DNA replication and thus lead to cell mutations. Radiation effects maybe countered by increasing the cell's protective antioxidant defenses, by enhancing the repair mechanisms that fix genetic base code damage or by increasing the body’s ability to identify and eliminate mutant or damaged cells. Many natural antioxidants have antimuta-genic properties that may prove useful in reducing long-term radiation effects. Furthermore, nutrients that help modulate endogenous antioxidants, such as superoxide dismutase, maybe useful in some radiotherapy protocols. [417]

Beta-carotene and other carotenoids found abundantly in microalgae, are known to be potent free-radical quenchers and lipid antioxidants. Natural beta-carotene (50 mg/kg diet), obtained from the unicellular alga, Dunaliella, was fed to rats exposed to a single high dose of whole-body radiation (4 Gy). Radiated control animals, not fed algal carotenoids. suffered a significant loss of body weight and decreased liver

concentrations of beta-carotene and retinol, compared to algal beta-carotene supplemented rats Normal increase in bodyweight and the absence of ill effects were noted in the groups of rats whose diet was supplemented by beta-carotene before and after irradiation. Furthermore the effect of irradiation was "partially cured" by supplementation with beta-carotene after irradiation in sub-group controls. These results suggest that beta-carotene (especially 9-cis isomers) and retinol protect in vivo against the cellular damage by free radicals induced after whole body irradiation[418]

However one possible contraindication of using beta-carotene extracts, versus giving the whole algae, is that high doses of beta-carotene treatment may markedly lower concentrations of vitamin E in tissues.[419] The use of mega doses of beta-carotene, like any isolated (pro-) vitamin extract, may contribute to other nutritional imbalances.

Foods rich in chlorophyll have been shown to offer radiation protective benefits. Tests performed by the U.S. Army showed that chlorophyll-rich foods may also be effective in decreasing the effects of radiation. In one controlled study it was found that a chlorophyll-rich diet doubled the life span of animals exposed to fatal doses of radiation.[277] Again, microalgae—in this instance. Chlorella—are cited as particularly rich sources of chlorophyll.

Chlorophyllin. a water-soluble derivative of chlorophyll, has demonstrated an ability to protect DNA against gamma-radiation-induced strand breaks exposing plasmid DNA in vitro. Researchers discovered that chlorophyllin effectively protected plasmid DNA against ionizing

radiation, independent of DNA repair or other cellular defense mechanisms, revealing direct evidence of free radical-scavenging properties of chlorophyll.[420]

Extracts of phycocyanin (the blue pigment) from blue-green algae helped to restore the efficiency of antioxidant defenses, dehydrogenase activity, and energy-rich phosphate levels in rats exposed to X-rays (dose of 5 Gy).[421]

Several animal and in vitro studies using microalgae have demonstrated remarkable radio-protective effects[422-426] When microalgae was administered orally to mice, radio-protective effects of microalgae were shown to occur both before and immediately after exposure to sub-lethal gamma-rays. [427] Significant benefits were observed in the number of bone marrow cells and the spleen weight.

According to Dr. John Apsley. “AFA has no less than 20 potent nutrients that limit and reverse the most common forms of radiation injury and may help to partly reverse genetic damage as well. [428] For example. "research has shown that salts of aspartic acid, which are present in AFA as potassium aspartate and magnesium aspartate, are useful for protection against radiation damage.” [429] The cyanophycin storage granules in blue-green algae are made of aspartic acid and arginine, which act to stimulate the thymus gland, promoting white blood cell activity.

As part of an attempt to evaluate and treat 709 children who had suffered long-term exposure to different doses of radiation during and

after the Chernobyl accident and subsequently moved to Israel, all of them underwent a medical examination. Of these children. 99 were provided with a beta-carotene powder of Dunaliella microalgae to take twice a day. The researchers found that irradiation increased the susceptibility of lipids to oxidation overall in the Chernobyl children. Yet they observed that microalgal beta-carotene may act as an in vivo lipophilic antioxidant or radio-protector in the supplemented group of children.[430]

Victims of the Chernobyl incident living in Russia and exposed to the long-term effects of radiation poisoning reported to medical relief workers that they experienced positive benefits after eating AFA blue-green. [431]

AFA MODULATION OF IMMUNE RESPONSE

"We may be different in gender, color of hair and skin, religion, and job, but we have a common bond; we are survivors. Our parents survived long enough to conceive us. Grandparents had the same claim for your parents. The thing that made this possible is that precious commodity; the immune system."

Schmidt. Smith, and Sehnert Beyond Antibiotics, 1993

This essay serves as a step in our direction towards answering questions about the efficacy of this particular nutritional supplement via scientifically proven data. First of all and before discussing our main subject we need to understand in brief how the complex immune system in the human body works.

The Immune System;[89,90]

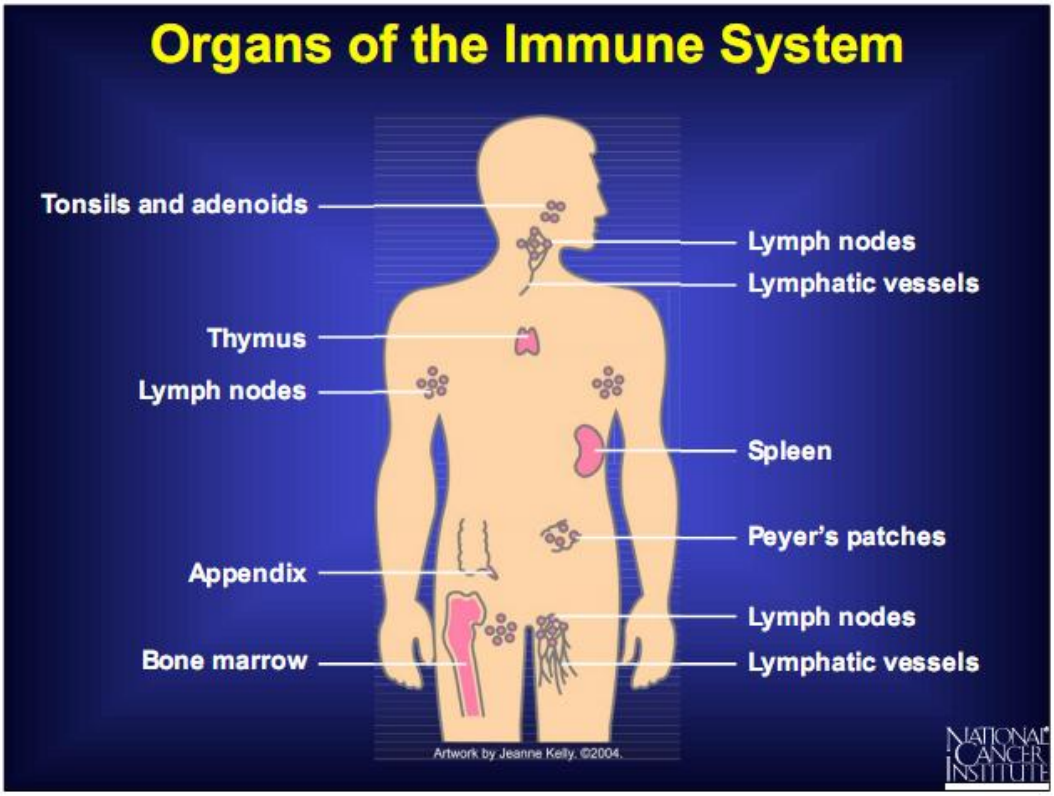


Fig. 11 ; Organs of the immune system.

The immune system is a very complex collection of white blood cells and lymphoid organs located in all parts of the body. This system has evolved to limit the pathogenicity of foreign organisms such as bacteria, viruses, fungi, and parasites. These organisms co-exist to a certain degree in the skin, the mouth, the respiratory tract, the intestinal tract and even the urinary tract. If these barriers are compromised, or if we are exposed to more highly infectious organisms, serious and sometimes lethal disease may result. [91,92]

Role of the immune system	Implications
Defense against infections	Deficient immunity results in increased susceptibility to infections; exemplified by AIDS Vaccination boosts immune defenses and protects against infections
Defense against tumors	Potential for immunotherapy of cancer
The immune system recognizes and responds to tissue grafts and newly introduced molecules	Immune responses are barriers to transplantation and gene therapy
The immune system can injure cells and induce pathologic inflammation	Immune responses are the cause of allergic, autoimmune, and other inflammatory diseases

Table 5; Importance of the immune system in health and disease. This table summarizes some of the physiologic functions of the immune system and its role in disease; AIDS, Acquired immunodefciency syndrome.

The immune system Consists of several different components that enables our bodies to combat different infectious and toxic agents. At the heart of the immune system blood leukocytes (the white blood cells) and tissue cells derived from these leukocytes. These cells all work together in two ways to prevent disease:

(1) by destroying invading agents by phagocytosis and other means (*innate immunity*) and

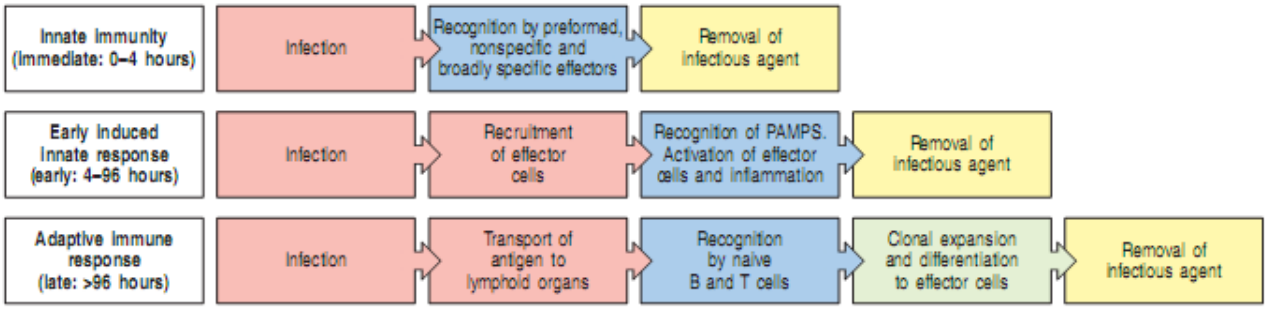


Fig. 12; The response to an initial infection occurs in three phases. These are the innate phase, the early induced innate response, and the adaptive immune response. The first two phases rely on the recognition of pathogens by germ line-encoded receptors of the innate immune system, whereas adaptive immunity uses variable antigen-specific receptors that are produced as a result of gene segment rearrangements. Adaptive immunity occurs late, because the rare B cells and T cells specific for the invading pathogen must first undergo clonal expansion before they differentiate into effector cells that migrate to the site of infection and clear the infection. The effector mechanisms that remove the infectious agent are similar or identical in each phase.

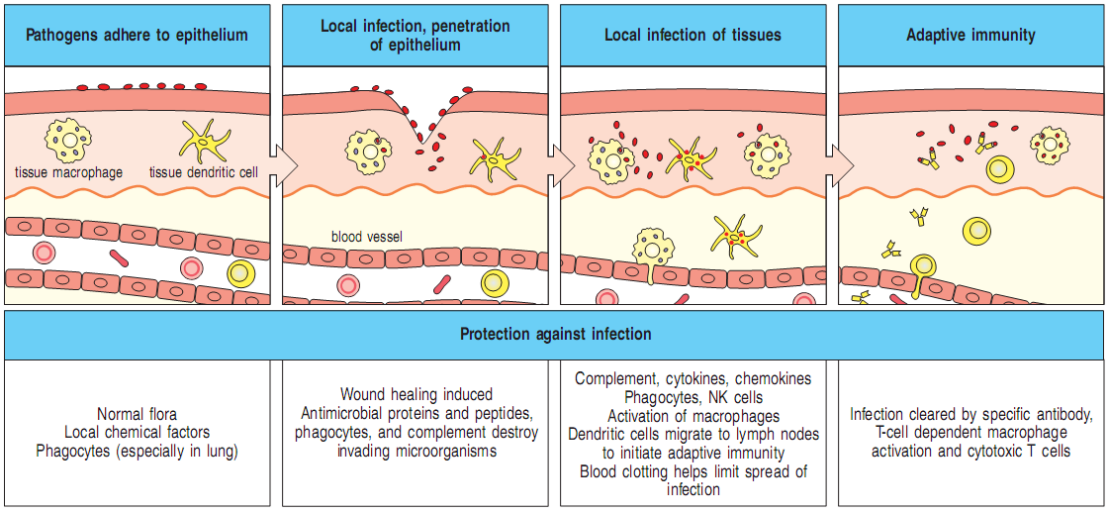


Fig. 13; An infection and the response to it can be divided into a series of stages. These are illustrated here for an infectious microorganism entering through a wound in the skin. The infectious agent must first adhere to the epithelial cells andthen cross the epithelium. A local immune response may prevent the infection from becoming established. If not, it helps to contain the infection and also delivers the infectious agent, carried in lymph and inside dendritic cells, to local lymph nodes. This initiates the adaptive immune response and eventual clearance of the infection.

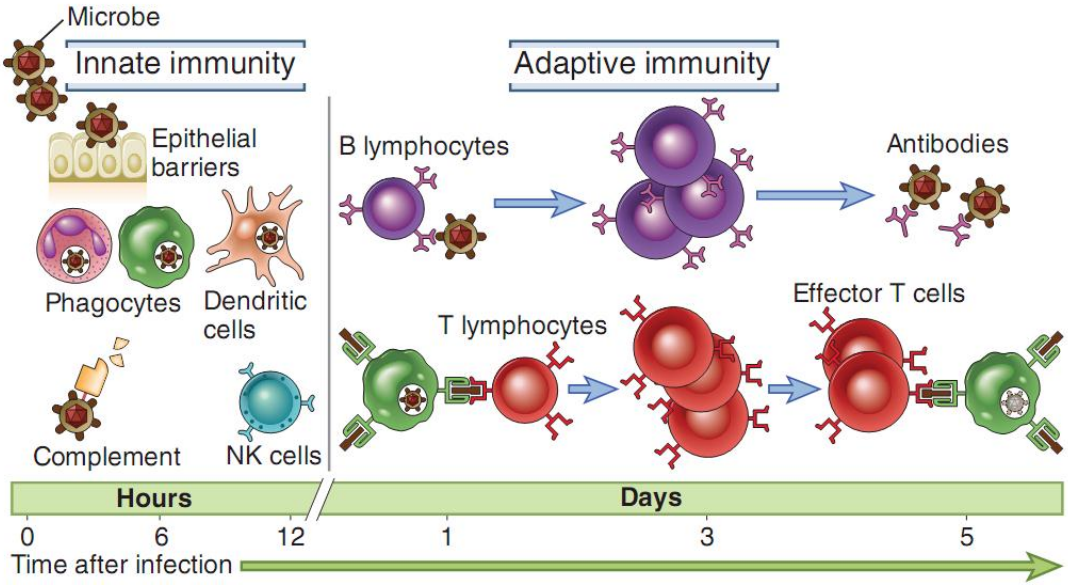


Fig. 14; Principal mechanisms of innate and adaptive immunity. The mechanisms of innate immunity provide the initial defense against infections. Some mechanisms (e.g., epithelial barriers) prevent infections, and other mechanisms (e.g., phagocytes, natural killer [NK] cells, the complement system) eliminate microbes. Adaptive immune responses develop later and are mediated by lymphocytes and their products. Antibodies block infections and eliminate microbes, and T lymphocytes eradicate intracellular microbes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections.

(2) by forming antibodies and sensitized lymphocytes, one or both of which may destroy or inactivate the invader (*acquired/adaptive immunity*).

The phagocytes (usually referring to neutrophils and macrophages) of the non-specific immune system provide a first line of defense against many common micro-organisms and are essential to the control of common bacterial infections. [89,90]

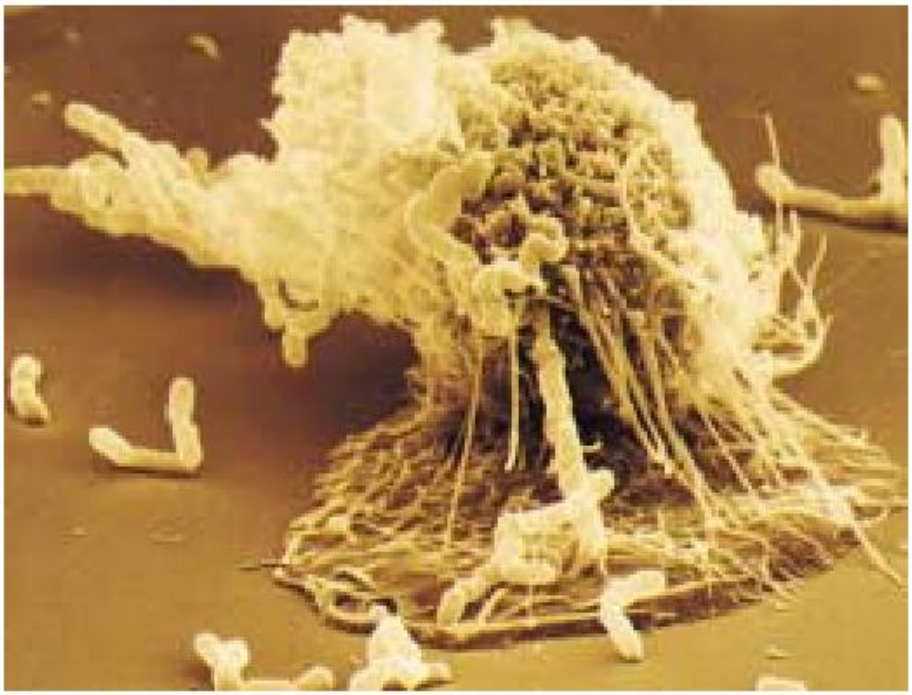
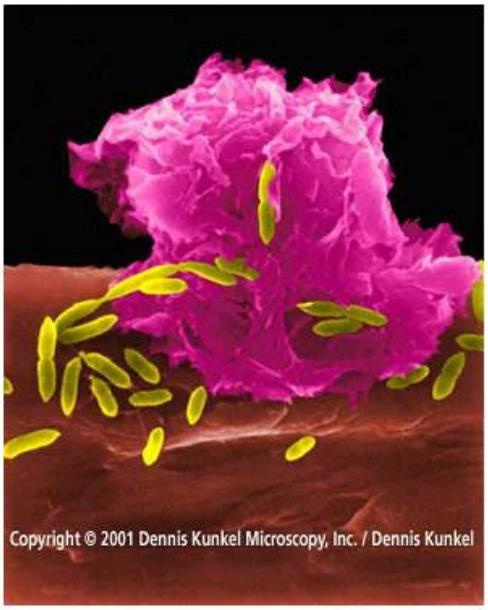
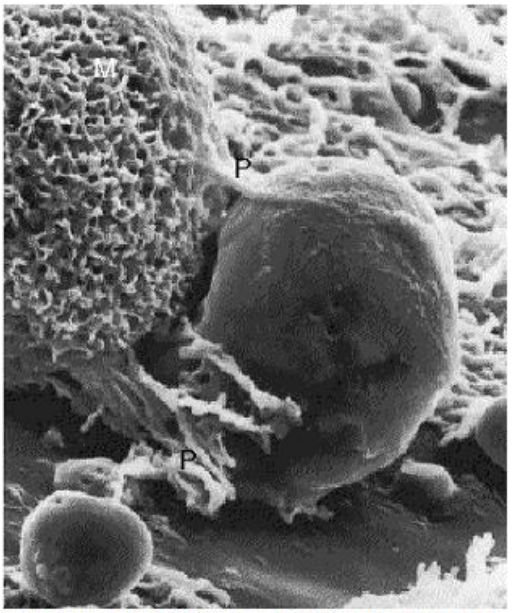


Fig. 15; A macrophage in action (1800x). In this scanning electronmicrograph, a macrophage is "fishing" with long, sticky cytoplasmic extensions. Bacterial cells that come in contact with the extensions are drawn toward the macrophage and engulfed.



Large macrophage and small cylindrical E. coli



Macrophage phagocytizing a spherical cell

Fig. 16; Phagocytosis by macrophages.

Normal circulating neutrophils do not express anywhere near their full microbicidal capacity. In order to carry out their full potential, they must be primed by one or more priming agents such as the cytokines TNF-α (tumor necrosis factor-alpha), GM-CSF (granulocyte/macrophage colony stimulating factor), and LPS (lipopolysaccharide) for example. **89,90**

These agents cause a dramatic increase in the response of these cells to an activating agent. The principle consequence of priming, aside from a direct effect on cell polarization, deformability, and integrin/selectin expression, is to permit secretagogue-induced reactive oxygen species generation (e.g. superoxide anion), degranulation and lipid mediator (e.g. leukotriene B4 and arachidonic acid) release. In addition, it is now recognized that most priming agents also serve to delay apoptosis and thus increase the functional longevity of neutrophils at the inflamed site. **89,90**

Neutrophils, however, cannot always eliminate infectious organisms, and there are numerous pathogens that they cannot recognize. The lymphocytes of the specific arm of the immune system (T cells and B cells) have evolved to provide a more versatile mean of defense in that they provide an increased level of protection to a subsequent re-infection with the same pathogen. These two arms of the immune system are not mutually exclusive however. The cells of the non-specific immune system play a crucial role in the initiation and subsequent direction of the specific immune response. **89,90**

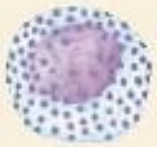
Cell Type	Function	
Helper T cell		Commander of the immune response; detects infection and sounds the alarm, initiating both T cell and B cell responses
Inducer T cell		Not involved in the immediate response to infection; mediates the maturation of other T cells in the thymus
Cytotoxic T cell		Detects and kills infected body cells; recruited by helper T cells
Suppressor T cell		Dampens the activity of T and B cells, scaling back the defense after the infection has been checked
B cell		Precursor of plasma cell; specialized to recognize specific foreign antigens
Plasma cell		Biochemical factory devoted to the production of antibodies directed against specific foreign antigens
Mast cell		Initiator of the inflammatory response, which aids the arrival of leukocytes at a site of infection; secretes histamine and is important in allergic responses
Monocyte		Precursor of macrophage
Macrophage		The body's first cellular line of defense; also serves as antigen-presenting cell to B and T cells and engulfs antibody-covered cells
Natural killer cell		Recognizes and kills infected body cells; natural killer (NK) cell detects and kills cells infected by a broad range of invaders; killer (K) cell attacks only antibody-coated cells

Fig. 17; Cells of the Immune System

Moreover, since there is a delay of four to five days before the initial specific immune response takes effect , the non-specific immune response has a critical role in controlling infections during this period. **89,90**

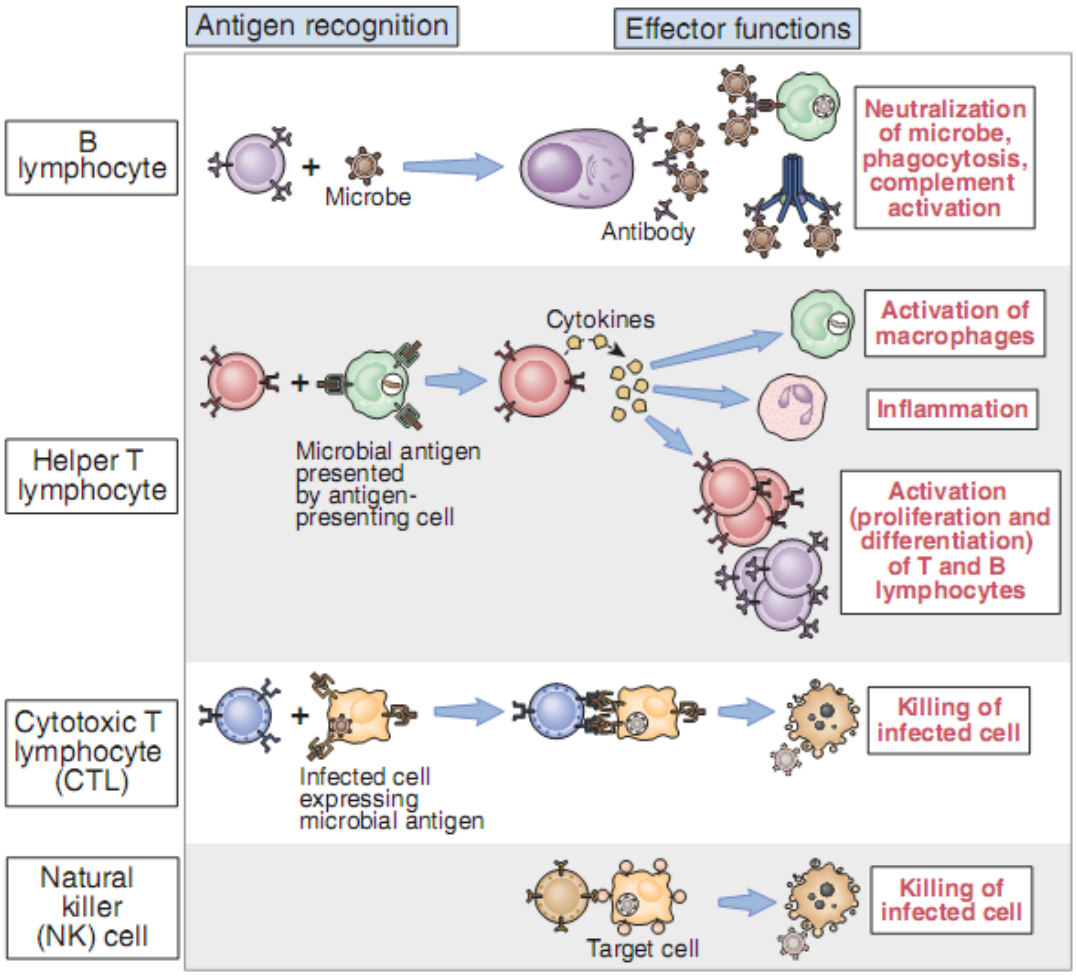


Fig. 18; Classes of lymphocytes. Different classes of lymphocytes recognize distinct types of antigens and differentiate into effector cells whose function is to eliminate the antigens. B lymphocytes recognize soluble or cell surface antigens and differentiate into antibody-secreting cells. Helper T lymphocytes recognize antigens on the surfaces of antigen-presenting cells and secrete cytokines, which stimulate different mechanisms of immunity and inflammation. Cytotoxic (cytolytic) T lymphocytes recognize antigens on infected cells and kill these cells. (Note that T lymphocytes recognize peptides that are displayed by major histocompatibility complex (MHC) molecules.) Natural killer cells recognize changes on the surface of infected cells and kill these cells. Regulatory T cells are not shown in the figure.

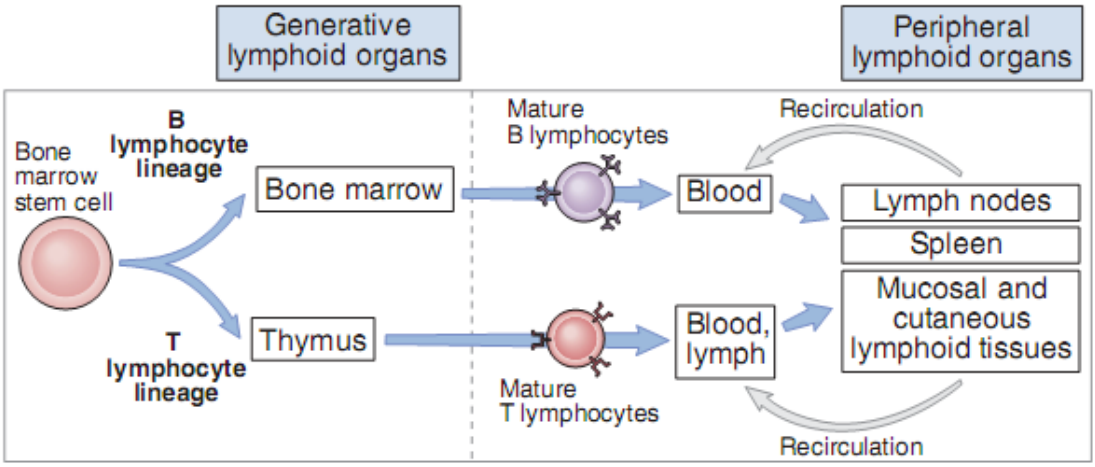


Fig. 19; Maturation of lymphocytes. Lymphocytes develop from precursors in the generative lymphoid organs (the bone marrow and thymus). Mature lymphocytes enter the peripheral lymphoid organs, where they respond to foreign antigens and from where they recirculate in the blood and lymph.

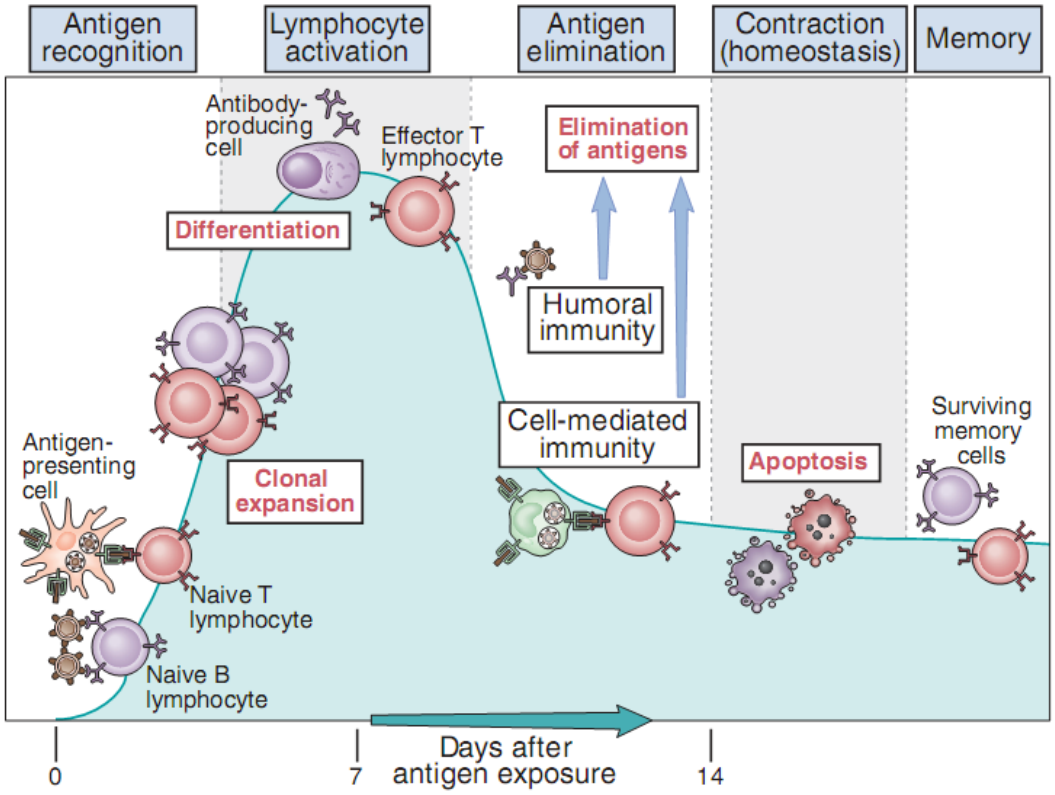


Fig. 20; Phases of an adaptive immune response. An adaptive immune response consists of distinct phases, the first three being the recognition of antigen, the activation of lymphocytes, and elimination of antigen (the effector phase). The response declines as antigen-stimulated lymphocytes die by apoptosis, restoring homeostasis, and the antigen-specific cells that

survive are responsible for memory. The duration of each phase may vary in different immune responses. The y-axis represents an arbitrary measure of the magnitude of the response. These principles apply to both humoral immunity (mediated by B lymphocytes) and cell-mediated immunity (mediated by T lymphocytes).

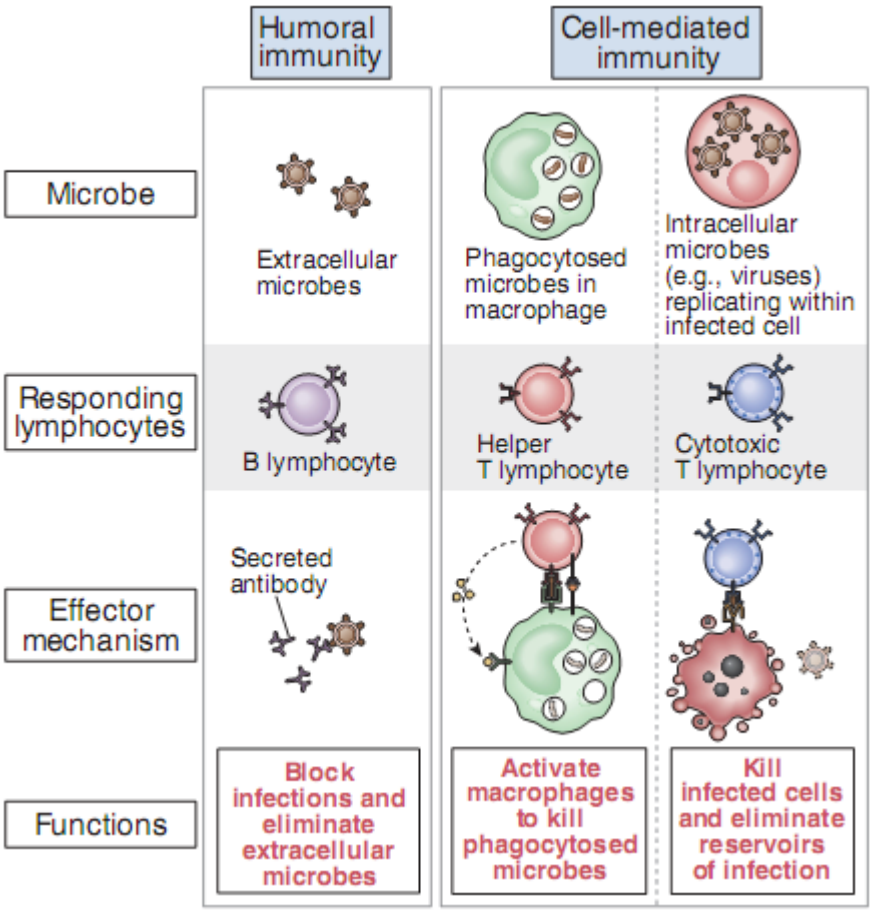
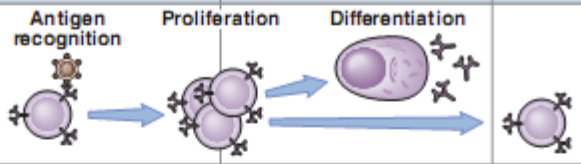
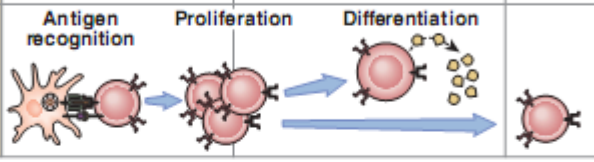


Fig. 21; Types of adaptive immunity. In humoral immunity, B lymphocytes secrete antibodies that eliminate extracellular microbes. In cell-mediated immunity, T lymphocytes either activate macrophages to destroy phagocytosed microbes or kill infected cells.

Feature	Functional significance
Specificity	Ensures that distinct antigens elicit specific responses
Diversity	Enables immune system to respond to a large variety of antigens
Memory	Leads to enhanced responses to repeated exposures to the same antigens
Clonal expansion	Increases number of antigen-specific lymphocytes to keep pace with microbes
Specialization	Generates responses that are optimal for defense against different types of microbes
Contraction and homeostasis	Allows immune system to respond to newly encountered antigens
Nonreactivity to self	Prevents injury to the host during responses to foreign antigens

Fig. 22; Properties of adaptive immune responses. The important properties of adaptive immune responses, and how each feature contributes to host defense against microbes, are summarized.

A

Cell type	Stage		
	Naive cells	Effector cells	Memory cells
B lymphocytes			
Helper T lymphocytes			

B

Property	Stage		
	Naive cells	Effector cells	Memory cells
Antigen receptor	Yes	B cells: reduced T cells: Yes	Yes
Lifespan	Weeks or months	Usually short (days)	Long (years)
Effector function	None	Yes B cells: antibody secretion Helper T cells: cytokine secretion CTLs: cell killing	None
Special characteristics			
B cells			
Affinity of Ig	Low	Variable	High (affinity maturation)
Isotype of Ig	Membrane-associated IgM, IgD	Membrane-associated and secreted IgM, IgG, IgA, IgE (class switching)	Various
T cells			
Migration	To lymph nodes	To peripheral tissues (sites of infection)	To lymph nodes and mucosal and other tissues

Fig. 23; Stages in the life history of lymphocytes. A, Naive lymphocytes recognize foreign antigens to initiate adaptive immune responses. Some of the progeny of these lymphocytes differentiate into effector cells, whose function is to eliminate antigens. The effector cells of the B lymphocyte lineage are antibody-secreting plasma cells (some of which are long-lived). The effector cells of the CD4+ T lymphocyte lineage produce cytokines. (The effector cells of the CD8+ lineage are CTLs; these are not shown.) Other progeny of the antigen-stimulated lymphocytes differentiate into long-lived memory cells. B, The important characteristics of naive, effector, and memory cells in the B and T lymphocyte lineages are summarized.

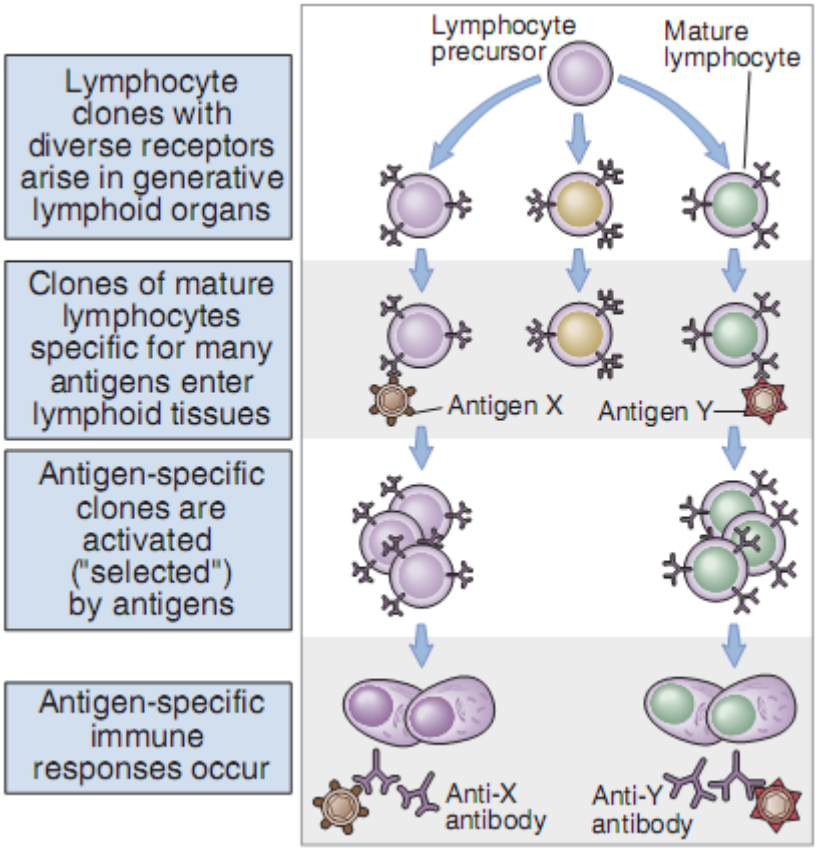


Fig. 24; Clonal selection. Mature lymphocytes with receptors for many antigens develop before encounter with these antigens. A clone refers to a population of lymphocytes with identical antigen receptors and, therefore, specificities; all these cells are presumably derived from one precursor cell. Each antigen (e.g., the examples X and Y) selects a preexisting clone of specific lymphocytes and stimulates the proliferation and differentiation of that clone. The diagram shows only B lymphocytes giving rise to antibody-secreting effector cells, but the same principle applies to T lymphocytes. The antigens shown are surface molecules of microbes, but clonal selection also is true for soluble antigens.

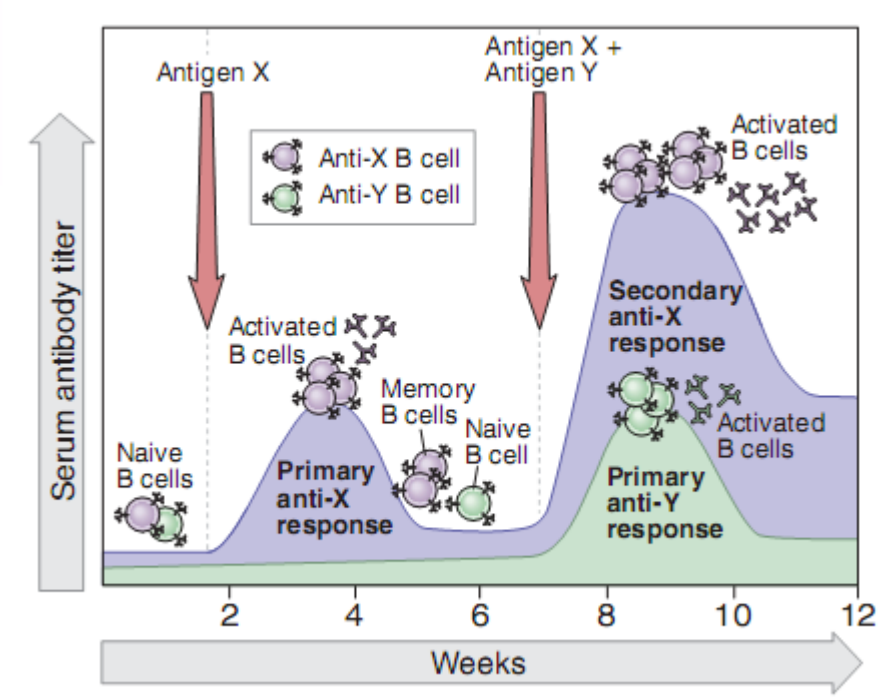


Fig. 25; Primary and secondary immune responses. Antigens X and Y induce the production of different antibodies (a reflection of specificity). The secondary response to antigen X is more rapid and larger than the primary response (illustrating memory) and is different from the primary response to antigen Y (again reflecting specificity). Antibody levels decline with time after each immunization.

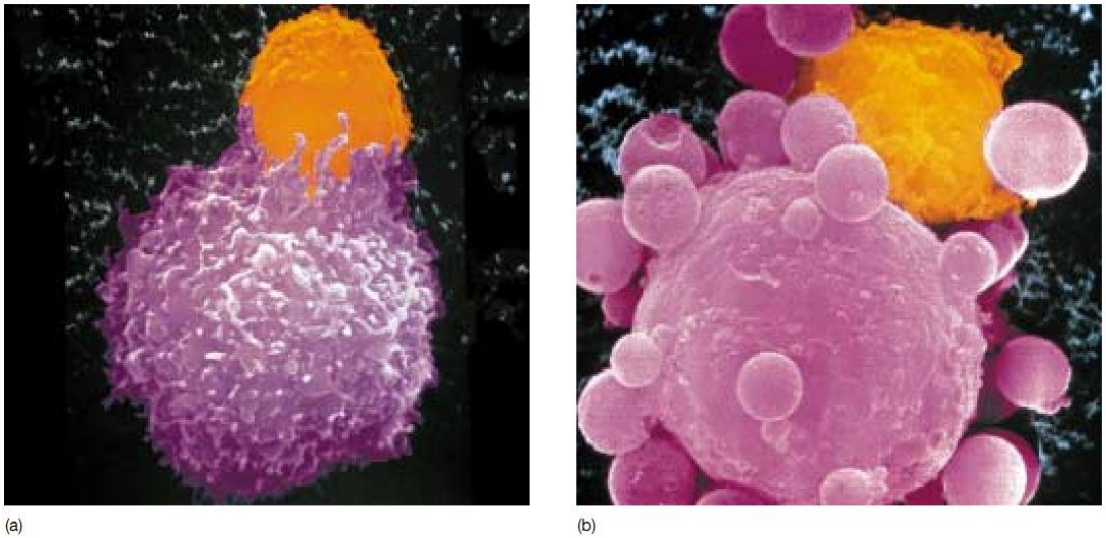


Fig. 26; Cytotoxic T cells destroy cancer cells. (a) The cytotoxic T cell (orange) comes into contact with a cancer cell (pink). (b) The T cell recognizes that the cancer cell is "nonself" and causes the destruction of the cancer.

Leukocyte Re-circulation [93,94]

The processes of both specific and non-specific immune surveillance very much depends on leukocyte access to tissue. It is clearly an advantage that leukocytes can re-circulate and enter tissues as part of a scavenging mechanism against invading pathogens and
infected or transformed cells. In order for immune cells to exit the blood and enter a new anatomical location however, they must be able to adhere to the endothelium of a blood vessel, and subsequently migrate through it. This process of adhering and migrating occurs via a very specific series of events:

(1) the cells are slowed down by forming loose adhesion (via the Selectin group of adhesion molecules) on the vessel wall, and subsequently roll along the endothelial surface,

(2) this loose contact enables further strong adhesion onto the endothelium via the integrin family of adhesion molecules, and

(3) the cells can then migrate through the endothelial barrier and underlying basement membrane.

As stated, these mechanisms are mediated by various adhesion molecules in addition to certain chemotactic factors. Circulating leukocytes are able to sense locales of cellular recruitment via such chemotactic factors (chemokines) which may be bound to the endothelium or secreted into the lumen of the blood vessel. The time frame for such changes in leukocyte trafficking to occur is relatively fast, and the monitoring of these changes can be used to evaluate the short-term changes in the immune system to various physical, chemical, and psychological stressors.

Role of chemokines in Leukocyte trafficking

Cytokine	Producing Cell	Target Cell	Function
GM-CSF	Th cells	progenitor cells	growth and differentiation of monocytes and DC
IL-1α IL-1β	monocytes macrophages B cells DC	Th cells	co-stimulation
		B cells	maturation and proliferation
		NK cells	activation
		various	inflammation, acute phase response, fever
IL-2	Th_1 cells	activated T and B cells, NK cells	growth, proliferation, activation
IL-3	Th cells NK cells	stem cells	growth and differentiation
		mast cells	growth and histamine release
IL-4	Th_2 cells	activated B cells	proliferation and differentiation IgG_1 and IgE synthesis
		macrophages	MHC Class II
		T cells	proliferation
IL-5	Th_2 cells	activated B cells	proliferation and differentiation IgA synthesis
IL-6	monocytes macrophages Th_2 cells stromal cells	activated B cells	differentiation into plasma cells
		plasma cells	antibody secretion
		stem cells	differentiation
		various	acute phase response
IL-7	marrow stroma thymus stroma	stem cells	differentiation into progenitor B and T cells
IL-8	macrophages endothelial cells	neutrophils	chemotaxis
IL-10	T h_2 cells	macrophages	cytokine production
		B cells	activation
IL-12	macrophages B cells	activated Tc cells	differentiation into CTL (with IL-2)
		NK cells	activation
IFN-α	Leukocytes	various	viral replication MHC I expression
IFN-β	fibroblasts	various	viral replication MHC I expression
IFN-γ	Th_1 cells, Tc cells, NK cells	various	Viral replication
		macrophages	MHC expression
		activated B cells	Ig class switch to IgG_{2a}
		Th_2 cells	proliferation
		macrophages	pathogen elimination
MIP-1α	macrophages	monocytes, T cells	chemotaxis
MIP-1β	Lymphocytes	monocytes, T cells	chemotaxis
TGF-β	T cells, monocytes	monocytes, macrophages	chemotaxis
		activated macrophages	IL-1 synthesis
		activated B cells	IgA synthesis
		various	proliferation
TNFα	macrophages, mast cells, NK cells	macrophages	CAM and cytokine expression
		tumor cells	cell death
TNF-β	Th_1 and Tc cells	phagocytes	phagocytosis, NO production
		tumor cells	cell death

Table 6 ; Cytokines

Many of the currently characterized chemokines mediate highly selective patterns of migration and recruitment of specific leukocytes subpopulations. [95] For example, lymphotactin is a chemokine that elicits a migratory response in natural killer (NK) cells and T cells, while having no effect on monocytes and neutrophils.[96] Of interest for some of the data presented in this essay are the chemokines involved in recruitment of NK cells into tissues: In addition to fractaline,[97] seven out of eight C-C chemokines studied induced chemotaxis of NK cells.[98]

Role of the circadian rhythm in leukocyte trafficking

The time of day also appears to play a role in the re-circulation pattern of leukocytes. That is, re-circulation patterns follow a circadian rhythm. In one study, a clear circadian rhythm was seen for T cell subsets, but not for NK cells,[99] where as another study reported a clear increase in NK cell numbers and activity in the morning.[100] It has been hypothesized that these phenomena exist as a result of daily neuro-endocrine signals. For example, it is thought that the increased levels of cortisol, which occur at the beginning of the day, interfere with interleukin-2 production and enhances the migration of lymphocytes from the blood into tissues. In fact, other mechanisms that are known to increase endogenous cortisol levels (stress, exercise), [101-103] as well as injection of exogenous hydrocortisone[104] have similar effects on lymphocyte migration.

Role of catecholamines in leukocyte trafficking

Another factor that modulates traficking patterns of leukocytes (especially NK cells) is catecholamines such as epinephrine. Epinephrine has a negative effect on the adhesion of NK cells to the vessel wall, and causes the NK cells to detach.[105] These changes in NK cell trafficking are not accompanied by changes in adhesion molecule expression, and the resulting accumulation of NK cells in the blood was identical in normal and splenectomized donors, indicating that the spleen was not the reservoir of NK cells.[106]

Role of the CNS in leukocyte trafficking

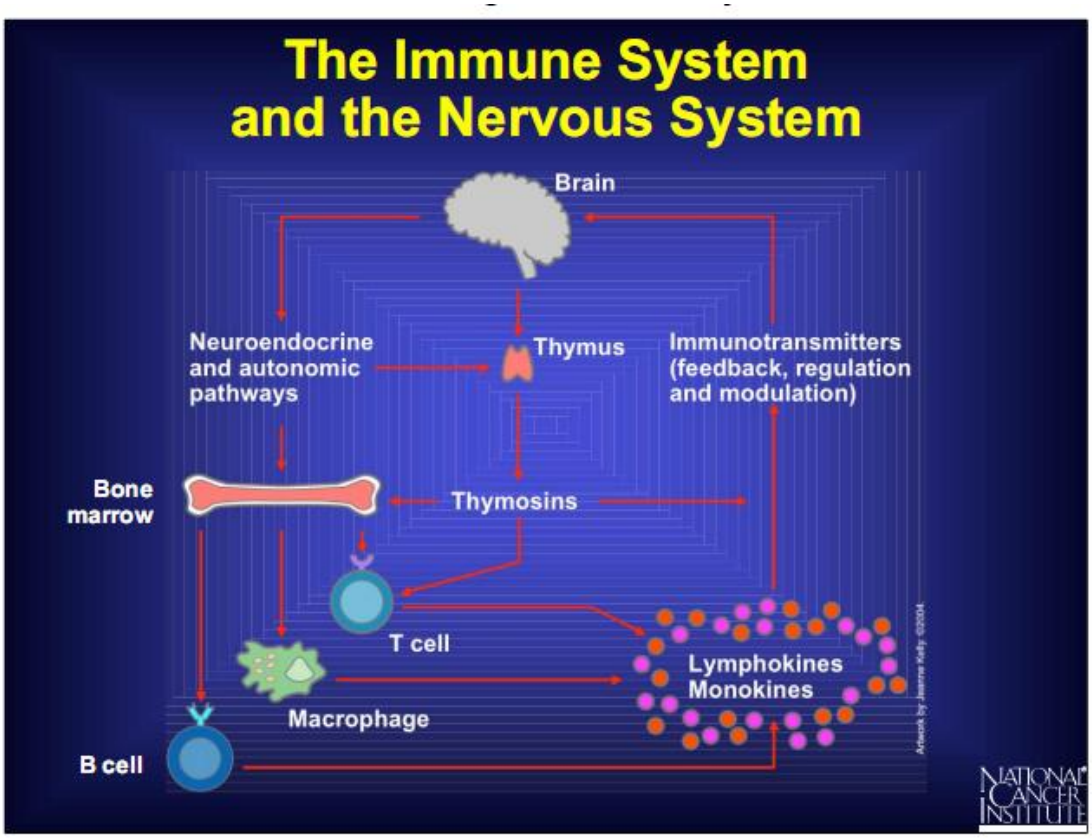


Fig. 27; The nervous system and the immune system.

The effects of catecholamines on leukocyte trafficking bring the possibility of central nervous system (CNS) control of the immune system into clearer focus. In combination with the knowledge that many nerve factors are able to function as chemokines and that some leukocytes express receptors for neurotransmitter molecules, it is easier to rationalize studies that have reported stress-mediated changes in the numbers of circulating leukocytes.[107,108]

One study in particular showed that when signaling from the sympathetic nervous system of mice was interrupted prior to injection of NK-sensitive tumor cells, the numbers of metastases were significantly increased. [109]As the NK activity was not altered, nor was the ability to respond to tumor antigens, one possible explanation is that the sympathetic nervous system regulates NK cell trafficking, thereby regulating the ability of NK cells to migrate to the vicinity of tumors. This was partially confirmed by demonstrating that the sympathetic nervous system modulates lymphocyte recruitment into lymph nodes.[110]

The relationship between the CNS and the immune system also extends to the lymphoid tissue proper. In addition to the well-characterized CNS regulation of the adrenal glands, nerve terminals invade all lymphoid tissue, and synapse-Like formations can be seen between nerve endings and leukocytes in bone marrow, lymph nodes, and spleen.[111] It is now known that there is a neuronal control of hematopoiesis, and that a complex feedback system exists involving multiple cytokines and neurotransmitters. [112,113] Neuropepetide Y is an example of a neurotransmitter that is directly able to up-regulate adhesion molecules on human endothelial cells. [114]

Self or nonself antigens:[90]

At the heart of the immune response is the ability to distinguish between "self" and "non-self". Every cell in our body carries the same set of distinctive surface proteins that distinguish you as "self". Normally our immune cells do not attack our own body tissues, which all carry the same pattern of self-markers; rather, our immune system coexists peaceably with our other body cells in a state known as self-tolerance.

This set of unique markers on human cells is called the major histocompatibility complex (MHC) proteins. There are two classes: MHC Class I proteins, which are on all cells, and MHC Class II proteins, which are only on certain specialized cells.

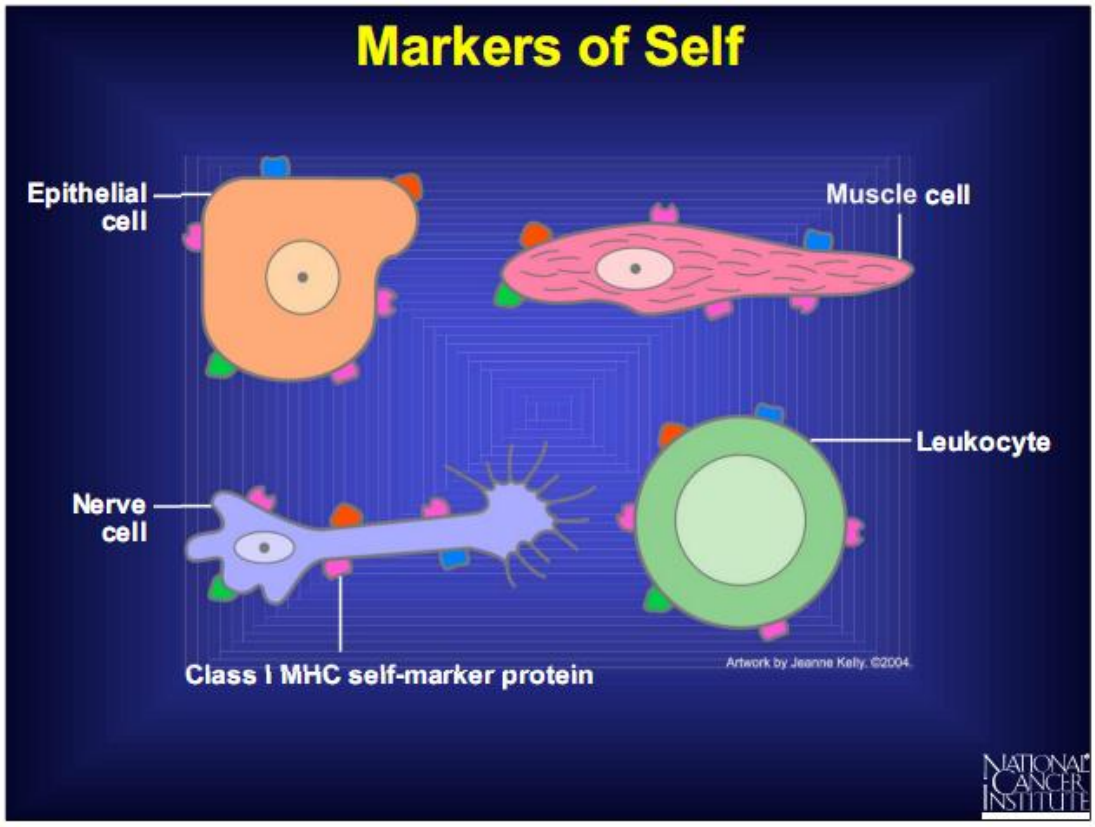


Fig. 28; Markers of self antigens.

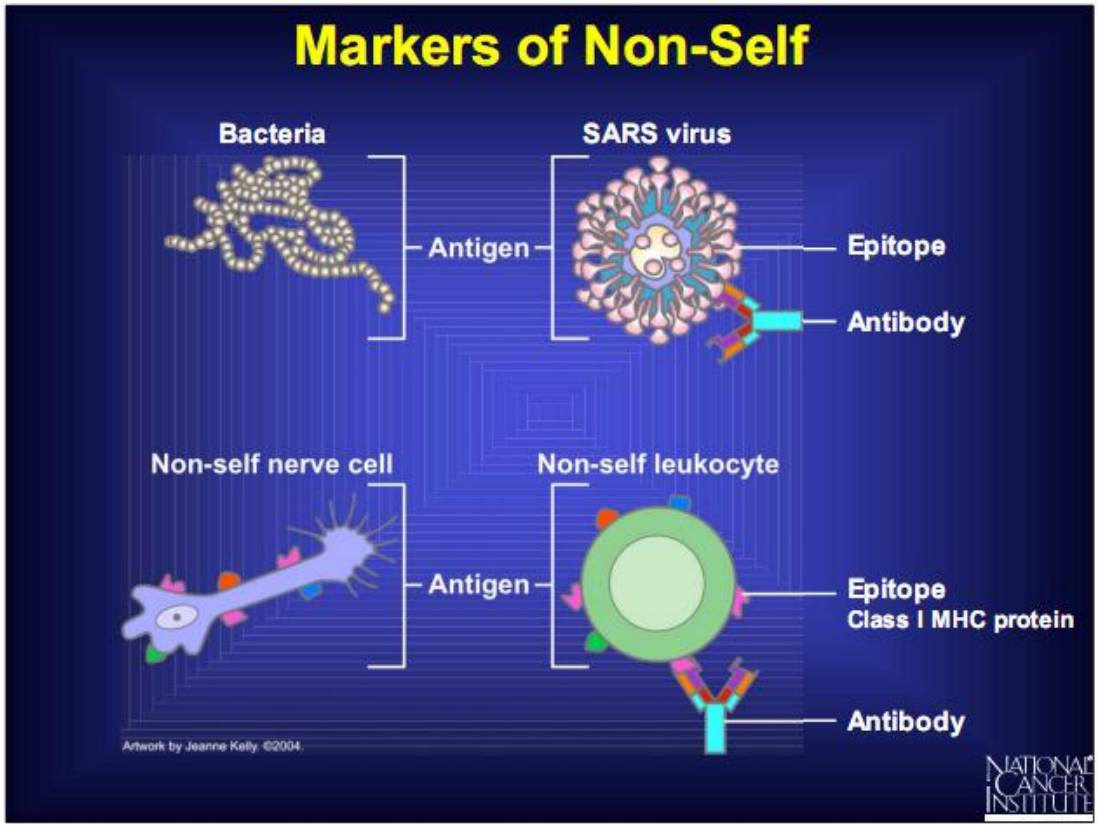


Fig. 29; Markers of nonself antigens

Any non-self substance capable of triggering an immune response is known as an antigen. An antigen can be a whole non-self cell, a bacterium, a virus, an MHC marker protein or even a portion of a protein from a foreign organism.

The distinctive markers on antigens that trigger an immune response are called epitopes. When tissues or cells from another individual enter your body carrying such antigenic non-self epitopes, your immune cells react. This explains why transplanted tissues may be rejected as foreign and why antibodies will bind to them.

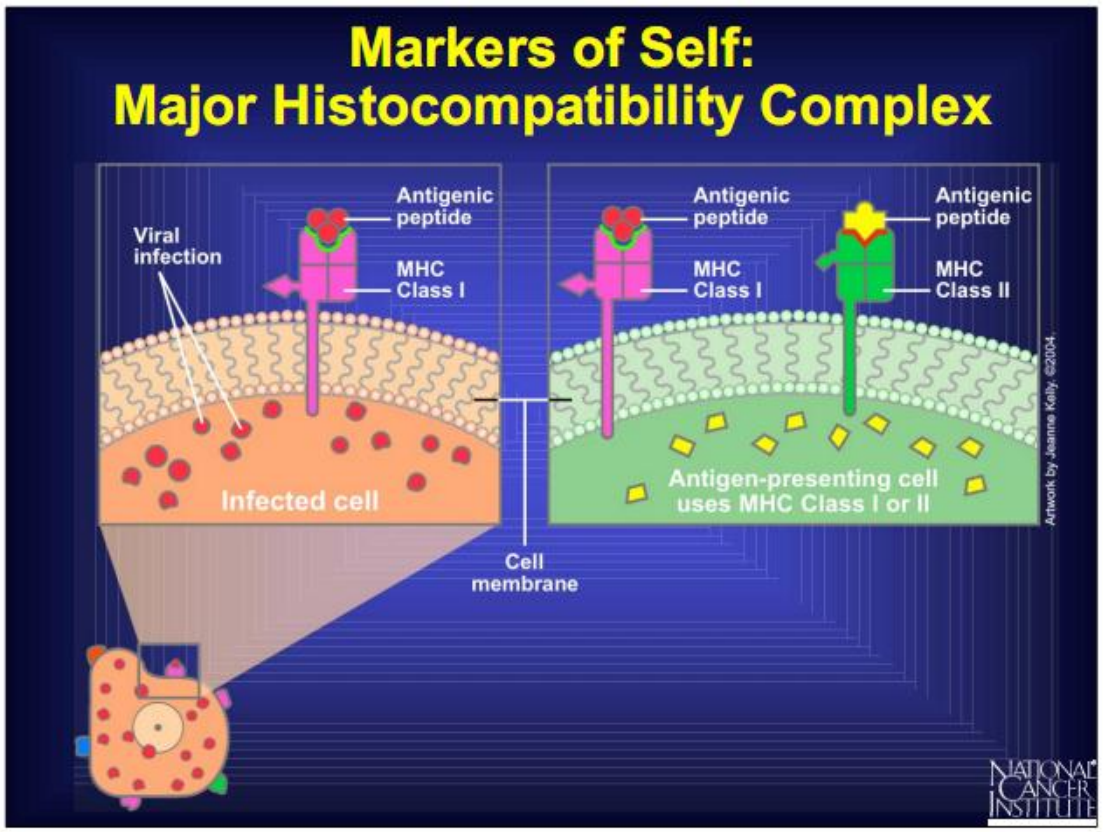


Fig. 30; Markers of self MHC

Our immune cells recognize major histocompatibility complex proteins (MHC) when they distinguish between self and non-self. An MHC protein serves as a recognizable scaffold that presents pieces (peptides) of a foreign protein (antigenic) to immune cells.

An empty "foreign" MHC scaffold itself can act as an antigen when donor organs or cells are introduced into a patient's body. These MHC self-marker scaffolds are also known as a patient's "tissue type" or as human leukocyte antigens (HLA) when a patient's white blood cells are being characterized.

For example, when the immune system of a patient receiving a kidney transplant detects a non-self "tissue type," the patient's body may rally its own immune cells to attack.

Every cell in our body is covered with these MHC self-marker proteins, and-except for identical twins-individuals carry different sets. MHC marker proteins are as distinct as blood types and come in two categories-MHC Class I: humans bear 6 markers out of 200 possible variations; and MHC Class II: humans display 8 out of about 230 possibilities.

Immune system and reactive oxygen species[115]

As stated before the immune system is comprised of innate (natural) and acquired (adaptive) immunity. Acquired immunity is composed of lymphocytes; these are highly active cells that constantly generate reactive oxidative products(ROS) as a part of their normal cellular activity. Oxidizing pollutants and many viruses also can induce ROS production by normal cells. The ROS are highly reactive and can destroy cellular membranes, cellular proteins and nucleic acids. One mechanism by which the innate branch of the immune system protects the animal is by phagocytizing and subsequently killing antigens through an oxidative bactericidal mechanism termed respiratory burst.

Phagocytosis of a foreign particle by a macrophage or neutrophil activates NADP oxidase, resulting in the production of a large amount of superoxide anion ($O2^-$) from molecular oxygen. The O2 is then rapidly converted to hydrogen peroxide (H2O2) by superoxide dismutase. Neutrophils contain myeloperoxidase that converts H2O2 to the highly potent bactericidal component, hypochloride ions(OCl^-).

Macrophages do not possess myeloperoxidase and, instead, depend on a myeloperoxidase-independent mechanism to generate other biological oxygen-derived free radicals. The latter include the generation of the hydroxyl radical (OH) through Fenton and/or Haber-Weiss chemistry. Even though the ROS are produced as part of the killing mechanism, nevertheless excessive phagocytic activity can lead to ROS-induced tissue damage.

As a defense mechanism, the body produces a number of endogenous antioxidants capable of scavenging these harmful ROS to maintain an optimal oxidant/ antioxidant balance, thereby maintaining normal cellular function and health. However, under conditions of high oxidative stress, the ability of these antioxidants to eliminate ROS are often exceeded and, therefore, dietary sources of antioxidants or drugs are required. The most widely used dietary antioxidants include vitamin E, vitamin C, carotenoids, flavanoids, zinc and selenium. The use of N-acetyl-L-cysteine as an antioxidant drug has gained popularity in recent years due to its ability to inhibit pro-inflammatory molecules and HIV replication.

The harmful effects of ROS are not unique to immune cells but affect all cell types. However, immune cells are particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of PUFA and they generally produce more ROS.[116]

Studies on the role of carotenoids on immune response have generally used several key immune function assays. These include:

(i) Immunoglobulin (Ig) production: Production of Ig has traditionally been used to assess B cell function in a humoral immune response. B cells produce Ig that circulate freely to protect the body against foreign materials. The Ig serve to neutralize toxins, immobilize certain microorganisms, neutralize viral activity, agglutinate microorganisms or antigen particles and precipitate soluable antigens. B cell function requires the help of helper (Th) cells.

(ii) Lymphoblastogenesis: Antigen-stimulated lymphocyte proliferation normally occurs in lymphoid tissues. However, the ability of isolated lymphoid cells to proliferate when cultured in the presence of certain mitogens has given researchers an important tool to assess both T and B cell function in vitro. Commonly used mitogens include concanavalin A that stimulates T cells, lipopolysaccharide that stimulates B cells, and pokeweed mitogen that stimulates T and B cells. This in vitro immune response correlates well with that observed in vivo. [117]

(iii) Lymphocyte cytotoxic activity: NK cells are a critical component of innate resistance against viruses, bacteria, fungi and parasites. They regulate the adaptive immune system and hematopoiesis, and serve as an immuno-surveillance system against tumors. The T cell cytotoxicity assay determines the efficiency of killing of target cells (e.g., cancer cells) by effector cells (lymphocytes) when cultured in vitro.

(iv) Cytokine production: Cytokines are soluble molecules that mediate cell-to-cell interactions. Cytokines commonly measured include IL-2, TNFα and IFNγ produced by the CD4 Th1cell subset, and IL-4, IL-5, IL-6 andIL-10 produced by the

Th2 subset. The Th1 cells mediate cytotoxic and local inflammatory reactions, and therefore play important roles in combating intracellular pathogens including viruses, bacteria and parasites. The Th2 cells are more effective in humoral immunity, i.e., they stimulate B cells to proliferate and produce antibodies against free-living microorganisms. Therefore, a normal immune response will require a balance between the Th1 and Th2 subsets.

(v) Delayed type hypersensitivity (DTH): This is a cellular reaction involving T cells and macrophages without involving an antibody component. Antigen-presenting cells (e.g., dendritic cells) present the antigen or allergen to T cells that become activated and release lymphokines. These lymphokines activate macrophages and cause them to become voracious killers of the foreign invaders. The DTH response is simple to conduct. More importantly, it is a good indicator of invivo cell-mediated immune response and is a predictor of morbidity and mortality in elderly human.[118]

(vi) Modern advances in flow cytometry technics have enabled researchers to phenotype blood lymphocyte subsets by identifying cell surface molecules. Identifying the population shifts for a given cell subset will provide additional supporting evidence for the immune responses assessed using the functional assays described earlier. Also, flow cytometry can be used to identify cell activation through the induction of certain surface markers. Availability of reagents has further extended the application of flow cytometry to include apoptosis, cell cycle progression and cell signaling.

(vii) Last, but not least, molecular bioscience technics have provided exciting new research tools for studying mechanism of action of carotenoids in regulating intracellular events.

Carotenoids, ROS and immunity

The traditional concept of ROS function is that they indiscriminately destroy cell components. However, exciting research has more recently elucidated the role of these reactive species in signal transduction, gene regulation, and disease etiology. This has infused new excitement and challenges into research on the possible role of carotenoids as antioxidants in disease prevention. This discussion will attempt to address the complex interaction of carotenoids and the immune response, and how this interaction may relate to cancer etiology.

Early studies demonstrated that dietary β-carotene prevented bladder, kidney, ear and gut infection in vitamin A-deficient rats[125] and reduced ear infection in young children. [120] Because of the provitamin A activity of β-carotene, these studies raised the possibility that the action of the carotenoid is due to its prior conversion to vitamin A. To circumvent this problem, the specific role of carotenoids can be demonstrated either by using carotenoids without provitamin A activity (e.g., lutein, lycopene, canthaxanthin, astaxanthin) or by using animals that cannot convert or are poor converters of carotenoids to vitaminA (e.g.,cats). Numerous studies using non provitamin A carotenoids and, more recently, using cats as the animal model have demonstrated the immuno-modulatory action of dietary carotenoids. It is recognized that cats can convert carotenoids to vitamin A, albeit very inefficiently.

Many earlier studies focused on β-carotene. [121] **Seifter et.al**. [122] reported a marked stimulatory action of β–carotene on the growth of the thymus gland and a large increase in the number of thymic small lymphocytes. The stimulatory activity of β–carotene on lymphocyte blastogenesis has similarly been demonstrated in rats[123], pigs[124, and] cattle[125]. Increased numbers of Th and T inducer lymphocytes have been reported in human adults given oral –carotene supplementation. [126,127] The number of lymphoid cells with surface markers for NK cells and for IL-2 and transferring receptors also was increased substantially in peripheral blood mononuclear cells (PBMC) from individuals supplemented with β-carotene. [127,128] Enhanced NK cell cytotoxicity was observed in human subjects given oral β-carotene. [129]

Similarly, long-term β-carotene supplementation to elderly but not middle-age men increased NK cell activity.[130] In vitro, β-carotene induced hamster macrophages to produce TNF.[131] Activation of TNFα by ROS increases the dissociation of IκB from NFκB and the subsequent translocation of this transcription factor to the nucleus, resulting in the production of cytokines, chemokines, cell adhesion molecules, and acute phase proteins; this activation also produces an anti-apoptotic effect.

Alternatively, intracellular ROS may directly increase NFκB. Therefore, ROS are important in primary immune response; conversely, antioxidants can produce the opposite effect. In fact, **Verhasselt et.al**.[132] reported that the antioxidanat molecule N-acetyl-L-cysteine can inhibit NFκB, and consequently down-regulate the production of cytokines (IL-6,IL-8,IL-12,andTNF), as well as down-regulate the expression of surface

molecules (HLA-DR, B7–2 and CD40) in human dendritic cells. Therefore, an antioxidant may impair the generation of primary immune responses through its inhibitory action on dendritic cells. While this scenario occurs in a normal cell, under conditions of high oxidative stress, excess ROS may be produced, resulting in the inhibition of NFκB Excess ROS is known to cause abnormal cell proliferation and to decrease apoptosis; both are undesirable responses in tumor cells. Therefore, antioxidants are desirable under conditions of high oxidative stress. Analogous to this situation, high concentrations of intracellular nitric oxide induced oxidative killing of isolated rat hepatocytes while low nitric oxide concentrations was protective. [133]

Besides cell-mediated and humoral immune responses, β-carotene has been shown to regulate non specific cellular host defense. Blood neutrophils isolated from cattle fed β –carotene had higher killing ability during the peripartum period. [134] The increased bacterial killing could be accounted for partly by increased myeloperoxidase activity in the neutrophils. **Tjoelker et.al**.[135] reported that dietary β –carotene stimulated phagocytic and bacterial killing ability of neutrophils from dairy cows during the stressful drying off period. In contrast, retinol and retinoic acid generally decreased phagocytosis and had no effect on killing activity.

A specific role of carotenoids on immune response was first reported by **Bendich and Shapiro**.[123] They showed that rats fed canthaxanthin, a carotenoid with no provitamin A activity, had a heightened mitogen-induced lymphocyte proliferation; dietary β –carotene showed similar action. Subsequent studies have similarly reported the immuno-enhancing action of carotenoids without provitamin A activity, notably lutein, lycopene, astaxanthin and canthaxanthin.

Canthaxanthin enhanced the expression of activation markers for Th and NK cells in human PBMC invitro.[136] **Jyonouchi et.al**.[137] reported that lutein and astaxanthin increased the exvivo antibody response of mouse splenocytes to T-cell antigens. **Schwartz et.al**.[138] reported increased cytochrome oxidase and peroxidase activities in macrophages incubated with canthaxanthin, β -carotene, and α-carotene compared with incubation with 13-cis retinoic acid. The stimulatory activity of canthaxanthin was greater than that observed with β –carotene and α -carotene. Phagocytosis also was stimulated by these carotenoids, even though to a lower degree. All of these changes indicate increased respiratory bursts by the macrophages when they are exposed to carotenoids.

The domestic dog and cat have recently been used in parallel studies using similar experimental designs to compare the immuno-modulatory role of carotenoids. These studies thus provide direct comparisons between carotenoids with (β -carotene) or without (lutein) provitamin A activity ,and also between species that can (dogs) or that are very inefficient converters (cats) of β –carotene to vitaminA. Dietary β -carotene[139] and lutein[140] stimulated DTH response, the number of CD4 Th cells, and IgG production in dogs, thus demonstrating that lutein, a carotenoid without provitamin A activity, exerts a similar immuno-modulating action as -carotene. In contrast, lutein but not β –carotene enhanced mitogen-induced lymphocyte proliferation in dogs, indicating species differences in the lymphocyte proliferation response to a given dietary carotenoids. Cats fed β –carotene (unpublished data, Park et al.) or lutein[141] also showed heightened DTH response, higher Th and B cell subpopulations, and increased plasma IgG concentrations. It can be concluded that the actions of both β –carotene and lutein in cats are not

due to their prior conversion to vitamin A because cats are poor converters of β –carotene to vitamin A.

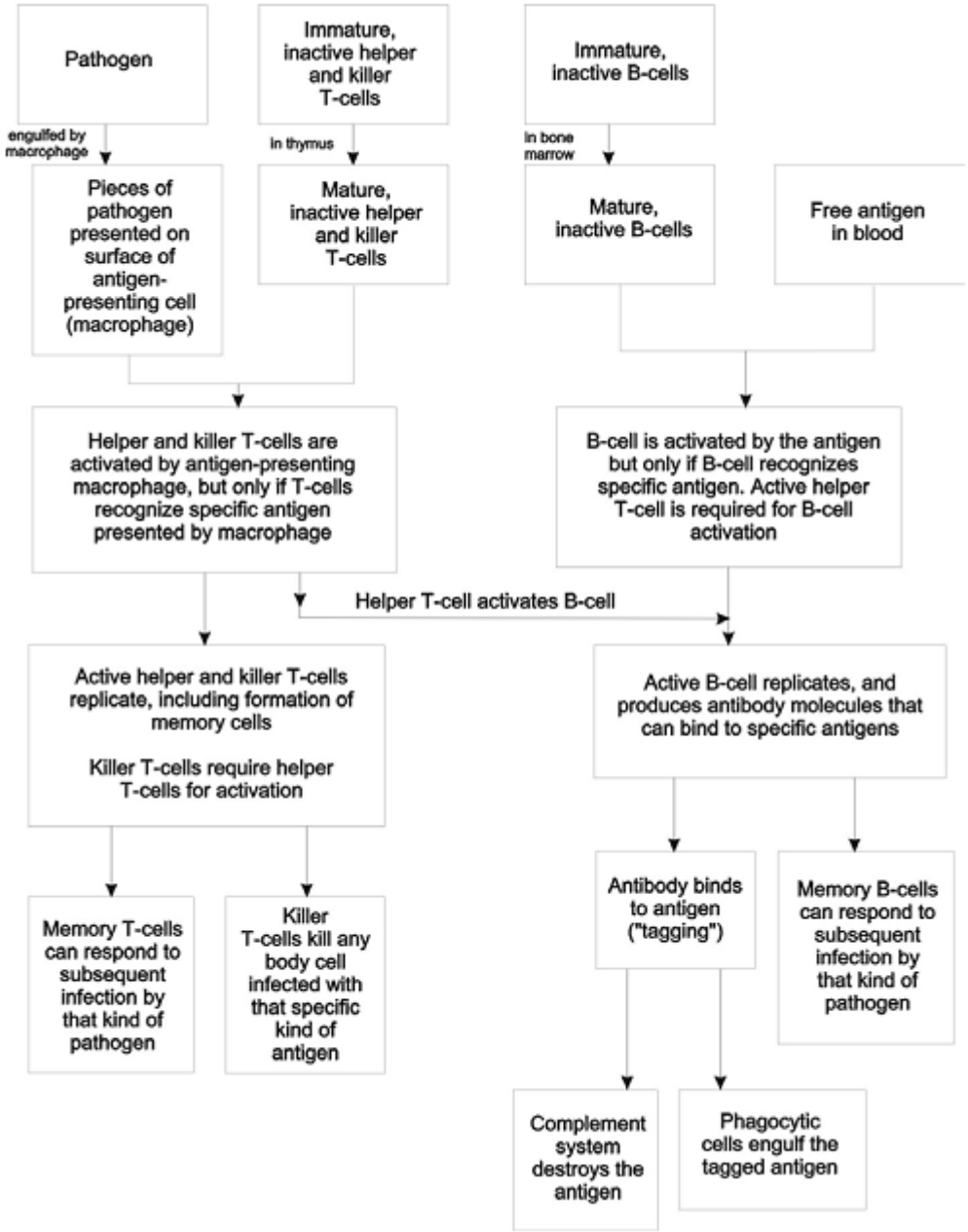


Fig. 31; Summary of the immune response.

Nutrition and the Immune System

In keeping with the concept that the functioning of the immune system comes to depend on a variety of endogenous and exogenous stimuli, it is pertinent in the discussion of the current essay to examine the influence of nutrition on the immune system. Indeed, the idea that nutrition is an important determinant of the immune response and adequate immune functioning has been generally accepted for some time. There are extensive amounts of epidemiologic and clinical data that suggest nutritional deficiencies alter immune-competence and increase the risk of infection. This concept applies not only to young children in developing countries but also to all age groups in all populations of the world, including the elderly, those with eating disorders, and patients with other primary diseases .[142]

Research into the effect of nutrition on the immune system has generally been subdivided into two components. Although not mutually exclusive, both macronutrient (e.g. protein) and micronutrient (e.g. vitamins, trace elements) deficiencies have been strongly linked to immune system dysfunction and malnutrition-associated infection.

Macronutrients: E.g. protein energy malnutrition

In addition to lymphoid organ atrophy, protein energy malnutrition impairs the majority of host-defense mechanisms, including both the specific and non-specific arms of the immune system. These findings are summarized in table No. 7.

Lymphoid Organs:
▪ Lymphoid atrophy and loss of corticomedullary differentiation
▪ Depletion in lymphoid cell numbers in spleen and lymph nodes
Non-Specific Immunity:
▪ Diminished phagocytic capacity
▪ Diminished bactericidal ability of neutrophils
▪ Decreased complement levels and opsonic activity of plasma
Specific Immunity:
▪ Depressed delayed type hypersensitivity reactions
▪ Decreased T-helper ($CD4^+$) and T-cytotoxic ($CD8^+$) cell counts
▪ Decreased $CD4^+$ to $CD8^+$ ratio
▪ Decreased B cell counts
▪ Decreased immunoglobulin secretion and affinity
▪ Diminished production of interleukins 1 and 2, and interferon gamma
▪ Diminished ability of T cells to respond to cytokines

Table 7; Effects of Protein Energy Malnutrition on the Immune System.

Micronutrients

Human malnutrition is usually a composite syndrome of nutrient deficiencies, and micronutrient deficiencies often complicate protein energy malnutrition. Nevertheless, several trace elements and vitamins on their own have an essential role in key metabolic pathways and immune cell function. Observations in animal models deprived of one dietary element and findings in patients with single nutrient deficiencies have confirmed the crucial role of several vitamins and trace elements in immunocompetence. The consequences of such deficiencies depend very much on both the subject, and the type, timing, and magnitude of the deficiency.[48]

Some of the micronutrients studied in detail in the context of the immune system include zinc, iron, vitamin A, B6, C, and E to name a few. A more detailed description of the effects of micronutrients on the

immune system can be found elsewhere.[143] Indeed, our knowledge about the nutritional demands of a well-functioning immune system has opened up exciting possibilities for nutritional intervention in both the primary and secondary prevention of infection in high-risk groups. Nutritional deficiencies are often seen in hospitalized patients, especially the elderly.[144] These individuals are particularly susceptible to developing life-threatening opportunistic infections.

Both experimental animal and clinical studies have highlighted the value of nutrient enriched diets in improving immune responses and survival following challenge of infection.[145] Similarly, modest supplementation of micronutients improve immune responses and reduce the incidence of respiratory infection and antibiotic use in the elderly. [146]

Pharmacologic and nutritional approaches to immune system modulation

The aforementioned studies introduce a concept that dietary intervention can alter susceptibility to disease and disease outcomes. This occurs not simply by repletion to correct dietary deficiencies, but by the pharmacologic effects of specific nutrients, a concept sometimes called **nutritional pharmacology**.[48]

It is clear that dietary composition can profoundly affect cell growth and function, metabolic and inflammatory responses to injury, cell-to-cell communication, the response to pharmacologic agents, and other important biological processes. Research done during the last decades in particular, has led to progress in the understanding of the influence of particular nutrients on both the specific and non-specific

arms of the immune system, ultimately leading to various therapeutic outcomes.[48]

Some of the nutrients studied in detail include proteins (both type and amount), arginine, glutamine, omega-6 and omega-3 fatty acids, short-chain fatty acids, the elements iron, zinc, and the vitamins E, C, and A.[48]

Although ubiquitous, these nutrients taken in the proper amounts and context show great promise for use in disease-specific nutritional products, and may be administered in conjunction with traditional methods of treatment. Some of the most encouraging areas in which nutritional modulation of the immune system may influence clinical outcomes include situations of burn injury, treatment of bacterial sepsis, prevention of gut-origin sepsis, prevention of post-surgical infection, autoimmune diseases, vascular disease, transplantation, and cancer.[147]

Blue-green algae and the immune system

As the connection between nutrition and the immune system becomes increasingly clear, there has been an intense interest in substances with potential immuno-modulatory effects. These are substances that can alter the manner in which the immune system can react to a challenge, be it a virus, bacterium, or cancer cell. There is now some evidence that certain species of blue-green algae may modulate the immune system.[48]

Nutritionally acquired immune deficiency syndromes (NAIDS) are found primarily in Third World, malnourished countries. Yet while "NAIDS is seen most frequently in children throughout the world, it is often seen in elderly individuals, and it is an all too common complication of severe medical or surgical diseases, malignancies, burns and other forms of trauma being treated in the most modern hospital centers. ...Children are highly susceptible to infection and each febrile infection causes them to lose vital body nutrients. Multiple, closely spaced, or severe infections are the most frequent cause of childhood malnutrition.[148]

Deficiencies in any of the large number of essential nutrients can produce dysfunctions in the immune system and other host defensive mechanisms. Single nutrients that impact importantly on protein synthesis can influence every aspect of immunity. Deficiencies of essential amino acids can also depress the synthesis of proteins, including those that contribute uniquely to host defenses. Trace [mineral] elements that function as the key component of metallo-enzymes are also known to

have some effects on the internal structure and function of lymphocytes.[149]

From a clinical point of view, important individual nutrients that support immunological functions include vitamin A, beta-carotene, zinc, iron, B-vitamins, amino acids (especially arginine and glutamine), polyunsaturated fatty acids, and nucleotides that are found abundantly in microalgae.

School records of children eating AFA blue-green algae showed a dramatic improvement in class attendance in two studies. Both research teams, along with school personnel reports, suggested that the increased attendance of students who ate blue-green algae was related to decreased sick days.[150, 151]

In a study of 100 children diagnosed with a zinc deficiency and given either zinc sulfate or blue-green algae tablets, those given blue-green algae demonstrated a superior immune response. The zinc found in blue-green algae may be about three times more effective than zinc from mineral sources.[152]

The immunological effects of zinc are many, well studied, with a variety of possible mechanisms. Zinc is required for nucleic acid metabolism and essential to over 100 metallo-enzyme activities. Also, zinc is a fundamental component of thymic hormones, helping to stimulate the activities of T-lymphocytes throughout the body and to increase antibody production.

Chemistry professor **Karl Abrams** describes how the carotenoids in AFA enhance the immune system by protecting the thymus gland and offering antioxidant protection to immune cells (e.g., white blood cells). "Beta-carotene and the other carotenoid compounds of AFA biostimulate our immune system by increasing the number, activity, circulation of antiviral thymus helper cells.[153] Once beta-carotene has been biochemically transformed into vitamin A, white blood cells may use it to increase their abilities to kill invading viruses. This vitamin A precursor is also known to increase B-cell activity, thus increasing antibody production- especially IgA- when necessary;" [154]

However, beta-carotene's immune-enhancing effects are maximized only in cases when it occurs in combination with a wider family of carotenoids, such as with microalgae.[155]

Microalgal carotenoids, such as beta-carotene, canthaxanthin, and astaxanthin have demonstrated immune-modulating activities using in vitro ("test tube") cell culture experiments.[156]

Researchers have found increased antibody production and enhanced immune function in animals supplemented with blue-green algae. [163-164]

Several studies, animal and human, have demonstrated the ability of microalgae to increase macrophage movement.[159-161] The dietary use of blue-green algae is reported to enhance the immune response in laboratory mice, by stimulating macrophage functions, phagocytosis and enhanced interleukin-1 production.[162, 163]

In a study of 463 calves, kept under the same conditions of tending and feeding, the experimental groups fed supplemental algae were the least likely to contract any disease. [164]

***Gitte Jensen, Ph.D.**, an immunologist at McGill University, working with a team of researchers at the Royal Victoria Hospital in Montreal, demonstrated improved trafficking of immune cells to be among the effects of AFA algae on the human immune system. Many immune cells (e.g., natural killer [NK] cells) do their primary work outside of the bloodstream in the tissues. AFA algae increased the number of white blood cells that moved from the bloodstream into the tissues to do their search-and-destroy mission.*[165]

In a follow-up double-blind study, Jensen's team replicated the initial results and also found that longer-term consumers of AFA demonstrated greater benefits than those taking algae for the first time. Yet even short-term consumers showed some benefits. Dr. Jensen's team found that within two hours of eating AFA there was a significant migration of natural killer cells from the blood into the surrounding tissues. Natural killer cells play a key role in our defense system as they "patrol for invading microbes and infected or transformed precancerous cells." This gentle immune boost was rapid, short-term, and cell-type specific.[160]

According to Dr. Jensen, these milder, killer-cell specific, episodic effects are preferable to a stronger, prolonged and more global immune activation. For example, it is possible to put something into the blood-stream that will provoke a hyper activation and a more global immune response. The results were recently published under the title: Effects of the Blue Green Algae Aphanizomenon Flos-Aquae on Human Natural

Killer Cells. It appears in Chapter 3.1 of the IBC Library Series, Volume 1911, Phytoceuticals: Examining the health benefit and pharmaceutical properties of natural antioxidants and phytochemicals. The results of this study are discussed in details later on in this essay.

It is also noteworthy that high doses of AFA are not required to realize these benefits. The positive immune effects were seen with low amounts of AFA algae (1.5 grams), available in food supplementation.

In a retrospective review of medical cases, researchers found positive evidence that AFA blue-green algae might be useful in the treatment of chronic fatigue, Epstein Barr infection, fibromyalgia, and AIDS. These diseases all involve significant immune system, and sometimes viral components. Such anecdotal evidence suggests that at least some autoimmune diseases may respond favorably to blue-green algae.[166]

Some species of microalgae, AFA in particular, have been shown to be rich sources of polyunsaturated fatty acids (PUFAs), which increase cell membrane fluidity and enhance immune function. Our immune system depends on optimal cell membrane fluidity. For example, when white blood cells in our immune system develop more rigid cell membranes, it becomes more difficult for them to maneuver through tissues to attack viruses, bacteria, and unhealthy cells. Additionally essential fatty acid (EFA) deficiency is known to substantially lower the number of thymus suppressor cells and thus impair overall immune function.[167]

Lipopolysaccharides and C-phycocyanin in blue-green algae have been shown to stimulate macrophage activation and stem cell

differentiation potential.[175-176] Also, cell wall fragments of lipopolysaccharide, lipid A, and glycolipoproteins are known to have immune-enhancing effects that strengthen the entire immune system.[170-171]

When blue-green algae, such as AFA, is freeze-dried and encapsulated, such complex polysaccharide compounds of the cell wall are broken down into easily absorbable fragments. AFA microalgae, like human breast milk, contain a peptide known as substance P, which also acts as an immune booster. [172]

Effects of blue-green algae on non-specific immunity

Several studies have examined the use of blue-green algae in the context of the normal functioning immune response.

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-tumour cell activity when compared to control animals.[157] The investigators in this study speculated that blue-green algae may increase NK cell activity via the production of cytokines such as interferon.

Many substances are known to stimulate NK cell activity, though the most potent are polysaccharides extracted from rice and mushrooms. In a recent study, the polysaccharide fraction of AFA was tested against polysaccharides extracted from rice, known as arabinoxylan, one of the most potent NK cell activator available. The polysaccharide from AFA

was found to be many times more potent than arabinoxylan. This research suggests that eating AFA daily may stimulate the immune system to help prevent cancer as well as illnesses associated with viral infections. The anticancer properties of AFA have already been established by its ability to prevent cancer in the Ames test.

Introduced as	Test species	Effects	References
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	
Extract	In vitro, rat	Activation of macrophages (NF-kappaB, cytokines)	**Pasco, in press**

Table 8; Immuno-Modulatory and Anti-Inflammatory Effects of AFA

Subject	Summary of results	Reference
Mouse	Dietary microalgae enhances IgM antibody production in primary immune response.	[Hayashi, 1994] [Hayashi, 1998]
Cat	Water-soluble extract of blue-green algae enhances macrophage phagocytic function in vitro.	[Qureshi, 1996]
Chicken	Dietary microalgae boosts humoral and cell-mediated immunity.	[Qureshi, 1996]
Rat	Dietary microalgae tones down allergic responses.	[Kim, 1998] [Yang, 1997]

Table 9; Summary of studies on immunologic effects of microalgae

Effects of blue-green algae on specific immunity

Hayashi et al. also examined the effect of the algae supplemented diet on the ability to mount a specific immune response to sheep red blood cells. After immunizing the 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.[162]

These findings however, have been more recently challenged by results of experiments involving chickens. Contrary to mice, no differences were 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.[157]

Hayashi et al. then examined other antibody classes such as IgA and IgE in the context of mice orally immunized with a crude shrimp extract. 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 if ingested along with or before a potential antigenic threat, blue-green algae may enhance IgA antibody levels to protect against food allergies.[163]

Along the same lines, further studies have indeed suggested that blue-green algae may inhibit mast cell-mediated type 1 allergic reactions and even the anaphylactic reaction in rats.[173,174] They showed that by

injecting blue-green algae extract intra-peritoneally (100-1000µg/g body weight) one hour prior to an allergic challenge, one could decrease mortality induced by the anaphylactic compound 48/80, inhibit local allergic reaction activated by anti-dinitrophenyl (anti-DNP) IgE, and decrease serum histamine levels. Similar in vitro experiments reported by this group revealed the same results.

In addition, analysis of the cAMP (cyclic adenosine mono-phosphate) levels of rat peritoneal mast cells (RPMC) exposed to blue-green algae extract in vitro showed a transient 70-fold increase of cAMP at 10 seconds compared with that of controls. Moreover, the extract had a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor-alpha (TNF-α) production in RPMC.

Thus, data exist to suggest that blue-green algae contain substances that can alter the way in which the immune system functions both in vitro and in vivo in animal models. Nonetheless, the exact identities of these substances and the mechanisms by which they generate their effect remain unknown.

A novel approach to nutritional mobilization of the immune system **Gitte S. Jensen, et.al**.,[160] (as mentioned before) examined the short-term effects of consumption of a moderate amount (1.5 grams) of the blue green algae Aphanizomenon flos-aquae (AFA), on the immune system.; Twenty-one non-hospitalized volunteers were analyzed in a double-blinded cross-over fashion.

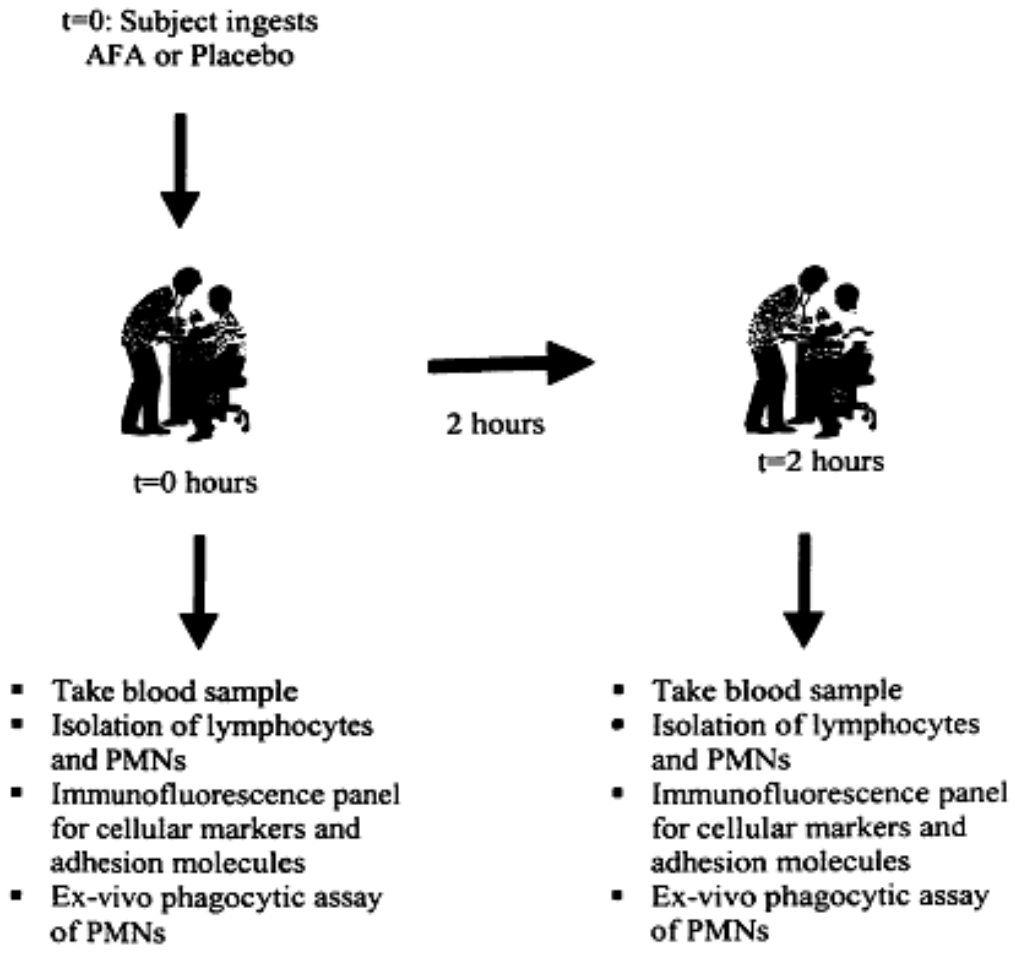


Fig. 32; Depiction of study design. Results from the two time points were compared. Changes, if any, were examined for statistical significance across all 21 subjects.

The volunteers had no known acute or chronic infections. Five were long-term AFA consumers, 2 were occasional AFA consumers, and the remaining 14 had never before consumed AFA. Eleven volunteers were male, and ten were female. The age range was 20-52 years. Each volunteer was studied on two separate days. Any volunteer was always studied at the same time on the two study days, to eliminate the circadian influence on the data. The volunteers were asked to consume the same breakfasts at the same times on the two study days, and not to consume any other vitamin preparations or nutraceuticals for at least 12 hours before the study. The volunteers were required to sit quiet for 45 minutes

prior to study start, so that any prior walking or other exercise did not affect the relative proportions of leukocytes. The first blood sample was taken, and the substance was given. Until the sampling of the second blood sample 2 hours later, the volunteer was required to remain quiet and avoid any extensive walking.[48]

The objective of that project was to study the role of blue-green algae in enhancing/modulating the immune system via the gastro-intestinal route. As a model they have used AFA, a species of microalgae researched extensively in terms of nutritional value, but relatively little in terms of potential therapeutic benefit.

A preliminary study from their own lab has shown that the ingestion of AFA has an acute effect on the circulation of NK cells.165

The current study consisted of examining several additional aspects of immune cell migration and function. These aspects include absolute and proportional changes in cell counts, changes in adhesion molecule expression, and alterations in the phagocytic potential of neutrophils. These changes were measured within two hours of the ingestion of 1.5g of AFA, the dose recommended for food supplementation. Analyses were performed after a time of two hours on the basis of the findings of a preliminary study. The data presented here show a moderate yet consistent effect on several aspects of the immune system. This effect may be explained either by the direct action of AFA on leukocytes and or the lymphoid organs, or by an indirect mechanism possibly involving the central nervous system. The following figures from 33 to 39 can summarize the results of that study. [48]

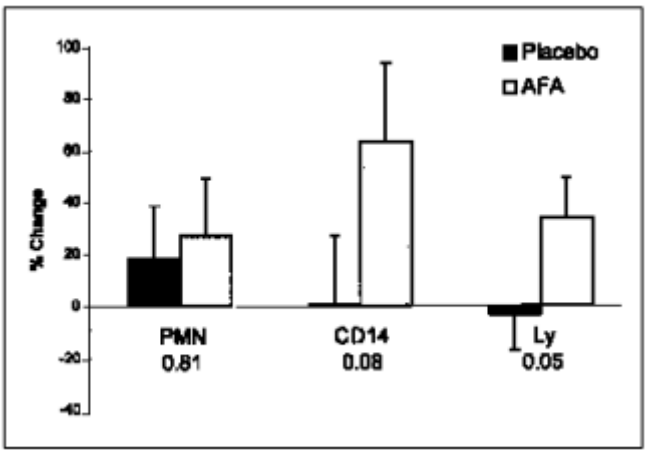


Fig. 33a; AFA-induced changes in blood leukocyte populations. The histogram shows the % change of polymorph nucleated cells (PMN), monocytes (CD14), and lymphocytes (Ly). Black columns represent the mean values of placebo, and the white columns represent the mean values of AFA. The bars indicate the standard error of the mean.

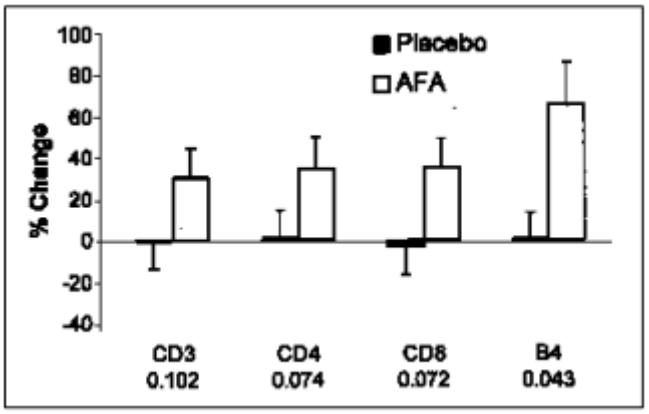


Fig. 33b; AFA-induced changes in lymphocyte sub-populations. The histogram shows the % change of total Tcells (CD3), T cell subsets (CD4, CD8), and B cell (CD19) lymphocyte populations. Black columns represent the mean values of placebo, and the white columns represent the mean values of AFA. The bars indicate the standard error of the mean.

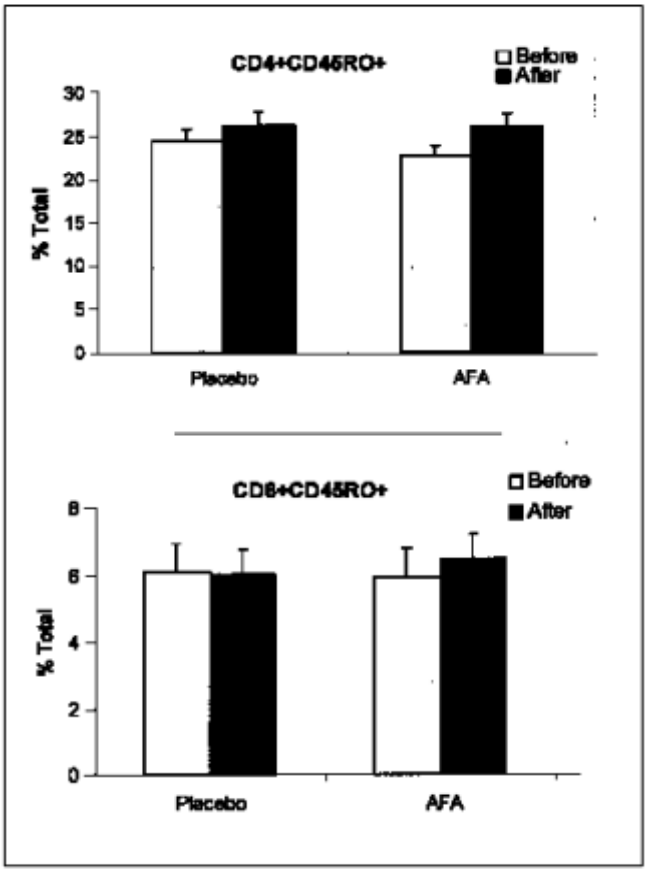


Fig. 34; The relative changes in subpopulations of Tcells is shown (mean and SEM for 21 volunteers). The helper (CD4+) T cell and cytotoxic (CD8+) Tcell populations only showed a slight shift towards less naive and more activated/memory Tcells in the circulation, and no statistical significance was reached.

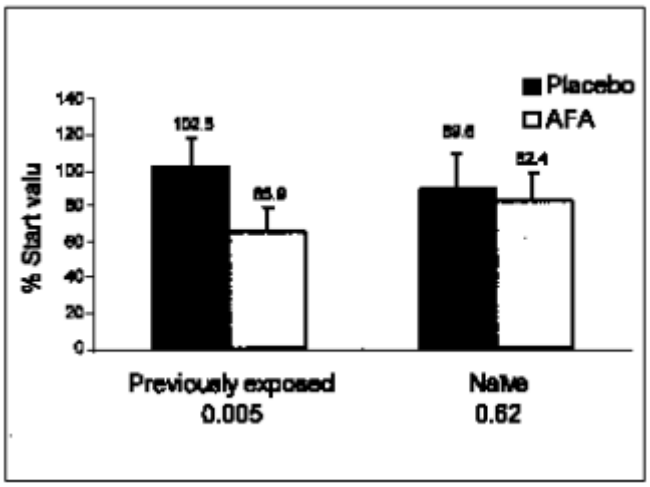


Fig. 35; Changes in natural killer cells (NK cells) in % of the starting value. Black columns represent the mean values of placebo, and the white columns represent the mean values of AFA. The bars indicate the standard error of the mean.

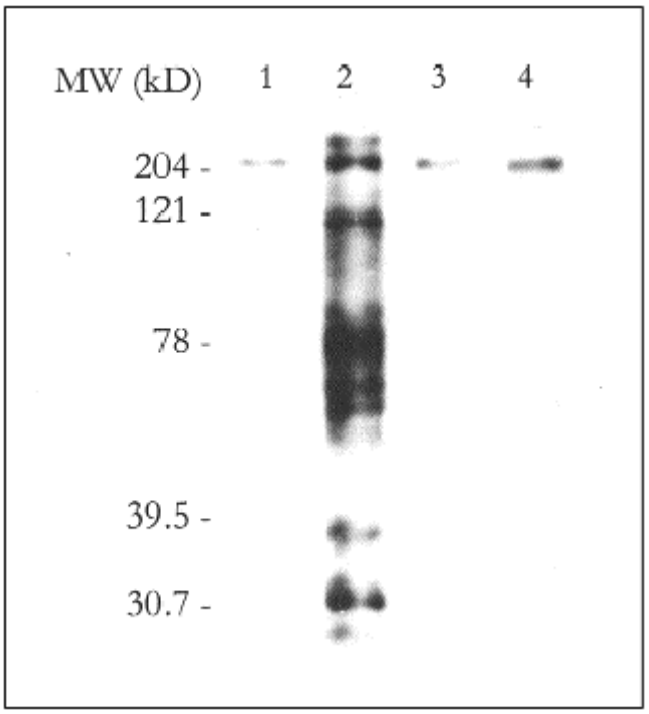


Fig. 36; Western blotting of tyrosine phosphorylation of proteins extracted from unstimulated lymphocytes (lane 1) versus lymphocytes incubated with Pokeweed Mitogen (PWM, positive control, lane 2) or AFA(lane 3: extract 1:5, lane 4: extract 1:25). Incubation of freshly purified human lymphocytes with AFA extract did not induce tyrosine phosphorylation. The data are representative of 4 similar experiments.

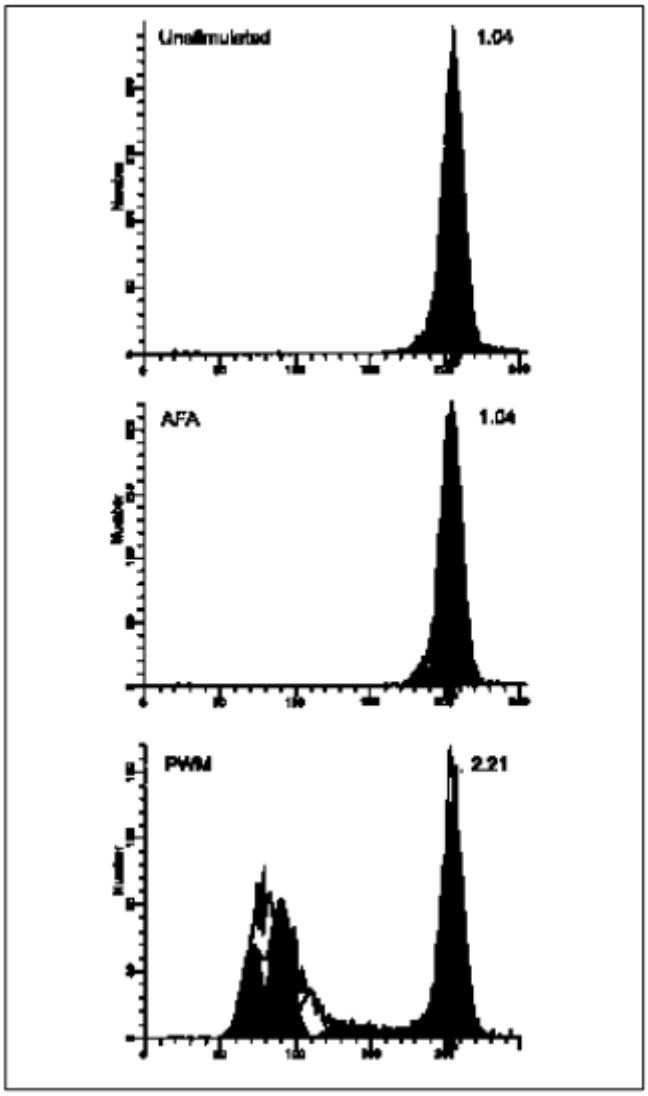


Fig. 37; Flow cytometric evaluation of lymphocyte proliferation after 5 days of culture with no stimulation (top), with AFAwater extract (middle), and Pokeweed Mitogen (PWM, bottom). The X axis displays fluorescence intensity, where loss of fluorescence corresponds to proliferative activity. The proliferative indexes for each culture condition is displayed in upper right corner of each histogram. The experiment was conducted three times, where all cultures were performed in triplicate.

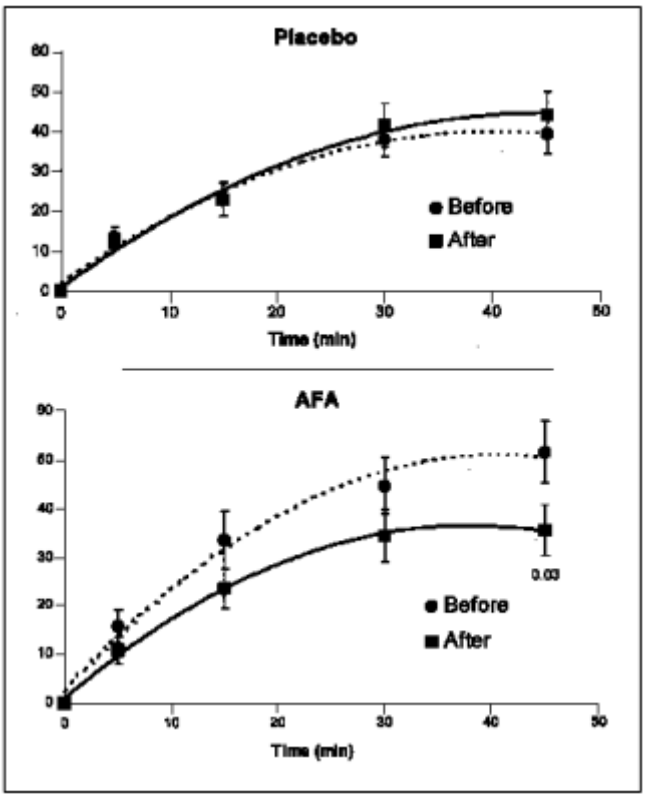


Fig. 38; Phagocytic activity of polymorph nucleated cells (PMN) from volunteers before and after placebo or AFAingestion. The phagocytic activity was unaffected by placebo, but was moderately reduced by AFA, thus resulting in a lower maximum phagocytic capacity, and a lower phagocytic rate.

Measuring the absolute numbers of circulating leukocytes after ingestion of AFA showed an increase in cell counts limited to lymphocytes and monocytes, whereby polymorphonuclear cell numbers were unaffected. This apparent selective mobilization of lymphocytes and monocytes is most likely from the primary and secondary lymphoid tissues, releasing more monocytes, B cells, and T cells into the blood circulation. When the CD3+ T cell population was further subdivided into subsets, no significant differences were found in the changes observed whether in the CD4+, CD8+, CD45RA,or CD45RO subsets.

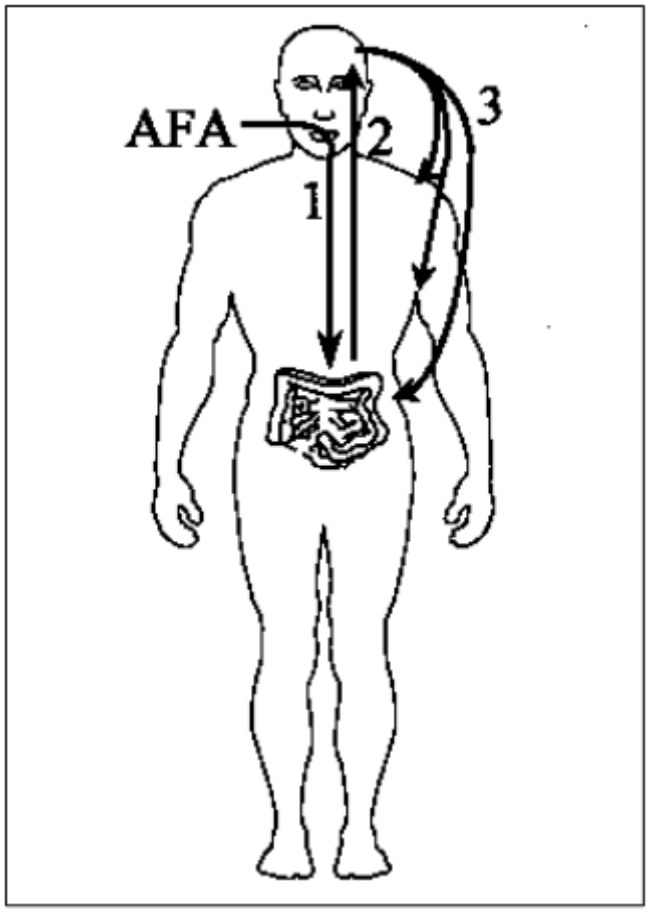


Fig. 39; Hypothetical model for AFA-induced immuno-modulation.
1: Ingestion of AFA, and release of bioactive phytochemicals in the stomach and/or upper intestine.
2: Release of cytokine(s) in the gut trigger vagus nerve signals from gut to CNS.
3: Central nervous system signals to the peripheral lymphoid tissues, resulting in altered immune cell trafficking.

Examining the effect of AFA on NK cell circulation, they confirmed the earlier pilot study on specific recruitment of small NK cells from the peripheral blood. They postulate that the decrease in NK cell counts in the peripheral blood translate into increased tissue recruitment. It could be argued nonetheless that the NK cells being 'recruited' out of the blood due to AFA are actually only marginating, that is, sticking to the vessel walls without transmigration. As margination is not a permanent phenomenon however, a certain proportion of cells would

likely 'unstick' and be obtainable for sampling. These cells would likely demonstrate altered adhesion profiles. Including shedding of L-selectin and up regulation of CD11, which was not found in the current study. Thus, the argument of margination is unlikely to be the cause. Furthermore, with the knowledge that the recruitment of cells from the circulation into lymphoid tissue is highly cell-type specific, mediated in part by cell-type specific chemokines, it seems more plausible that transmigration is the reason for altered NK cell counts.

The data presented here are generated based on different rationale and strategy than most other studies on blue-green algae. It had evaluated the migration and adhesion profile of immune cells, whereas most other studies had evaluated the function of subsets of white blood cells.

One manner in which AFA may function centerally in this pathway involves AFA containing one or several neuro-modulatory substances. These substances could directly stimulate vagal afferent fibres, relay signals to the CNS, and in turn produce the observed effects.

On the contrary, AFA might contain substances come into contact with other cells in the GI tract such as epithelial cells or intraepithelial leukocytes and trigger the release of cytokine-type molecules. Cytokines, such as IL4 beta, have been show to mediate a put-to-brain communication via the abdominal nervous network.[175-176]

Subsequent CNS signals to lymphoid tissue recruitment sites would then result in the observations presented here. An important finding of this study was that the changes in leukocyte re-circulation

observed after AFA consumption were greater in the long-term AFA consumers. These results point towards a possible conditioned type response to the ingestion of AFA. This response was not seen with the ingestion of placebo in the same subjects, indicating a true effect. The phenomenon of a conditioned biological response can be seen in many of the human systems, namely the CNS for the context of this discussion.

Given that the CNS is capable of modulating the immune system and that CNS is a highly plastic system. it seems plausible that a conditioning may have been established in which the CNS may recognize the stimulation by AFA from prior occasions and adapt to react to further exposures. In other words, previous regular exposure may lead to an increased immuno- modulatory response.

In addition to the observations of increased NK cell recruitment, the preliminary study mentioned before also showed an increase in the expression of certain adhesion molecules. The present study represents a more thorough evaluation of adhesion molecule expression. Indeed, when examining the profile of adhesion molecules on circulating lymphocyte subsets, they found occasional shifts in expression confirming earlier observations, but overall they found no consistent differences induced by AFA in vivo. The exact timing of when an effect could be detected in the bloodstream could vary between individuals, and the timing of the second blood sample (post-AFA) could contribute to the differences seen (i.e. it may take more time to alter adhesion molecule expression). It is also possible that AFA may have induced a changed adhesion profile, but that the affected cells leave before blood is sampled.

Examination of adhesion molecule expression in response to incubation with AFA in vitro revealed a significant reduction in the ability to stain for L-selectin on PMNs and lymphocytes after the cells had been incubated with MA extract for a short time (20-60 minutes). This phenomenon was not caused by shedding of L-selectin as similar results were obtained when the structural elements of the cell membrane were artificially fixed to prevent shedding. In addition, the observed effect was specific for L-selectin as no other classes of adhesion molecules were affected, including the beta-l integrins (CD29, CD49d), the beta-2 integrins (CD11b, CD18), and CD44 To our knowledge, this is the first study examining adhesion molecule expression in response to blue-green algae.

As part of the in vivo study of AFA's effects, neutrophil phagocytic capacity was evaluated on cells obtained from volunteers before and after ingestion of AFA or placebo. As indicated in the results, consumption of AFA induces a mild decrease in the ability of neutrophils to phagocytose Staph aureus bacteria in vitro. This data seems in contrast with other studies that showed increased phagocytic activity in tissue macrophages.[157]

When comparing data of these studies, it may be important to note that different cell types, derived from different anatomical locations and tissues were used. The **Hayashi study** also differed in the choice of phagocytic substrate (latex particles), the feeding regimen (ten percent of total diet for >10 weeks), and the subject (mice). These in vitro data are therefore not easily related to our in vivo data.

Nonetheless, the observations prompted us to further investigate AFA's effect on neutrophils in order to elucidate possible mechanisms that could explain these findings.

In order to better characterize some of the observed in vivo effects of AFA on separate components of the immune system, various in vitro experiments were performed on freshly isolated human leukocytes. It is very difficult to define the functional relevance of such in vitro data, but they may provide some information about possible in vivo mechanisms, or help exclude certain interpretations. AFA is normally taken orally as a food supplement, and it is not known whether in vivo leukocytes come in contact with the equivalent soluble extract (used in vitro), a digested form of AFA (i.e. particular fractions), or if they contact AFA at all. In addition, the concentrations used for in vitro experiments probably do not correlate to levels of AFA extract found in the bloodstream.

Using the appropriate dose of AFA extract in vitro, they found that AFA did not have a direct stimulatory effect on cultured human lymphocytes as measured by proliferation and tyrosine phosphorylation. In other words, AFA does not contain a mitogenic or super-antigenic substance, This study addressed the question of a mitogenic component in blue-green algae, which would warrant caution if found.

The mouse study examined the magnitude of response after altering the nutrition of the experimental animals. Cells exposed in vitro to blue-green algae extract is very likely to be different from the in vivo exposure to blue-green algae with subsequent isolation and mitogen induced proliferation of such cells.

AFA-induced changes in neutrophil function in vitro were assessed using a ROS (reactive oxygen species) formation assay. In vivo observations demonstrated that ingestion of AFA produced a decrease in PMN phagocytic ability. Examining PMN ROS formation revealed that AFA does not induce an inflammatory reaction as it does not induce neutrophil ROS formation in vitro. They found unchanged ROS levels within neutrophils exposed to AFA when compared to baseline control levels. As a positive control for induction of ROS, they used TNF-α. Interestingly, when neutrophils were co- incubated with AFA and TNF-α, results showed a reduction in the TNF-α induction.

Taken together, these data indicate that while AFA has no effect on baseline ROS formation, it can moderate the inflammatory activity of activated neutrophils. This pattern of reduced cellular responses after exposure to blue-green algae has been show before in studies of allergic reactions in rats. In particular, when rat peritoneal mast cells were CO-incubated with blue-green algae and sensitizing IgE antibodies and then challenged with antigen, the resultant TNF- α secretion (measure of mast cell activation) was decreased when compared to incubation with sensitizing antibody alone plus challenge.[173]

Although the data available in humans is testimonial, people taking blue-green algae supplements have seen improvements of allergic, asthmatic, and arthritic conditions. The findings presented here and elsewhere may point towards a possible mechanism by which chronic inflammatory conditions may be controlled using blue-green algae.

ln accordance with these testimonials, phycocyanin, one of the pigments found in blue- green algae, was recently found to possess antioxidant properties in vitro.

It is postulated that open chain tetrapyrrole structure enables the molecule to scavenge for free radicals. In addition, the same group has showed that phycocyanin exhibits significant anti-inflammatory activity in different animal models of inflammation.[177,178] These models include arachidonic acid-induced ear edema in mice, and carrageenan-induced paw edema, cotton pellet induced granuloma formation, and acetic acid-induced colitis in rats.

Phycocyanin makes up approximately 15% of the dry weight of AFA. Considering the data on its anti-oxidant and anti-inflammatory activity mentioned above, it is possible that phycocyanin is the constituent of AFA that may explain our current findings. These findings include a decrease in the phagocytic capacity of neutrophils, and the blunting of ROS formation in neutrophils primed with TNF-α.

In an attempt to study phycocyanin's putative role in our findings, we repeated some of the in vitro experiments using an extract of AFA that we believed to contain pure phycocyanin. Interestingly, the results of some of these experiments are similar to the results presented in this study (ROS data and L-selectin data). They require further confirmation however, because they were unsure of the purity and concentration of the phycocyanin used in these experiments; they recommended to repeat these experiments using properly purified phycocyanin in known doses in order to examine whether it is the putative compound that can explain the results show here.

IMMUNITY RELATED DISORDERS

A- Antiviral, Antibacterial, and Antifungal Effects of AFA:

"We have given too much attention to the enemy and have to some extent overlooked our defenses"

—M. Behar,World Health, February-March, 1974

Role of AFA in infections:

On its Microorganisms, bacteria, and fungi have been exploited for almost a century to provide useful drugs, antibiotics, and other pharmacologically active compounds.[275] Antibiotics, active against bacteria, fungi, and even viruses, have been isolated from marine algae, especially macroalgae.[276] Microalgae as well as macroalgae are able to produce a wide variety of pharmacologically active compounds.

Beneficial effects in leprosy were first observed in the 1940s. "**Jorgensen and Convit** fed a soup made from concentrated Chlorella to eighty patients at a treatment colony in Venezuela. The improvement in those patients' physical condition was the first documented evidence of the potential of microalgae as a health supplement." [277]

Subsequent positive results in the treatment of leprosy were documented in India with the use of AFA blue-green algae. [278] Leprosy is a bacterial infection; in some as yet unknown way microalgae appears to improve that condition.

Antibacterial, antiviral, and antifungal properties have been found in dozens of micro-algae species. [279-281] A variety of unique and different biochemical properties might be involved. The antibacterial agents of blue-green algae often appear different from known cyanotoxins as well as known antibacterial substances. While some microalgal species extracts show specific and targeted antiviral or antibacterial effects, extracts of other species demonstrate an inhibitory effect across a wider range of viruses, fungi, and bacteria. A few of the species used in research are known to be toxic, while other species are edible.[282-284]

Because of the affinity of some bacterial species for sulphated glycoconjugates exposed on the epithelial cells of susceptible host animals, researchers have hypothesized that sulphated exopolysaccharides of microalgae can be used in anti-adhesive therapies against certain bacterial infections, both in cold- and warm-blooded animals.[294]

AFA blue-green algae shows an inhibitory effect on the growth of Salmonella bacterial strains, in amounts greater than 2 mg. [297] A hot water extract of the green alga, Chlorella, given to mice infected with Listeria mono-cytogenes. Significantly increased the survival rates of mice, as well as demonstrating an increased immune cellular response. [298,299] Unicellular green algae have also been shown to increase resistance against E. coli and cytomegalovirus infections. [300-303]

According to Kenneth Bock, M.D., beta-carotene affects the immune function by: “Enhancing lymphocyte production: increasing macrophage cytotoxicity and cytotoxic T cell activity; helping detoxify pollutants; enabling the growth and development of cells; maintaining the membrane

receptors that are essential for immune function; and modulating the release of prostaglandins and leukotrienes.[304] Microalgae provide the richest source of the best quality and most easily absorbed beta-carotene.

Beta-carotene, which is plentiful in microalgae, may also decrease susceptibility to respiratory infections. As beta-carotene is transformed into vitamin A, deficiencies associated with vitamin A—such as increased risk of respiratory disease—might be reduced. [305,306]

Chlorophyll, for which microalgae constitutes a rich source, appears to have antibacterial components.[307] Traditionally, before the advent of modern antibiotics, chlorophyll was often used to prevent infections and accelerate wound healing. [308]

THE IMMUNE SYSTEM AND THE HEPATITIS C VIRUS

Introduction[228]

Because the immune system typically protects against infection and kills viruses, it is unusual that it is unable to clear the hepatitis C virus (HCV) when infected. Most people infected with HCV experience persistent infection whereby the virus evades, subverts, and/or weakens the immune system and survives for the life of the infected person. Such chronic infection with HCV often results in liver damage, which can lead to cirrhosis, liver failure, liver cancer, and/or premature death.

Approximately 5 million Americans and at least 180 million people worldwide are chronically infected with HCV, with a significant

proportion developing progressive liver disease. This makes HCV the most common reason in the western hemisphere for liver transplantation. Clearly, the stakes are high for conducting research that leads to the development of a vaccine to protect against HCV. The basic approach to begin this work is to unravel the type of immunity that naturally overcomes HCV then to design vaccines and/or therapies aimed at stimulating and amplifying that immunity. Strategies could conceivably involve stimulating specific immune cell types, such as lymphocytes, and/or blocking natural influences that decrease the body's response to infection with HCV. In other words, we are looking for a way to firmly push the immune system's "on' button while blocking all attempts to trigger the "off" button. My intent here is to introduce you to aspects of the immune system that seem central to this endeavor at the present time.

At the outset, it is essential to recognize that some fundamental questions relating to the immunology of hepatitis C remain unanswered. For example:

- How is HCV cleared from the body naturally, and in response to interferon-based therapy?
- Can the immune response itself contribute to the progression of liver disease?

Background Information

HCV Persistence Versus Clearance

A virus that is not cleared from the body by the immune system is said to persist. Persistence is unusual for viruses like HCV, which are made of a short single strand of RNA (a close but distinct chemical

relative of DNA). The most infamous RNA virus, HIV, ultimately persists by inserting itself among the infected person's genes where it permanently avoids detection by the immune system. We do not believe HCV hides from the immune system in this way. [228]

The viral and patient (called the host) factors that are responsible for the persistence of HCV have yet to be fully explained. However, it is clear that the fate of hepatitis C (whether it is cleared or persists) is nearly always determined during the early (acute) phase of infection, that is, within the first 6 months after exposure.[229] Although it can sometimes take longer, most people who naturally (spontaneously) clear HCV from their body experience this clearance within the first 6 months of infection.

Timeframes are important in HCV infection. Natural clearance of HCV by the immune system prevents disease progression and returns liver health. Therefore, early HCV infection has become the focus of intense attention for some immunologists. If we understand the type of immunity that allows people with acute hepatitis C to clear infection, we can devise methods of stimulating the same type of protective immune response in others. That is, we can make a vaccine. [228]

HCV Clearance Has Been Difficult to Study

Acute hepatitis C has been difficult to study because it is rarely diagnosed. Curiously, most people have few or no symptoms with acute hepatitis C. In the absence of symptoms, people feel well and are unaware they've been infected. As a result, very few doctors are likely to see even one case of acute hepatitis C per year.

This situation has severely limited research regarding the predictors and rate of viral clearance in natural HCV infection.
In recent years, researchers have begun studying special groups of people to gain a window into early HCV infection: those who experience high rates of new HCV infections. [228]

INJECTION DRUG USERS EXPERIENCE A HIGH RATE OF ACUTE HCV INFECTION

While modern blood-supply screening practices have reduced the number of new HCV infections in the West, cases continue to arise particularly among injection drug users (IDU's). In recent years, diligent work by scientists who analyze the incidence, spread, and control of diseases in populations has defined study groups (called cohorts) of IDU's who experience a high incidence of acute hepatitis C. These precious cohorts now provide an unprecedented opportunity to investigate acute HCV infection. [228]

A Smoldering Immune Response May Underpin Liver Disease Progression

While unraveling the details of successful immune responses is of great interest to aspiring HCV vaccine developers, the details of host immunity in people with chronic hepatitis C may provide clues for halting the progression of liver scarring (fibrosis). [228]

For chronically infected people, interactions between the immune system and HCV-infected liver cells may determine the amount and rate of liver fibrosis and therefore the rate of liver disease progression. In this

regard, the liver injury associated with persistent HCV may be similar to that incurred in chronic hepatitis B virus (HBV) infection. Like HBV, HCV probably causes little or no liver damage if ignored by host immune cells. When HCV stimulates but defeats waves of immune attack, the immune system may actually cause liver injury. [228]

Certain immune defense strategies—which may be genetically influenced—might be particularly damaging. Some people with hepatitis C seem particularly prone to form scar tissue. A variety of other factors may further contribute to the cascade of events that leads to liver injury and scarring, such as alcohol consumption and, conceivably, stress and diet. To complicate the plot further, each contributing factor may have immunological effects. [228]

Liver Damage Is Not Universal [228]

A central question is why liver inflammation converts to scar tissue only in some people. It will be important to work out the immune defense tactics deployed in these ill-fated battles, with the intent of distinguishing pathways that promote liver fibrosis. The eventual goal is to develop therapies aimed at blocking, dampening, or diverting harmful interactions. Thus, in the chronic setting, the immune response might be more detrimental to the person infected than to the virus.

Passive coexistence with HCV may prove more harmonious than unsuccessful attempts at eviction.

Successful Interferon-based Therapy May Need a Healthy Immune System [228]

An accumulating body of evidence suggests that in order to work, interferon-α (IFN) and ribavirin probably need to boost a critical arm of the immune system. Therefore, studying the components of natural HCV immunity could also hold implications for understanding the mechanism of viral clearance in response to IFN and ribavirin. A possible role for the immune system in treatment-induced HCV clearance will be discussed later in the chapter.

Thorough study of the immunology of hepatitis C will undoubtedly require further collaboration between doctors and scientists from different disciplines. In this chapter, we will review what is known about the immune response to HCV and for reasons described previously, we will pay particular attention to the way in which the immune system interacts with the virus during the acute phase of infection.
A coherent discussion of these issues is not possible without initially devoting some attention to the hepatitis C virus itself.

What Is HCV Made Of? [230]

To understand the concepts of HCV immunity, it is helpful to appreciate the basic structure of the virus. HCV is a relatively small virus made from ribonucleic acid (RNA) genetic materials, not DNA, which is the building material of human genes. The entire genetic component (the genome) of HCV is made of a single strand of RNA of only 9,600 units.
By comparison, the average length of a single human gene is 27,000 units.

Each RNA genetic unit (called a base because of its chemical property) is made up of a sugar unit (ribose) fused to a molecule of either guanine (G), adenine (A), cytosine (C) or uracil (U) arranged in specific sequence. Each sequence of 3 bases constitutes a code that usually specifies a particular amino acid. For example, AUG specifies the amino acid called methionine. Each set of 3 bases is called a codon. A series of codons specifies a series of amino acids.

Amino acids are the individual units that when strung together build proteins. In nature, the vast majority of proteins are assembled from only 20 amino acids that are arranged in diverse combinations, as genetically instructed. The structure of a particular protein is determined by the specific sequence of amino acids that are strung together. HCV has ten major proteins (see Figure 40). The structure of all ten individual HCV proteins is determined by the specific sequence of bases in the HCV RNA. The ten HCV proteins taken together are called the HCV polyprotein.

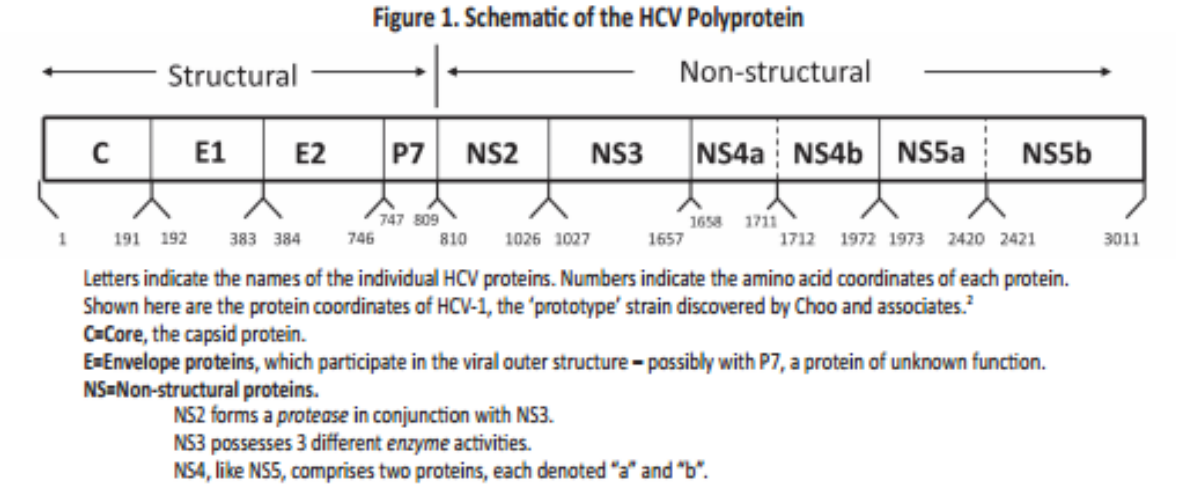


Fig. 40; Schematic of the HCV polyprotein [228]

HCV Is a Virus With a Highly Variable Structure [231,232]

Remarkably, HCV seems to change its genetic and protein sequences with relative ease. This is an important concept and we will devote some time to it.

The act of switching one genetic building block (a base) to another is called a mutation. In general, RNA viruses like HCV have much higher rates of mutation than DNA viruses. Recall that HCV possesses a single strand of RNA. In a liver cell, this positive sense or {+) strand is copied into a complementary (-) strand, which acts as the blueprint for making more (+) strands. However, this process is error prone.

On average each daughter HCV RNA strand will possess at least one different base from its parent and siblings. This is depicted in Figure 41. It was estimated that approximately one trillion replications of HCV occur each day in a single person infected with HCV. Because mutations occur largely at random, this huge number of replications implies that every single base in the HCV RNA strand can theoretically undergo mutation daily. In reality, many mutations will not lead to a fully functioning virus because they injure some aspect of the lifecycle. Nevertheless, many capable mutant viruses emerge to face the host's immune defenses. [231,232]

The main point is that no one infected with HCV is infected with merely a single virus, but instead with a mixture of related viral sequences. Remarkably, the HCV sequences in a single person can exhibit a greater percentage of genetic difference than that distinguishing major mammalian species, for example, such as a human and a chimpanzee. Scientists describe these circulating families of close viral relatives as quasispecies. In essence, each infected person harbors a group of HCV quasispecies, not a single entity. [233]

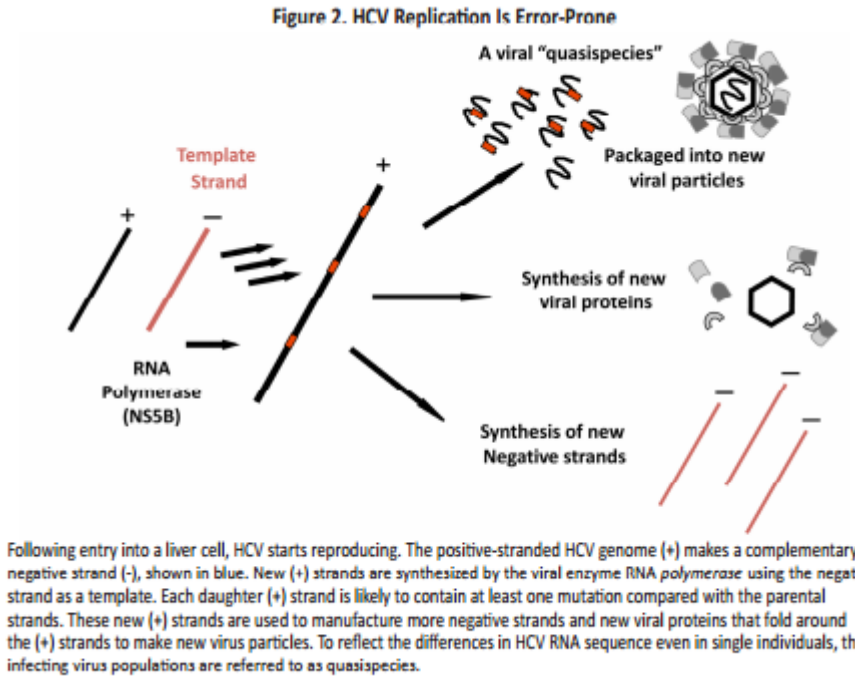


Fig. 41; HCV Replication is Error Prone [228]

Genetic diversity results in diversity among the encoded proteins, which creates the basic material on which natural selection can operate when a population is placed under environmental pressure. This holds true for all living things. In comparison to humans and other animal species however, RNA viruses play out the evolutionary game by producing remarkable numbers of genetically diverse offspring in relatively brief timeframes. This creates a rich opportunity for natural selection to operate quickly. Thus it is notable that many of the observed amino acid mutations in HCV proteins are clustered at sites targeted by the immune system, indicating they have not emerged merely by chance. Chance would not favor clustered mutations. We know that these mutations can disrupt the viral targets of immune attack, which in scientific jargon are called epitopes. The important point is that if mutation alters the structure of an epitope, the mutant virus is liberated from that source of immune attack. [234,235]

Natural selection works like this: mutations occur randomly, and when a certain mutation creates an advantage for its owner, that individual (officially called a carrier) is set apart from the pack. If the genetic mutation fails to injure the function or reproductive success of its carrier, the imbued privilege can be passed to its progeny. Extending the example of the HCV epitope mutation described above, viruses carrying such mutations will become more abundant in the host as their fitness outstrips that of other viruses neutered by the immune response. We are, therefore, able to readily observe them. This phenomenon is called escape mutation and represents evolution on the mini scale. Freed from immune attack, viruses with escape mutations can thrive. [234,235]

As we have implied, examples of escape mutation by HCV have already been observed and are popularly invoked as a principal means of HCV persistence. Indeed, so talented is this changeling virus that perhaps no HCV epitope can be guaranteed to remain permanently intact - a consideration that might confound vaccine development. It is important to emphasize, however, that the true extent by which mutation underlies HCV persistence remains uncertain. Because of the implications for vaccine design, some researchers are eager to measure the effect of HCV mutations on the overall efficiency of the immune response. The virus may, for example, experience more difficulty in surviving with some mutations than others. If a hierarchy of HCV mutations exists, discovery of epitopes that are problematic for the virus and therefore advantageous to the immune system could significantly enhance prospects for vaccine development. [234,235]

HCV Mutation Has Resulted in Genotypes and Subtypes [228]

The same genetic plasticity that results in HCV diversification has resulted in the evolution of even more divergent forms of HCV. The most profound HCV genetic divergence is observed between hepatitis C viruses from different human populations. Such comparisons reveal at least six major HCV genetic families, called genotypes, numbered 1 through 6 in their order of discovery (see Figure 42). Genetic sequence variability has resulted in each genotype being further divided into subtypes, which are denoted by lower case letters (a, b, c and so on). Hence, we routinely specify that someone is infected with HCV genotype la or genotype 2b, etc. In the North America, HCV genotype 1 infection is most common with genotypes la and lb occurring with approximately similar frequency, except in African Americans among whom genotype la is predominant. Next most common are infections with genotypes 2 and 3 viruses. The tree shown in Figure 42 depicts the relationship between the HCV genotypes and subtypes. [228]

It is possible that the natural history of infection may differ between HCV genotypes in different ethnic groups, though little evidence in support of this possibility is yet available. However, differences in interferon-based treatment outcome according to HCV genotype and ethnicity are now well described. [248] For example, genotype 1 causes most HCV infections in the West, where it is also the most difficult type of HCV to successfully treat Furthermore, genotype 1 is significantly more treatment resistant in African Americans. Overall, infections with genotypes 2 or 3 are the most treatment receptive, at least in western populations, with genotype 2 being most susceptible. While

treatment responsiveness is discussed in more detail in different sites, these differences remain largely unexplained and imply interplay between host and viral factors. We suspect that genetically determined differences in the immune response play an important role, but a discussion of this is beyond the scope of this chapter. [228]

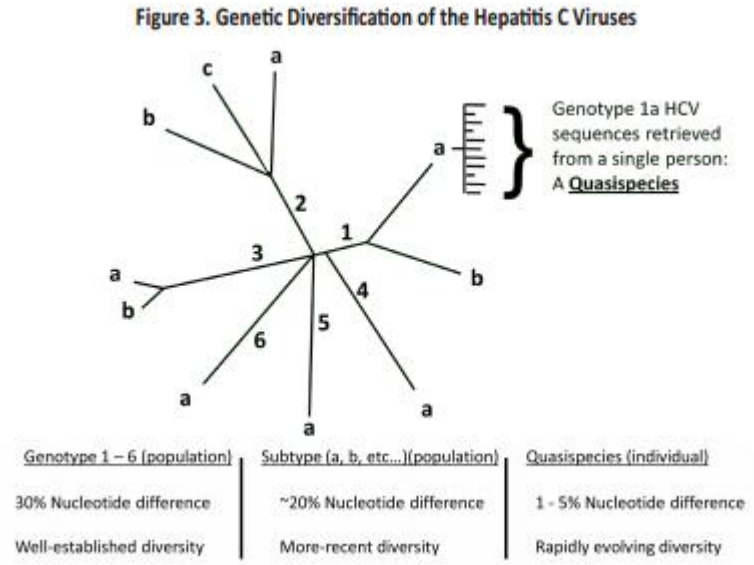


Fig. 42; Genetic Diversification of the HCV: [228]

Just as family trees depict the relatedness of human families, phylogenetic trees such as the one shown here, depict relatedness between different genetic sequences. A computer program has compared corresponding positions of many HCV genetic sequences and represented the degree of correspondence graphically. All members of each of the 6 major branches (the genotypes, numbered 1-6) are defined by shared genetic characteristics. Genetic variability exists at three levels. HCV genotypes (numbered 1-6) represent the highest level of divergence and probably the most ancient splits among HCV sequences. Each of the 6 major genetic groups contain a series of more closely related subtypes, typically different from each other by ~20% compared with >30% between genotypes. Genotypes la, lb and 3a are now widely distributed due to unscreened blood transfusion and shared injection drug use, and now represent the vast majority of infections in Western countries. Each person is infected with a broad range of viral variants referred to as quasispecies, which exhibit up to 5% sequence differences.

Finally, it should be stressed that viral mechanisms other than mutation are likely to also play a role in immune subterfuge. [249-252] We suspect that HCV mutation may only be a factor in viral persistence, which requires further study. [231,232,241]

SUMMARY OF HCV IMMUNOLOGY [242-255]

Chimpanzee and human studies suggest an inverse relationship between the extent of CTL (cytotoxic T cell) response in early infection (which may have quantitative and qualitative components) and the likelihood of viral persistence.

Fundamentally, the effectiveness of a T cell vaccine will depend upon efficient induction and maintenance of an adequate repertoire of HCV-specific memory cells. Given that the principal site of HCV replication and probably antigen presentation is the liver cells, this may require or (at least benefit from) preferential memory cell induction and / or maintenance in the liver. This could be problematic because the liver is the site where T cells could be tolerized or removed, properties that perhaps contribute to viral persistence. [242-255]

Several studies have examined multiply HCV exposed but seemingly uninfected individuals and found apparent HCV-specific CD4+ and CD8+ T cell response following stimulation with HCV protein antigens. Although this suggested the possibility that HCV-specific T cells could endure following subclinical exposure, concern existed that such T cells may have been low affinity, cross reactive cells rather than true HCV-specific memory cells that could respond naturally to

processed antigens. CTL characterized from liver biopsies of chimpanzee studied during and after acute self-limited infection confirmed that HCV-specific memory cells could endure for at least 18 months. The latter study hinted that CD8+ T cells might reside preferentially in liver tissue. More recently, HCV-specific memory CD8+ T cells have also been shown to endure in humans who clear the virus. Persistence of HCV-specific memory CD8+ T cells following self-limited infection suggest that virus-specific CD4+ T cells similarly persists. [242-255]

While antibodies appear to select HCV envelop protein variants, clear evidence that antibodies protect against natural infections remain elusive. Although many experiments suggested that high titer antibodies directed against certain structural antigens, especially envelop hypervariable regions (HVR) might at least modulate or even potentially protect against HCV infection, conclusive evidence that antibodies reliably afford protection is still lacking, particularly against different HCV strains, and putatively protective antibody titers may yet prove to sustain. So while antibodies appear to exert selective pressure upon HCV, the ability to terminate infection remains uncertain, particularly against different strains and genotypes. [242-255]

In up to 50% of people, HCV infection is associated with the presence of antibodies that bind to each other and precipitate out of solution when cooled below body temperature. These are called cryoglobulins. When these antibodies deposit in small blood vessels, inflammation add blockage can occur (a condition known as vasculitis) and give rise to related damage and clinical syndromes. Vasculitic damage is most commonly apparent in the skin and kidney. [242-255]

HCV in Egypt

It appears that there are endemic strains which have persisted in specific locations for many centuries. These can be readily identified by viral genotype. There have been iatrogenic outbreaks leading to massive spread of specific subtypes in countries such as Egypt (genotype 4A)[257,258] Clinically genotype information on the virus is of major importance in defining response to conventional as well as newer therapies.[259]

HCV infection is a major health problem in Egypt. [260,261] The current standard of care for hepatitis C infection is peginterferon/ribavirin, it gives higher sustained response rates in genotype 2/3 infected individuals . [262] But in Egypt the infection is due to genotype 4A for which is less affected by this type of therapy, beside its high cost and numerous serious side effects.[263] The lack of vaccine for HCV infections, the high cost of the drugs specially in low-income countries with a high prevalence of HCV, ineffective therapy and the rapid emergence of new drug-resistant viruses have urged a growing need for developing new, more effective less costly chemotherapeutic agents with less side effects for successful HCV treatment as HCV infection is more common in patients with low socioeconomic status. In addition, they are immunocompromised by parasitical infections such as Schistosomiasis and pollution of food by aflatoxins and toxic carcinogenic insecticides.[256]

Role of AFA in HCV infection[310]

Several compounds were extracted from blue green algae including a protein called Cyanovirin-N (CV-N) which appears to irreversibly inactivate diverse strains of HIV virus.[264] CV-N has antiviral activity against HCV as it inhibits HCV entry into host cells at low nanomolar concentrations.[265]Also, blue green algae compounds selectively inhibit the penetrations of enveloped viruses (Herpes simplex, human cytomegalovirus, measles virus, mumps virus, influenza virus and HIV virus) into host cells thereby preventing replications.[266-268] Also, consumption of AFA has rapid effect on the circulation of immune cells in humans.[269] The WHO approved that the daily intake of AFA algae is 2 g/day (WHO).[259]

Takeshita et al. (2009) at the university of Miyazaki in Japan believed that since HCV is localized in the liver and can take 20 years or more to developed into disease, dietary supplement might help slow or stop disease progression.[270] Consumption of AFA has rapid effects on the circulation and function of immune cells in humans. **Jensen et al.** (Chapter No.9)[271] conducted a randomized, placebo-controlled study to examine the short-term effects of consumption of 1.5 grams of the blue green algae Aphanizomenon flos-aquae (AFA), on the immune system.

They found that consumption of an AFA results in rapid changes in immune cell trafficking i.e. generalized mobilization of lymphocytes and monocytes, but not polymorph nucleated cells. This included

increases in CD3+, CD4+, and CD8+ T cell subsets and CD19+ B cells with reduction in relative proportions and absolute numbers of Natural Killer (NK) cells. The polymorph nucleated cells showed a mild but significant reduction in phagocytic activity. In vitro, AFA did not induce a direct activation of lymphocytes, as evaluated by tyrosine phosphorylation and proliferative activity. They concluded that AFA increases the immune surveillance without directly stimulating the immune system.[269]

Recently there have been many scientifically-controlled studies analyzing the immune enhancing properties of Aphanizomenon flos-aquae (AFA) from Klamath Lake, Oregon. On the basis of the Provisionally Tolerable Daily Intake (PTDI) I value established by the WHO, a guidance value of 1 μg microcystin-LR/g AFA algae was issued for AFA algae in the USA and the daily intake of AFA algae is 2 g per person weighted 60 kg.[271]

Many patients in Egypt including those who are not eligible for IFN/ribavirin, cannot afford treatment, or fail to respond to IFN, use natural products as alternative treatment for HCV infection.

Baicus and Tanasescu(2002)[272] studied the effect of one month treatment of Spirulina (extract of blue green algae) on serum aminotransferases and general state, compared to placebo, in 24 patients with chronic HCV. They found no effect on the level of aminotransferases with improvement in the general status. However, these results may be due to the very short duration of treatment. **Yano et al.(2007)**[273] found that three nutrients β-carotene, linolenic acid(both are

present in considerable amounts in AFA) and vitamin D2, inhibited HCV RNA replication in a cell culture system and that their combination caused additive and/or synergistic effects on HCV RNA replication.

The first study designed to evaluate the effect of combination of natural products in Egyptian patients with chronic HCV, conducted by **Zaki Sheir, Gamal Badra et al.(2013)** [274] in collaboration between **Mansoura University and National Liver Institute, Menoufya University, Egypt** showed that combination of AFA (although the dose used is less than 10% of WHO recommendations for economic causes most propably!!!!!!!????), Vitamin D, black seeds, olive oil and honey and chloroquine may have a role to achieve SVR in combination with recent direct acting antiviral drugs for HCV infection.

Methods: Patients with detectable HCV RNA with different stages of fibrosis and cirrhosis refusing or unfit for combined Interferon Ribavirin (INF/RBV) therapy or patients who failed to achieve sustained virological response to INF/RBV from April 2009 to March 2012 were included in this study. All the patients received combination Blue green tablet(each tablet contains 50mg AFA); 2 tablet/30 kg once daily; Vitamin D: 1000 IU/day; tea spoon fled paste made of linolenic acid, black seeds powder and honey; and 250 mg chloroquine once daily for 10 days and then every 3 days through the duration of therapy.

This study enrolled 195 HCV naive patients refusing INF/RBV therapy. Also, patients who failed to achieve sustained virological response to combined Interferon Ribavirin therapy (INF/RBV) whether PEGylated or conventional interferon were included if they stopped the antiviral treatment at least 3 months before recruitment. [274]

This study included patients with different stages of fibrosis and patients with compensated cirrhosis. All the patients were treated first from co-infections as schistosomiasis, helicobacter pylori and bacterial infections. Also, nutritional deficiencies were treated before starting treatment of HCV viral infection with a combination of natural products of (Blue green® tablet one tablet/30 kg, vitamin, tea spoon field paste of black seeds, olive oil, and honey) and chloroquine. In early phase of the study the effect of two tablets daily was not satisfactory so we increased the dose in weight base to 4 tablets daily (equivalent to 100 mg AFA/ 30 kg BW) This dosing was more convenient to the patients with less cost. Patients included in the study fulfilled the following inclusion criteria: their age 18 years or older, positive Anti-HCV antibody, detectable serum HCV-RNA by quantitative PCR. While patients were excluded if there is evidence of HCC, severe concurrent medical disease such as severe hypertension, heart failure, significant coronary heart disease, poorly controlled diabetes, chronic obstructive pulmonary disease and pregnant women were excluded. [274]

All patients were subjected to full history taking and thorough clinical examination and the following tests before treatment: abdominal ultrasonography, AST, ALT, serum bilirubin, serum albumin, prothrombin concentration, complete blood count, serum creatinine, viral markers (HCV Ab, HBs Ag). IHA for schistosomiasis and helicobacter antigen in stool were tested for before treatment. Detection of HCV RNA level by PCR quantitative measurements by COBAS Amplicor 2.0, Roche Molecular Diagnostics, Pleasanton, CA, USA (lower limit of detection of 50 IU/mL) was done before treatment. All patients had been followed up every three months with physical examination,

hematological studies, liver and kidney function tests and abdominal ultra-sonograms. Quantitative HCV-RNA by PCR test was done for each patient after 3, 6, and 12 and 18 months of treatment.[274]

Variables	Value
Age at presentation (years): Range Mean ± SD	20-76 47.8 ± 9.03
Gender (M/F)	132/63
Bilharziasis n (%)	57 (10.8)
Ascites n (%)	6 (3.4)
DM n (%)	17 (9.8)
Liver n (%) (Abdominal ultrasound) Normal Fatty Cirrhotic	 80 (40.8) 91 (46.9) 24 (12.2)
Spleen n (%)(Abdominal ultrasound) Normal Mild enlarged Huge Splenectomy	 125 (64.2) 62 (31.9) 5 (2.4) 3 (1.5)
Previous IFN therapy n (%)	8 (4.1)

Table 10: Demographic data of the included patients.

Variables	Mean ± SD
ALT range	7-275 54.31 ± 40.26
AST range	4-261 56.66 ± 39.97
Total bilirubin range	0.1-7.0 1.01 ± 0.73
PC range	31-100 77.11 ± 19.09
Creatinine Range	0.7-1.2 0.93 ± 0.14
HB Range	7.8-16.8 12.52 ± 2.06
WBCs Range	2000-11000 4879.41 ± 1983.08
Platelets Range	1000-302000 143416.69 ± 72938.82
Initial HCV RNA Range	280-11000000 505538.30 ± 1208201.18

Table 11: Initial liver functions, serum creatinine and complete blood picture of the studied cases

Variables	Mean ± SD	Paired *t* test	p- value
ALT before treatment	52.87 ± 36.97	3.28	<0.01*
ALT after treatment	38.12 ± 16.47		
AST before treatment	53.46 ± 36.11	2.69	<0.01*
AST after treatment	41.61 ± 17.27		
Bilirubin before treatment	0.96 ± 0.29	0.63	>0.05*
Bilirubin after treatment	0.98 ± 0.34		

*Wilcoxon test

Table 12: Differences between liver functions before and after treatment.

Variables	No (%)
Rapid virological response (3 months) Responder	 67 (34.4)
Early virological response (6 months) Responder	 82 (42.1)
virological response at 12 months of treatment Responder	 107 (54.9)
End treatment response (18 months) Responder	 125 (64.3)
Two log decrease of initial HCV RNA Positive Negative	 129 (66.1) 66 (33.9)

Table 13: **Virological response of the studied cases.**

Results: 195 patients with chronic HCV were included; mean age 47.8 ± 9.03 years, 67.7% males. All patients have chronic hepatitis. 24 patients had cirrhosis. 82 (42.1%) achieved negative HCV RNA after 6 months of treatment. After 12 months of treatment, 107 (54.9%) patients had negative HCV RNA. 125 (64.3%) patients achieved ETR after 18 months of treatment. Moreover, 4/6 (66.6%) patients with combined HCV and HBV showed undetectable HBV after 3 months. Two out of 8 (25%) patients who failed to achieve SVR with previous (INF/RBV) have ETR.

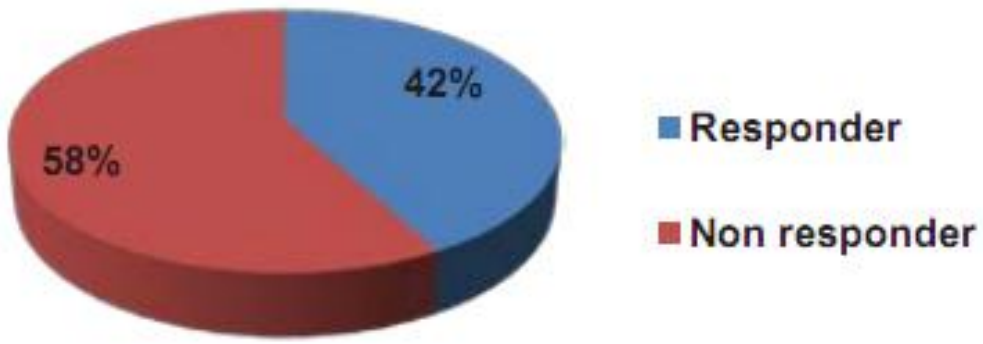


Fig. 43: **Virological response after 6 months of treatment.**

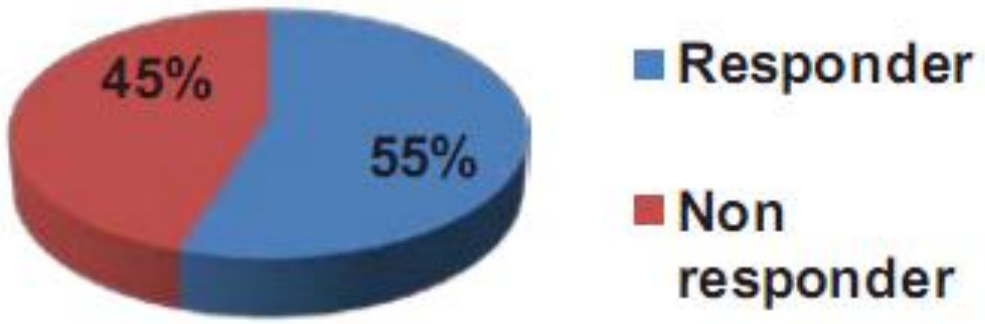


Fig. 44: Virological response after 12 months of treatment.

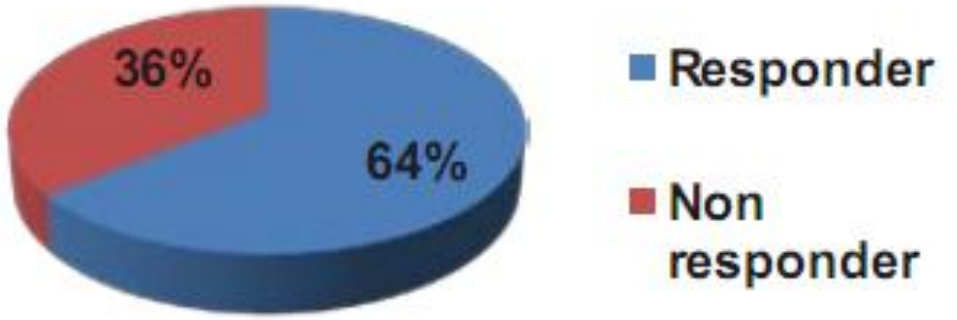


Fig. 45: Virological response after 18 months of treatment.

Variables	Mean ± SD	p- value
Age	47.81 ± 8.53	>0.05
ALT	52.51 ± 39.96	*>0.05
AST	51.49 ± 32.93	*<0.05
Bilirubin	0.93 ± 0.32	*>0.05
Serum Albumin	3.99 ± 0.34	>0.05
INR	81.25 ± 25.94	>0.05
HB	12.43 ± 1.55	>0.05
WBCs	4600.00 ± 2103.97	*>0.05
Platelets	121000.05 ± 74365.56	*>0.05
Initial HCV RNA	347851.32 ± 109928.55	*<0.01

*Mann Whitney test

Table 14: Univariate analysis of age, liver functions, CBC and initial HCV RNA in relation to end treatment response.

Variables	Mean ± SD	p- value
Age	46.32 ± 7.64	>0.05
ALT	50.45 ± 24.04	*>0.05
AST	58.80 ± 30.82	*>0.05
Bilirubin	1.15 ± 0.49	*>0.05
Serum Albumin	3.94 ± 0.34	>0.05
INR	59.57 ± 4.67	>0.05
HB	12.33 ± 3.37	>0.05
WBCs	4825.00 ± 1558.58	*>0.05
Platelets	172750.00 ± 97448.70	*>0.05
Initial HCV RNA	1462923.71 ± 205800.24	*<0.01

*Mann Whitney test

Table 15: Univariate analysis of age, liver functions, CBC and initial HCV RNA in relation to 2 log decrease of initial HCV RNA.

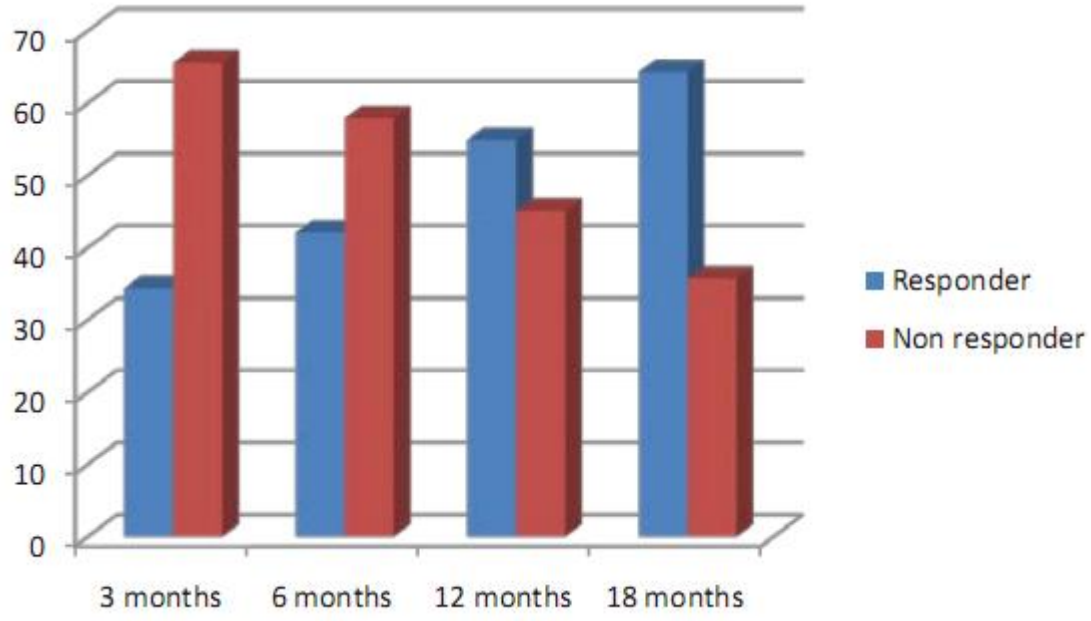


Fig. 46: Virological response after treatment.

Introduction to HIV immunology:[89,90]

There are two types of this virus: HIV-1, which is the primary cause of AIDS worldwide, and HIV-2, found mostly in West Africa.

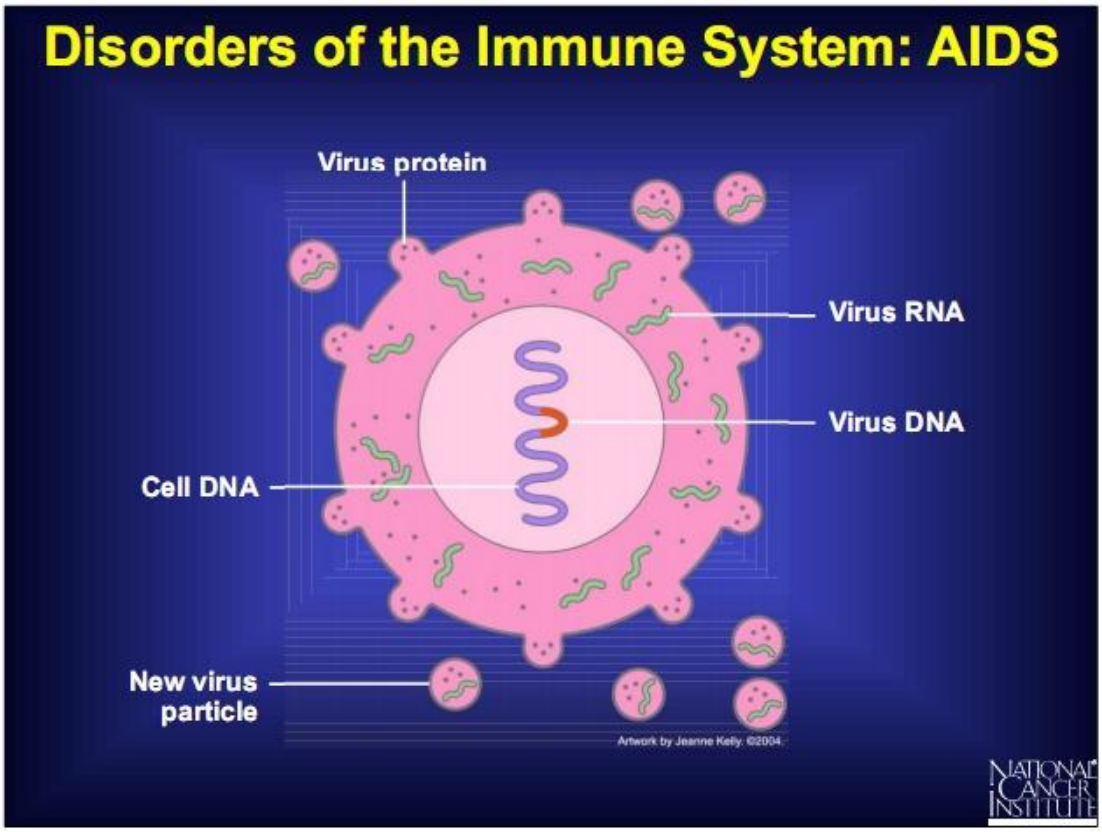


Fig. 47; **Disorders of the immune system, AIDS**

When the immune system is lacking one or more of its components, the result is an immune-deficiency disorder. These disorders can be inherited, acquired through infection, or produced as an inadvertent side effect of drugs such as those used to treat cancer or transplant patients. AIDS is an immune-deficiency disorder caused by a virus that destroys helper T cells. The virus copies itself incessantly and invades helper T cells and macrophages, the very cells needed to organize an immune defense. The AIDS virus splices its DNA into the DNA of the cell it infects; the cell is thereafter directed to churn out new viruses.

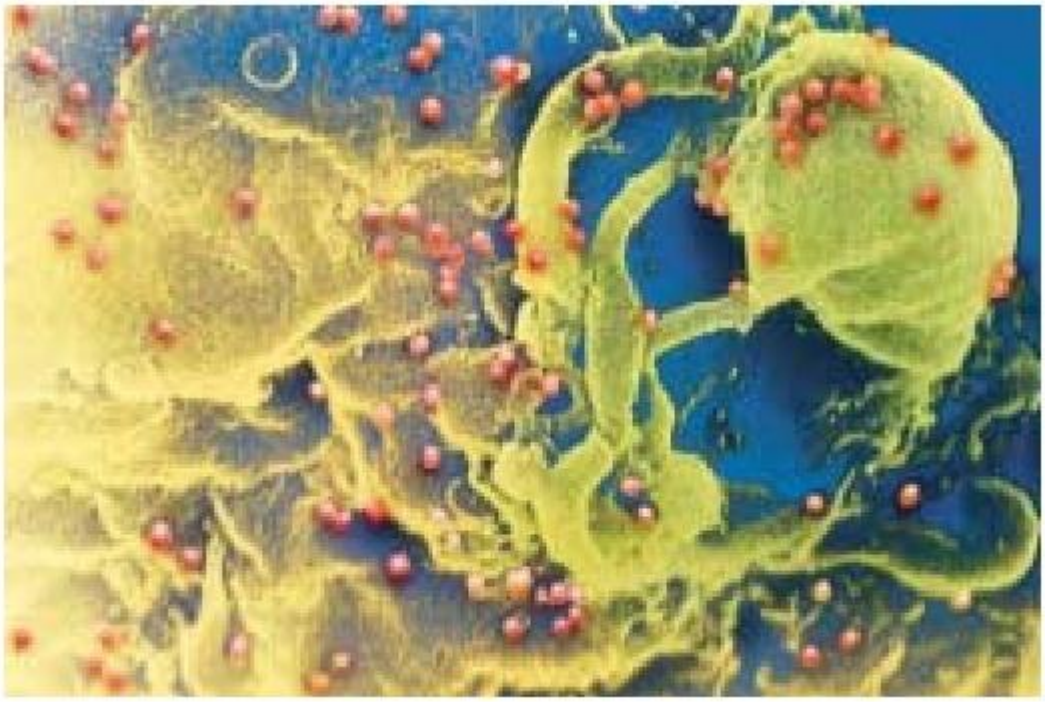


Fig. 48; HIV, the virus that causes AIDS. Viruses released from infected CD4+ T cells soon spread to neighboring CD4+ T cells, infecting them in turn.The individual viruses, colored blue in this scanning electron micrograph, are extremely small; over 200 million would fit on the period at the end of this sentence. AIDS destroys the ability of the immune system to mount a defense against any infection. HIV, the virus that causes AIDS, induces a state of immune deficiency by attacking and destroying CD4+ T cells. Antigen shifting refers to the way a pathogen may defeat the immune system by changing its surface antigens and thereby escaping immune recognition. Pathogens that employ this mechanism include flu viruses, trypanosomes, and the protozoans that cause malaria. Autoimmune diseases are produced when the immune system fails to tolerate self antigens.

Role of AFA in AIDS

In 1989, the National Cancer Institute (NCI) announced that a number of extracts from blue-green algae were found to be "remarkably active against the AIDS virus.[285] NCI has searched the world for natural plants and organisms that display active anticancer, antiviral, and immune-enhancing effects. A relatively high percentage (about 15%) of

aqueous extracts from freshwater and marine algae, cyanobacteria, marine invertebrates, and terrestrial plants have exhibited activity in the National Cancer Institute's primary AIDS-antiviral screening program. [286]

Upon further investigation, a new class of HIV inhibitory compounds called the sulfonic acid- containing glycolipids was isolated and the pure compounds were found to be strikingly active against the HIV virus in the p24 viral protein and syncytium formation assays.[285] Since this discovery, there has been Mer 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 seems to inhibit cell-to-cell and virus-to-cell adhesion.[309]

Other studies using a water soluble extract of blue-green algae have found a novel sulfated polysaccharide named calcium spirulan (Ca-SP) that acts as 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.[289,288,266]

Numerous studies indicate that cultured or harvested blue-green algae may represent a novel source of compounds that inhibit reverse transcriptase viral activity, including that of HIV-1.[287,288] For instance, naturally occurring sulfolipid portions of glycolipids in blue-green algae have demonstrated powerful antiviral properties. Through in vitro and in vivo experiments, a sulfated polysaccharide derived from blue-green

algae was demonstrated to be superior to dextran sulfate (DS) against human immunodeficiency virus type 1 (HIV-l). [288-290]

Extracts of microalgal polysulfates appear to: (1) block HIV replication in cell cultures at low concentrations without toxicity to the host cells; (2) inhibit cell-cell adhesion of HIV at its primary binding sites; (3) possibly act synergistically with anti-HIV drugs; (4) be very slow to cause virus-drug resistance and show activity against HIV mutants that become resistant to reverse transcriptase inhibitors, such as AZT and others; and (5) have the potential to be effective in a vaginal formulation to protect against HIV infection.[268] The efficacy of microalgal polysulfates in therapy or prevention of retroviral and opportunistic infections needs to be further demonstrated in animal models and humans.

Compounds and extracts from blue-green algae, as well as other microalgae, showing HIV inhibitory activity are often active against other retroviruses such as Herpes simplex virus types 1 & 2, simian immunodeficiency virus (SIV), cytomegalovirus, measles virus, mumps virus, and influenza A virus. [266,289-294]

Marine microalgae were screened for in vitro inhibition of viral replication for African swine fever virus and viral hemorrhagic septicemia virus of salmonid fish. Two out of ten species of marine microalgae tested produced a significant inhibition of both viruses in a dose-dependent manner. These two viruses were used because of their major economic importance. Thus, extracts from marine microalgae may have prophylactic utility against fish and mammalian viral disease. [295]

A team of medical researchers reviewed over 200 documented medical cases and concluded that AFA blue- green algae may be helpful in the treatment of chronic fatigue, Epstein Barr infection, chronic ear infections, AIDS, and other conditions involving viral infections. [166] An earlier retrospective study (1996) documented numerous improved case outcomes related to viral mononucleosis and Candida albicans (yeast) infection. [296]

B-Allergy and Asthma

"If you are sitting on a tack, it takes a lot of aspirin to make it feel good.If you are sitting on two tacks, removing just one does not result in a 50 percent improvement."

−Sidney Baker.Detoxification and Healing. 1997

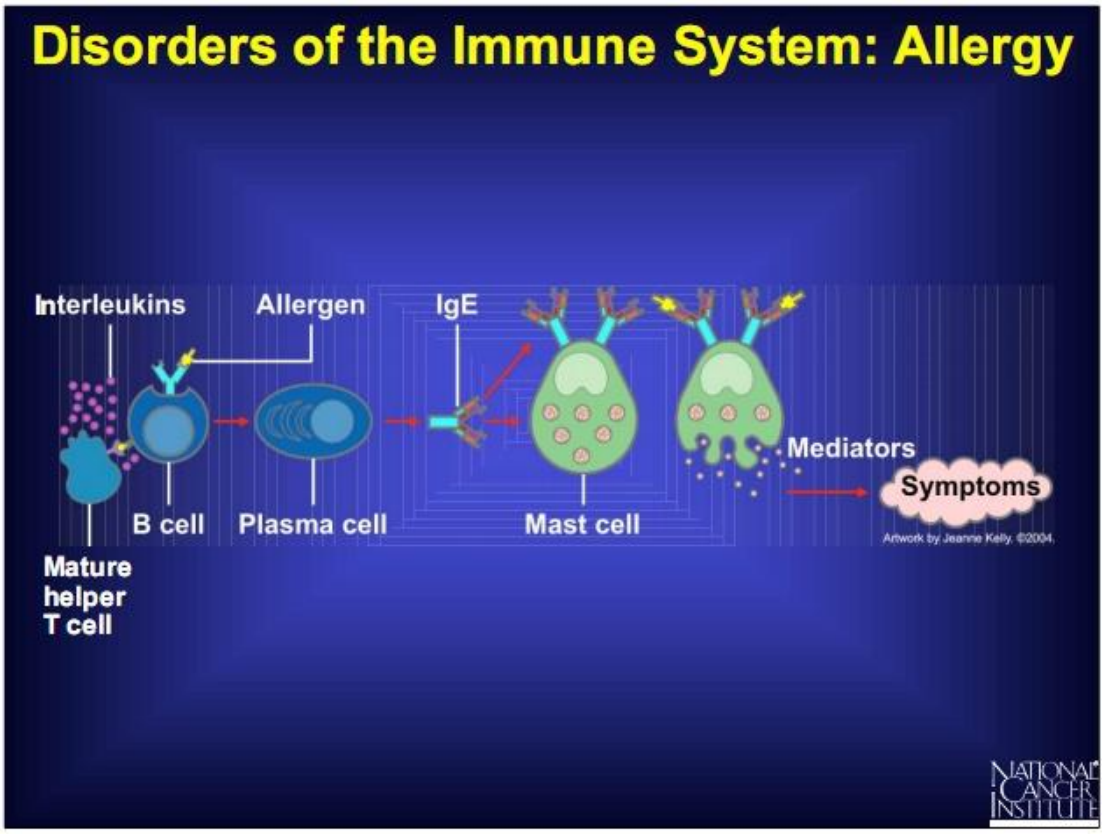


Fig. 49; Allergy

When your immune system malfunctions, it can unleash a torrent of disorders and diseases. One of the most familiar is allergy. Allergies such as hay fever and hives are related to the antibody known as IgE. The first time an allergy-prone person is exposed to an allergen--for instance, grass pollen--the individual's B cells make large amounts of grass pollen IgE antibody. These IgE molecules attach to granule-containing cells known as mast cells, which are plentiful in the lungs, skin, tongue, and linings of the nose and gastrointestinal tract. The next time that person encounters grass pollen, the IgE-primed mast cell releases powerful chemicals that cause the wheezing, sneezing, and other symptoms of allergy.[89,90]

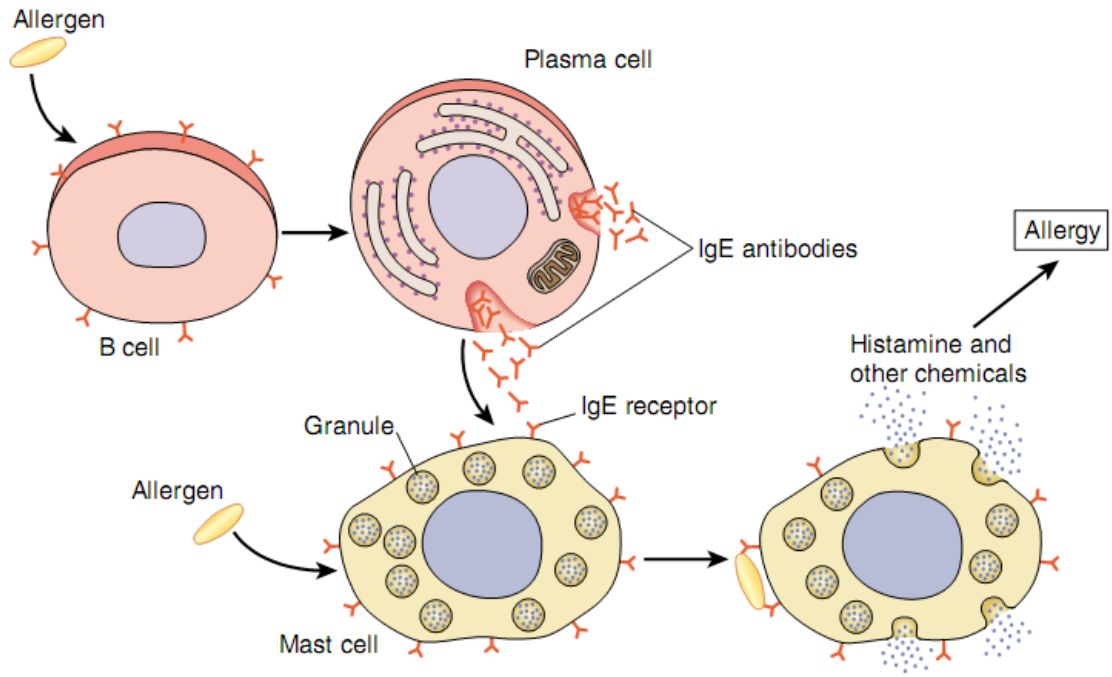


Fig. 50; An allergic reaction. This is an immediate hypersensitivity response, in which B cells secrete antibodies of the IgE class. These antibodies attach to the plasma membranes of mast cells, which secrete histamine in response to antigen-antibody binding.

According to **James Breneman**. former chairman of the Food Allergy Committee of the American College of Allergists. "The incidence of food allergy is greater than the incidence of any other type of illness affecting

mankind. By some estimates. 60 percent of the population have unknown food intolerances or allergies:[311]

Pediatric studies published in the Japanese journal Pediatric Clinics in 1962 reported the use of decolorized green algae in the diets of infants sensitive to milk and soybean formulas. The algae reduced allergic sensitivity and provided a range of nutritional factors found in ordinary milk products. Algae powder has also been used in the United States to reduce seasonal pollen allergies. [312]

Two studies have found that the inclusion of blue-green algae in the diet contributes to a reduction of anaphylactic and immune type allergic reactions in animal studies. These results suggest that blue-green algae may contain compounds that act to inhibit mast cell-mediated, immediate-type allergic reactions. [373,313] Positive effects were found in both in vitro studies with lymphoid cells from the spleen and mesenteric lymph nodes, and in vivo studies with live animals. [334]

Researchers at Massachusetts General Hospital, affiliated with Harvard Medical School, found that algal oils significantly reduced blood levels of arachidonic acid in rats.[314] Arachidonic acid produces molecules (leukotrienes) that trigger allergic reactions and contribute to water retention (edema) and puffiness. These molecules may be 1.000 times more problematic than histamine in contributing to asthmatic bronchial constriction.[315]

A daily dose of beta-carotene from an algae extract, demonstrated a protective effect against exercise-induced asthma. Of thirty-eight patients given 64 mg of algal beta-carotene extract daily for one week. 55%

were protected from exercise-induced asthma. All of the patients in the placebo condition showed a significant post-exercise reduction of breathing in a forced expiratory volume test. [316]

A pilot study that used a survey developed by the National Center for Health Statistics (1996) reported fewer allergies, skin problems, and asthma among AFA consumers. The algae eaters scored significantly better than average on numerous measures of health, when scores were compared to normative baseline data.[296]

In another research, a significant reduction was observed in parents' reports of their children's allergy-type symptoms during a three-month trial on AFA supplementation. This study included 26 children and used a standardized health symptom checklist as well as a number of other test instruments.[151]

In Japan blue-green algae is reported "to forestall pancreatic exhaustion and return balance to the flow of enzymatic secretions.[317] Good digestion requires that the body secrete sufficient hydrochloric acid and pancreatic enzymes into the stomach to process foods. Certain food allergies can be traced to poor digestion combined with "leaky gut syndrome" that allows undigested proteins to enter the blood; the immune system reacts to these large molecules as foreign invaders. "AFA blue - green alga contains carotenes and chlorophyll, both of which are able to dramatically stimulate specialized cells around the intestinal walls to secrete lubricating material and thus help to prevent this type of allergic reaction.[318] The omega-3 fatty acids are likely to be helpful as well.

3- Auto-immune diseases

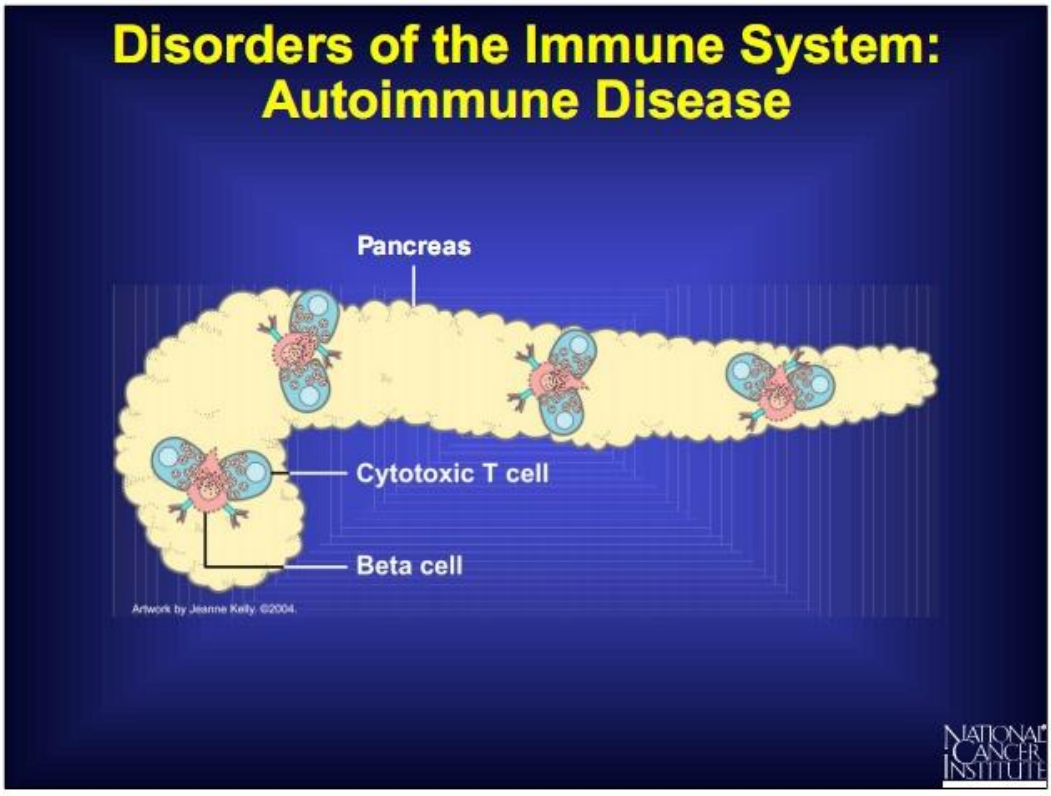


Fig. 51; Autoimmune disease

Sometimes the immune system's recognition apparatus breaks down, and the body begins to manufacture antibodies and T cells directed against the body's own cells and organs. Such cells and auto-antibodies, as they are known, contribute to many diseases. For instance, T cells that attack pancreas cells contribute to diabetes, while an autoantibody known as rheumatoid factor is common in persons with rheumatoid arthritis.[89,90]

AFA effects on the auto-immune diseases is multifactorial;

- Immune system modulation i.e. normalizing the immune system.
- Anti-inflamatory and anti-oxidant effects (phycocyanin and other anti-oxidants) i.e. reducing inflammation and tissue damage.
- Stem Cell mobilization i.e. regenerating dead cells.

Each of these effects is discussed in its specific chapter.

4- Immune complex disorders

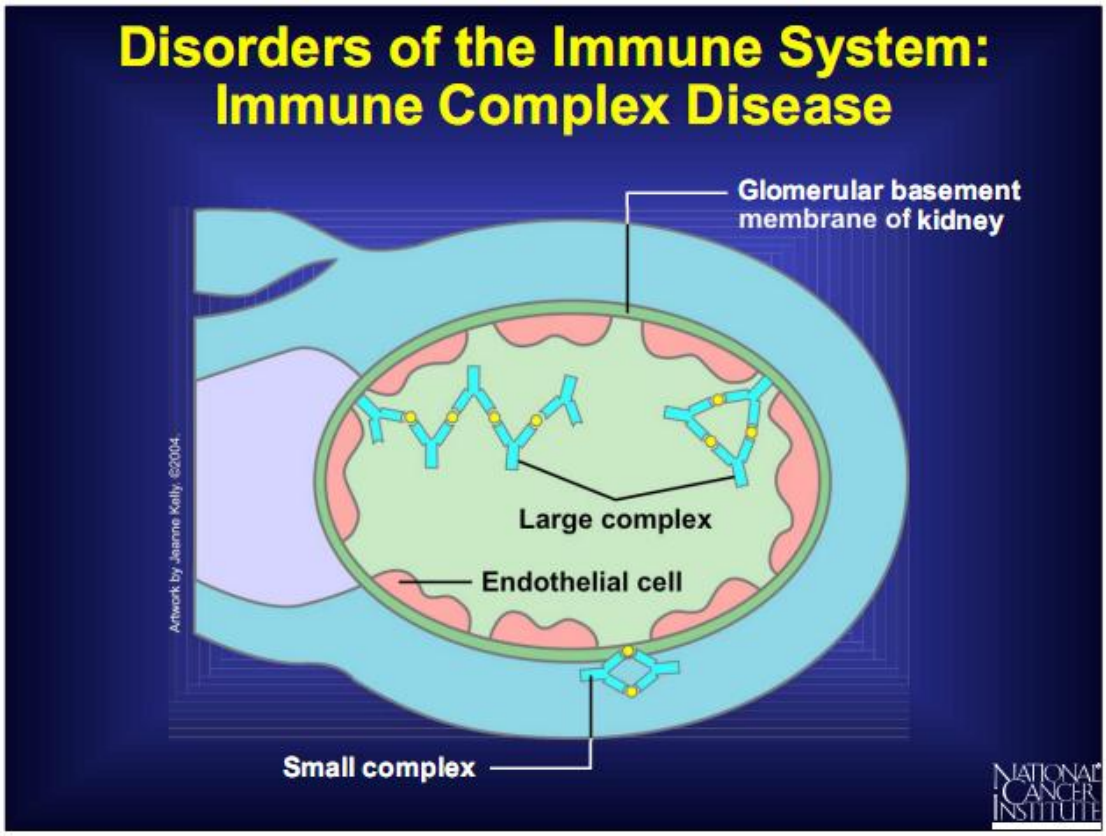


Fig. 52; Immune complex disorders

Immune complexes are clusters of interlocking antigens and antibodies. Normally they are rapidly removed from the bloodstream. In some circumstances, however, they continue to circulate, and eventually they become trapped in, and damage, the tissues of the kidneys, as seen here, or the lungs, skin, joints, or blood vessels.[89, 90]

AFA by providing immune system modulation, anti-inflammatory and antioxidants effects and stem cells mobilization can protect the body from its sequalae..

STEM CELL MOBILIZATION BY AFA

Introduction

Numerous studies performed by various scientific teams throughout the world, including the National Institute of Health[325] have clearly established that the higher the levels of circulating adult stem cells the better the ability of the body to maintain optimal health. A recent publication in the New England Journal of Medicine[468] reported that the level of adult stem cells in the blood was one of the best indicators of cardiovascular health. Elevating the number of adult stem cells in the blood has been shown to improve health in many ways. [370,469-471]

Many different types of stem cells are being used in research and clinical practice, on a global basis. Examples of these stem cells include, Human Embryonic Stem Cells (ESC), Human Cord Blood or Placental Stem Cells, Adult Stem Cells (ASC) and even animal stem cells (live cell therapy). Spurred by political considerations, much attention has recently been brought to the issue of using embryonic stem cells for research purposes and for the development of treatments for various diseases.[319-321]

Embryonic stem cells (ESC) are cells harvested from embryos that have nearly unlimited capacity to regenerate and become any kind of cell in the body. In the embryo, they are the initial precursors of all the cells destined to become the brain, heart, muscles, skin, bones, etc. When transplanted into an adult, embryonic stem cells have the ability to heal and repair any organ in which they are transplanted, providing an

extremely useful tool for the treatment of various degenerative diseases.[319-321]

Treatment with embryonic stem cells has either been shown or is suspected to improve various degenerative diseases such as Parkinson's diseases, diabetes, heart disease, as well as degeneration of the nervous system. Knowledge of the potential of embryonic stem cells in treating various health conditions emerged in the 1960s and gained significant momentum in the 1980s. However, research involving ESC received significant opposition over the years because of the obvious ethical nature of harvesting cells from live human embryos and because of the door it opens to research involving genetic manipulation of humans.[322]

Although the use of human umbilical stem cells tends to overcome ethical problems, work in this field poses special technical challenges.[320] These circumstances have led to major interest in the use of ASC. In ASC procedures stem cells are harvested, grown, manipulated and reintroduced.[320] The overall objective of ASC treatments is to implant ASC which are often coaxed down a pathway of differentiation toward a specific adult somatic cell type in order to replace diseased or ailing tissues. [319]

Adult Stem Cells are pluripotent and they have different degrees of versatility in their ability to engraft and replace damaged tissues. While some ASC appear limited in their ability to differentiate into different cell types, many recent studies show that such cells (especially stromal bone marrow or adipose tissue-derived) may have great versatility, in some circumstances[319-342].

Elegant biotechnology research is being undertaken to transform harvested ASC into highly specialized "functional" cell types for the potential treatment of disorders such as Parkinson's disease.[320]

There has been a great deal of high quality research in the application of ASC treatments, especially in the field of human bone marrow transplantation. However, there have been reports of poorly performed ASC treatment in off shore locations which are alleged to be poorly equipped or devoid of important ancillary services to make ASC treatments safe and effective. Recent revisions to U.S. laws that govern stem cell treatments (Obama legislation) have produced widespread interest in the development of stem cell treatment facilities, especially in centers of healthcare excellence. While stem cell therapies are advancing in a meteoric manner, all current "classic" stem cell treatments provide a series of disadvantages or limitations.[320]

Innovative scientists have been working on the potential use of mobilized in-situ ASC, as a non invasive form of stem cell treatment.[322,343] This is the process of Induction of Adult Stem Cell Recruitment (IASCR). A decade of research has led to current proposals that endogenous or in situ ASC (most notably bone marrow stem cells) can be mobilized from their niches in the body, with the result that they may migrate to various organs and engage in tissue repair or regeneration.[322, 343,344] While somewhat futuristic, it has been proposed that there may be several means whereby endogenous ASC could be released and promoted to differentiate into desired cell types to treat specific organ damage.[344] This proposal is supported by major advances

in the characterization compounds that can induce stem cell differentiation to specific cell types in-vitro.[320]

The proposed, non invasive technology of IASCR has obvious advantages over the processes of harvesting and reintroduction of ASC. These processes form part of current complex treatment programs.[343] It has emerged that several pharmaceuticals or natural substances are capable of mobilizing ASC from human bone marrow deposits, but some uncertainty surrounds the ability of mobilized ASC to home into damaged tissue and undertake a process of recruitment that will produce the desired treatment outcome of tissue repair or regeneration. However, diseased or damaged tissues provide complex signals to attract regenerative stem cells and the body has a built in homing system that it utilizes when ASC are part of an "internal repair kit" [320,322,343,344]

The development of IASCR represents a new horizon in stem cell treatments that could make stem cell therapies more portable and cost effective.

Adult Stem Cells at Work

Adult Stem Cells are ubiquitous in the body and they live in "niches" in many organs[320]. Most research has been performed with ASC of bone marrow origin, where stem cells are encouraged to proliferate to support the presence of blood components, often following marrow ablation. It has been stated that the very presence of ASC in adults poses questions concerning the exact definition of a stem cell.[320] While scientists have no problem in discussing the potential of several stem or progenitor cells to form new cell types or engage in tissue renewal, the concept of "stemness" emerges.[320] In brief, "stemness" is the ability of a

stem cell to produce different cell types and their ability to engage in self renewal.[320]

There is a large body of clinical and scientific literature that demonstrates the pluripotential of bone marrow ASC.[347-365] Bone marrow ASC have been harvested and reinjected into patients, following varying degrees of laboratory manipulation, in order to treat the consequences of degenerative disease. Variable success is apparent in some anecdotal reports on the internet. The results of many of these studies are reported in animal experiments in great detail, but many human experiences remain quite anecdotal in their descriptions of clinical outcome[368].Bone marrow ASC are engaged in the long term replenishment of all blood elements, but they are composed of a group of non-hematopoietic ASC (stromal cells) which are precursors of bone, cartilage and skeletal tissues.[343, 344]

This limited view of ASC has been overturned by many observations of the ability of these ASC to form a much wider range of specific cell types that may play a pivotal role in tissue healing and cellular replacement or regeneration.[324-342] In simple terms, ASC in a variety of niches in the body could migrate and translocate to a site of tissue damage where they may undergo cellular differentiation that improves organ structure and function.[320,324-342]

For example, **Goodell et. al. (2001)** [345] recently described how ASC can migrate from the bone marrow to the heart and contribute to cardiac muscle repair and the formation of new blood vessels after ischemic injury (cardiac infarct).

In brief, highly purified bone marrow stem cells were genetically modified to produce a fluorescent protein. The mice's innate stem cells were killed through irradiation. Then the genetically modified stem cells were transplanted into their bone marrow, leaving the fluorescent bone marrow stem cells as the sole source of available stem cells. Cardiac arrest was subsequently triggered in the mice by coronary artery occlusion.

A few weeks later, the engrafted fluorescent stem cells had differentiated into cardiac muscle and endothelial cells, which contributed to the formation of functional cardiac tissue, as well as new blood vessels.

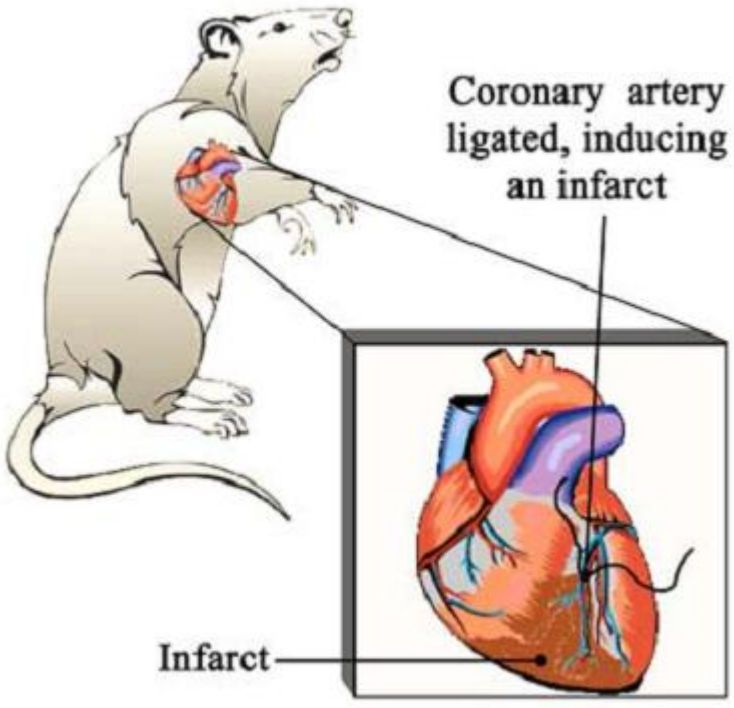


Fig. 53; Stem cells carrying a gene encoded for a fluorescent protein were trans-planted into irradiated mice. Cardiac arrest was triggered by ligating the coronary artery. Three weeks after the cardiac arrest, up to 68 percent of the necrotic area was filled with functional fluorescent cardiomyocytes.

Orlic et al. (2001)[325] carried an experiment that clearly demonstrated that the simple fact of enhancing the release and circulation of adult bone marrow stem cells could lead to significant healing. The researchers induced cardiac infarct in two groups of rats. The control group was left to recover on its own, while the experimental group received injections of ASC-releasing cytokines during 5 days after the infarct. After one month the survival rate in the control group was 17% and upon functional and histogical analysis the animals showed severe signs of cardiomyopathy. In the experimental group in which circulation of ASC was stimulated during 5 days post-infarct, the survival rate was 73%. Upon functional and histological analysis it was determined that newly formed cardiac tissue had developed, along with full functional blood vascularization, and that the cardiac functions were virtually normal. The simple fact of stimulating ASC release during 5 days post-infarct was sufficient to bring near complete recovery.[2]

Similar migration of bone marrow stem cells and subsequent regeneration of tissue was also suspected to take place in the brain. In a double-blind study including 40 patients suffering from Parkinson's disease, injection of stem cells derived from seven- to eight-week-old embryos slowed the progression of the diseases in all of the 20 patients.[334]

Likewise, there is evidence indicating that stem cells could reverse symptoms of Alzheimer's disease. [346]

Studies were therefore conducted to investigate whether stem cells injected intravascularly or endogenously released from the bone marrow could cross the blood-brain barrier, migrate, then differentiate into brain

cells. Bone marrow stem cells, along with monocytes and macrophages, were shown to have the ability to cross the blood-brain barrier and reach the brain.[335,347-349]

Intravascular delivery of genetically marked adult mouse bone marrow stem cells into lethally irradiated normal adult mice led to the development in the central nervous system of donor-derived cells having neuronal properties (neuronal phenotypes). [336]

After eight to twelve weeks, it was estimated that about 0.2 to 0.3 percent of the total number of neurons in the brain were derived from the bone marrow. The authors wrote, "Our results clearly show that adult cells from the marrow can gain access to the adult brain and assume characteristics of central nervous system neurons." [336]

Similarly, **Mezey et al. (2000)** showed that in a strain of mice incapable of developing cells of the myeloid and lymphoid lineages, transplanted adult bone marrow stem cells migrated into the brain and differentiated into cells that expressed neuron-specific antigens. Between 2.3 and 4.6 percent of all neurons were donor-derived. Some neurons were observed with axonic projections and apparent dendritic trees. The authors suggested that bone marrow stem cells might naturally migrate into certain regions of the brain and give rise to a variety of neural cell types, thus implying a greater potential for regeneration of the central nervous system than had been traditionally expected.[335]

Based on information produced by various scientific teams, **Jensen et al. (2002)**[322] recently proposed the Stem Cell Theory of Healing, Regeneration and Repair (Figure 54). This break-through theory suggests that bone marrow stem cells would leave the bone marrow and travel throughout the body, providing for healing and regeneration of damaged organs during the entire lifetime of an individual. In other words, adult bone marrow stem cells may be the natural mechanism that the human body utilizes to heal, regenerate and repair.

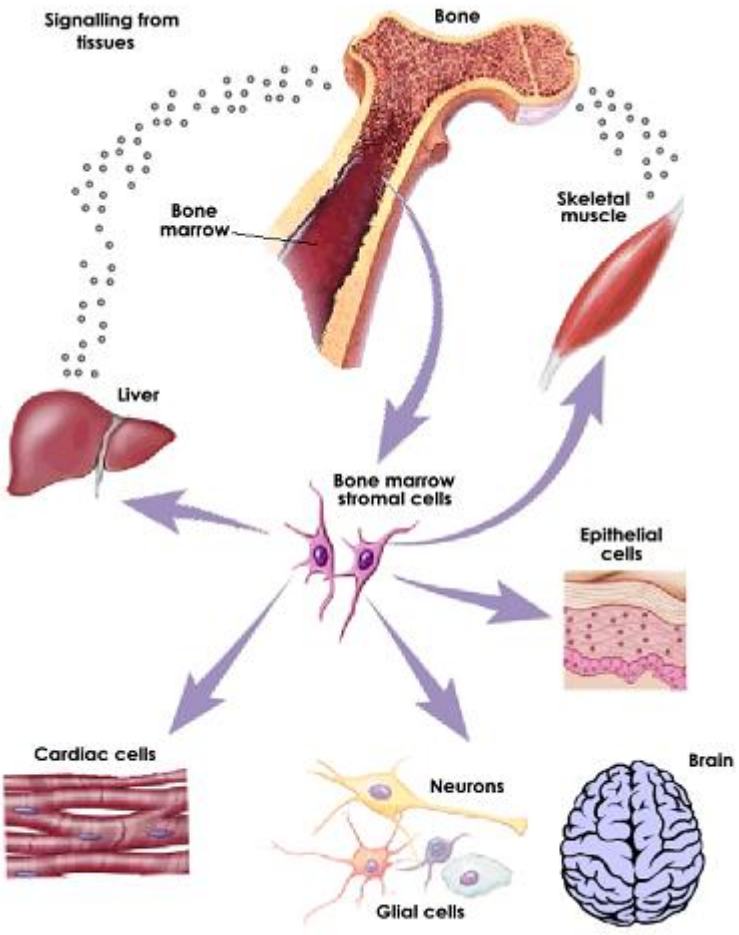


Fig. 54; Stem Cell Theory of Healing, Regeneration and Repair

According to this theory, there is no need to harvest embryonic stem cells, manipulate them, then re-inject them into individuals. Regeneration can take place simply by stimulating the release of stem cells from the bone marrow and stimulating their migration into tissues. The task is therefore simply to find natural compounds able to stimulate stem cell release and migration. Such compounds could be used for the daily enhancement of the body's natural mechanism of healing and regeneration.[319-349]

The only such natural compound known to date is AFA, which has been recently shown to stimulate stem cell release and migration. A dose of 5 grams of AFA was shown to increase the number of circulating stem cells. Based on this information, a patent has been filed and recently obtained regarding the use of AFA for the treatment of Parkinson's disease, Alzheimer's disease, diabetes, multiple sclerosis, cardiac arrest recovery, and regeneration.[2]

More recently, AFA was shown to contain an L-selectin blocker that acts as a mild stem cell mobilizer. Consumption of a proprietary extract of AFA that concentrates the L-selectin blocker was shown to lead to an average increase of 25% to 30% in the number of circulating stem cells within one hour of consumption.[269]

The ability of bone marrow stem cells to differentiate into a wide variety of specialized somatic cells such as cardiomyocytes, hepatocytes, pancreatic ß-cells, skin cells and skin appendages, and even neurons has been well documented. [350-355]

In essence, bone marrow stem cells constitute the natural repair system of the body. [356] *It is not surprising, therefore, that a higher number of circulating stem cells has been linked to greater health outcomes.* [356-366]*When tested in an animal model, this proprietary extract of AFA was shown to enhance tissue repair after cardiotoxin-induced injury to the tibialis muscle.* [367]

In their classic article in Medical Hypothesis **(2002)**, **Jensen** and **Drapeau** describe a hypothesis to support some of the components of what is termed IASCR. In brief, these scientists highlight the importance of the ability of ASC to target and grow at locations of tissue damage and the ability for this migration to occur after induced mobilization of ASC, most notably from bone marrow. There are other steps to be applied to the concept of IASCR which may include the use of agents to protect stem cells or improve their functionality and enhance their ability to differentiate into the desired types of adult somatic cells. Many authors confirm the pluripotent properties and ability of ASC to migrate within the body. [319-350]

Such studies include the ability of bone marrow ASC to become functional myocytes, hepatocytes, osteocytes and cells of the central nervous system. Available scientific information permits a clear conclusion that ASC have an ability to migrate from their tissues sites of origin and undergo cellular differentiation that results in a variable degree of repair of many different tissues.[320]

The laboratory identification of ASC involves a check for the presence of three characteristics that are hallmarks of "stemness". First, the cells must be able to renew themselves.[320] Second, the cells must have an ability to differentiate into specific cell types and, third, the cells must be transplantable with functional engraftment. A characteristic of all stem cells is the presence of telomerase, an important marker found in cancer cells1. The presence of telomerase has led to proposals that many types of malignancies originate in stem cells, as a consequence of disorganized cell division. [320]

Summarizing the Concepts of IASCR

• Mobilize ASC from bone marrow and other niche locations
• Increase circulation of ASC with semi-continuous, safe stimuli. (Herbs, botanicals and nutrients are preferred to drug approaches, (they cost less, and have less side effects)
• Protect ASC from oxidative damage
• Encourage homing to desired target organ (?)
• In-vivo assistance in the differentiation of ASC to replace cell types of the diseased organ has to be developed (The "human petri-dish" approach).
• A body of research demonstrates that human bone marrow ASC are able to "home in" on diseased organs and differentiate into many cell types.

Table 16. The concepts of how to utilize the Induction of Adult Stem Cell Recruitment (IASCR) (Holt, 2009) [344]

The residual argument that promoting ASC activity in humans could lead to the development of cancer is not supported by current scientific knowledge or experimentation. At present, it appears to be a

reasonable and safe proposal that ASC may be mobilized in the human body without significant adverse effects. [343] The use of adult stem cell technology has been perceived as widely acceptable in medical practice, because it rests on the relative safety and effectiveness of human bone marrow transplantation, but regulatory issues concerning the use of adult stem cell therapy remain to be defined with clarity. [320]

The discovery of AFA stimulatory effect on stem cells was reported by Christian Drapeau[368] in an article named The Discovery of StemEnhance published in HEALTHSPAN CANADA winter 2009/2010:

"It all started in the late 1970s when the cyanophyta Aphanizomenon flos-aquae, usually abbreviated as AFA, was discovered by schoolteacher who was looking for a source of exceptional nutrition to improve the academic performance of children. He began testing AFA, and as this natural blue-green algae plant was shared with a growing number of people, many began reporting testimonials and stories of how the consumption of this seemingly benign plant had transformed people's health and life. When the Dietary Supplement Health and Education Act (DSHEA) was passed in 1994, the Act required companies to provide a scientific basis for any claim made about a dietary supplement. I was hired to investigate the mechanisms of action behind the health benefits experienced by hundreds of thousands of consumers of AFA. The starting point for our research was to review the health benefits reported by consumers, with the intention of bringing the thousands of testimonials down to a list of body systems that might be affected by consuming AFA. In other words, we wanted to try to reduce all the reported benefits to just a few aspects of human physiology, and then design scientific protocols

to study the effect of AFA on these physiological processes. When our review was completed, the evidence on AFA's effects pointed to benefits for the body's inflammatory function, as well as for the nervous and immune systems.[368]

Over the course of the next five years, through numerous studies, we did identify three compounds produced by AFA that specifically affect these aspects of human health. Specifically, we described in AFA the presence of phycocyanin, a compound well known for its positive effect on the inflammatory function. We also discovered that AFA was an exceptional source of the biogenic amine, Phenylethylamine (PEA), a compound well known for its effect on mood, mental clarity and the sense of mental energy. Finally, we discovered a polysaccharide that supports various aspects of the immune function.[368]

But while we were identifying these compounds in AFA and their effects on health were being described in various studies, a small number of people were also reporting benefits that could not be explained by the presence of these compounds. The most challenging part of this whole scientific venture was actually the wide variety of the reported benefits.[368]

People were reporting benefits touching virtually every system of the body! How could one single plant affect so many aspects of human health? This mystery persisted for a number of years. Then one day a colleague gave me an article entitled “Turning Blood into Brain” (published in Science Daily, December, 2000). This article discussed how stem cells originating from the bone marrow could naturally migrate to the brain and become brain cells. Soon after, other scientific articles were published describing the ability of bone marrow stem cells to become

heart cells and liver cells. We then began thinking that if stem cells can become cells of the brain, liver and heart, then why not other types of cells? And more importantly, if this process was truly taking place in the body, then it had to be with a purpose: it had to be the natural renewal system of the body. We were especially interested in this renewal concept because it occurred to us that if a substance were to support stem cell function in the body, then a wide variety of health benefits would be seen, because stem cells travel to different tissues in different people. Maybe we were onto something! [368]

So our goal for this new research was to answer two questions:

1. What if stem cells constituted the natural renewal system of the body?

2. What if AFA supported the release of stem cells from the bone marrow?

Our in-depth review of the scientific literature published over the past few years clearly revealed that, indeed, bone marrow stem cells constitute the natural renewal system of the body. Then we went into the lab and studied the effect of AFA on stem cells and were able to show that AFA supports the release of stem cells from the bone marrow. [368,,]

Difference between AFA and StemEnhance

Difference from AFA StemEnhance is a patented and clinically studied blend of two concentrates from (AFA) : An L-selectin ligand that supports the release of stem cells from the bone marrow, and a polysaccharide that supports the migration of stem cells out of the blood into tissues. In the process of making StemEnhance, they also concentrate phycocyanin and PEA, as well as many vitamins and minerals. These compounds are concentrated approximately 5:1, which means that on average one gram of StemEnhance contains 5 times more of these compounds than one gram of AFA.[369]

Mechanism of AFA stimulatory effect on SCs[269]

Much recent research has focused on the role of selectins and their ligands in mobilization of bone marrow stem cells. L- selectin belongs to the selectin family of cell adhesion molecules involved in cellular migration during normal immunosurvillence and inflammatory conditions. L-selectin is best known as a homing molecule for recirculating lymphocytes to recognize high endothelial venules during the process of extravasation and for leukocytes to recognize and home inflamed tissues. However L-selectin plays significant roles in other physiologic cell adhesion processes as well including the retention vs. release of bone marrow stem cells into the blood circulation. [269]

Of special importance are the findings that engagement of L-selectin by some ligands will modulate the expression of CXCR4 chemokine receptor. The CXCR4 receptor specifically recognizes the chemokines stromal derived factor 1 (SDF 1), which act as a potent chemoattractant for stem cells and assists in retaining stem cells within the bone marrow environment.

The chemoattractant properties of SDF-1 on stem cells has been shown invitro as well as invivo to be directly associated with recruitment of stem cells in to kidney and liver. The mobilization of recruitment of stem cells is associated with repair of the CNS, heart and other tissues.[269]

Stem cells mobilization and homing involve a series of G-protein-coupled receptor that can interact with each other as well as with adhesion molecules. It proposed that loss of responsiveness twards CXCR4 may be one of several contributing mechanisms that allow some bone marrow stem cells to detach and leave the bone marrow as a part of embolization process. The use of selectin ligands has been proposed as a mechanism for stem cell mobilization. [269]

The study involving human SC mobilization was triggered by a few cases of empirical evidence that consumption of an extract from AFA enriched for LSL, resulted in unexpected extent of recovery after traumatic injury to CNS. The mobilization of SC is complex but involves two key features; [269]

1- Interference with the adhesion of SCs to the BM via L-selectin and
2- A reduction of the chemotactic response to SDF-1 via the CXCR4 chemokine receptor.

We found that AFA contain a novel compound that specifically bind to the ligand-binding area of human L-selectin. It is composed of two subunits with apparent molecular weight around 54-57 kDa under reducing conditions. This compound differs from the 100,000 kDa from AFA previously described. This ligand for human L-selectin, precipitated from AFA water extract (AFA-W) was able to modulate the functional response on human lymphocytes in vitro and interfered with up-regulation of CXCR4 when BMSCs were exposed to another LSL , fucidan. In parallel to human BMSCs, the primitive $CD34^{bright}$ KGIa cell line was responsive to L-selectin-mediated up-regulation of CXCR4, possibly due to its stage of differentiation being comparative to a subset of BMSCs. The ability of AFA-W to down regulate the expression of CXCR4 on BMSC and KGIa, but not K562, suggests that this ligand could play a role in SC mobilization from BM. **[269]**

This may specifically have relevance for mobilization of SC from BM to the circulation, as it has previously been shown that interference with SDF-1 CXCR4 axis is a primary mechanism of stem cell mobilization from BM. The LSL may have a direct role on SC release, as LSLs have been proposed as a therapeutic method for SC release and increase of the number of circulating SCs.

A double blind placebo-controlled crossover study involving 12 healthy subjects showed that consumption of StemEnhance (AFA extract enriched with this ligand) resulted in small but significant increase of the number of circulating $CD34^{+}$ SCs peaking at one hour after consumption. **[269]** The effect was statistically significant ($p<.0001$). when tested on one

individual on many occasions, the increase in number of circulating SCs after consumption of StemEnhance averaged 52 + or – 16% and varied greatly from 96% to 333% of baseline value. Interestingly, the average response in the one individual tested repeatedly on 16 different study days and the average response to StemEnhance in the double blind randomized study involving 12 people was similar, with an increase in $CD34^+$ cells at 153% vs. 125% respectively. The hypothesis that StemEnhance transiently increases the level of circulating $CD34^+$ cells is supported by significance for difference between the two treatments and the interactions of this difference with person and time. This suggests a significant consistency in the response, despite day-to-day fluctuations, which may contributed to an under-estimation of the response to StemEnhance in the double blind study.

The increase in the number of circulating $CD34^+$ cells peaked within 1 h after consumption of StemEnhance. This is in contrast with the response time seen with the known mobilizer granulocyte colony-stimulating (G-CSF), factor the response of which peaks after a few days of injection. It is believed that G-CSF triggers SC mobilization by activating proteolytic activity in the marrow, which degrades SDF-1, interfering with the SDF-1⁄CXCR4 axis.

More comparable to StemEnhance is the response to CXCR4 antagonist AMD3100 that peaks around 6 hours after injection. This supports the view that the effect of StemEnhance on SC mobilization may be caused by LSL, down-regulating the expression of CXCR4. The magnitude of the mobilization obtained with StemEnhance (18%-25%) is

much smaller than what is seen with G- CSF and AMD3100. Recent studies using the aforementioned factors have added evidence for the potential role of SC mobilizers in the mitigation of different diseases such as cardiomyopathies, kidney failure, multiple sclerosis, stroke, wound healing as well as many other health conditions. Such compounds, however, can only be used for short periods due to their severe side effects moreover the extreme increase in the number of circulating SCs is not required for achieving health benefits. [269]

Tamoda and Aoki (2003) quantified the level of circulating SCs in the victims of AMI and reported that individuals with more SCs showed greater recovery of ejection fraction 6 months after the incident.[370]

Werner et al. (2005) related the risk of cardiovascular incidents in 519 patients with coronary artery disease and concluded that the level of circulating $CD34^+$ endothelial progenitor cells predicted the occurance of cardiovascular events and death from cardiovascular causes. [371]

Another study carried out by **R. Douglas Shytle et al. (2010)**[372] to examine the effect of AFA extract on the proliferation of ASC in vitro concluded that AFA-C (AFA cellular concentrate) ethanol extract when studied in combination with NT-020 (a nutraceutical formulation promotes proliferation of hematopoietic SCs in vitro and protects SCs from oxidative stress when given chronically to mice in vivo) had an additive effect on both human BMCs and $CD34^+$ SCs in vitro. When examined alone the various AFA extracts have modest effects to promote

the proliferation of human BM or human $CD34^+$ SCs in vitro. This preliminary preclinical study suggest that NT-020 plus an ethanol extract of AFA cellular concentrate may promote the proliferation and health of human SC population.

Improved Cellular Repair

"The cell is immortal. It is merely the fluid in which it floats that degenerates. Renew this fluid at intervals, give the cells what they require for nutrition and. as far as we know, the pulsation of life may go on forever."

—Alexis Carrel, 1924

[Carrel, winner of two Nobel Prizes, kept the cells of a chicken alive for 38 years.The cells eventually died because of a custodial error made by his lab technician.]

"On the one hand, immortality releases us from the biological push to survive, which is the basis of egotistical struggle. On the other hand, mortality lends life a time-limited sweetness and preciousness. How interesting that we mortal organisms are composed of 75 trillion cells which have some connection with immortality? How possible is it to connect to both sides of this polarity by embodying both the human and the cellular levels of consciousness?"[373]

—Susan Aposhyayi, 1999

Blue-green algae, scientifically called "cyanobacteria," like other bacterial forms, lack a nucleus. In other words, its genetic material is not surrounded by a nuclear membrane, like that of higher plants and animals.

As Earth's first photosynthesizers, cyanobacteria had to depend on sunlight for their food supply, yet were also vulnerable to damage from that same energy source. "Ultraviolet (UV) radiation has provided a challenge for the evolution of life on Earth. On the one hand. UV

radiation is a mutagen, arguably the most important naturally occurring mutagen. Genetic novelties, the result of mutations, are the raw material of evolution. On the other. UV radiation is a selective agent because it affects metabolic processes from photosynthesis to vitamin D formation, and causes DNA damage. [374]

The effects of solar radiation were especially powerful around 2.5 to 3.8 billion years ago—when blue- green algae were one of Earth's only living inhabitants—and the protective atmospheric ozone levels were less than 1% of present levels. Without today's protective "ozone shield." blue-green algae had to develop a host of powerful antioxidant pigments and protective agents, including carotenoids. and phycobilins. Today all plants can trace their chloroplast genetic structure—their ability to photosynthesize—back to cyanobacteria[375] However, while solar radiation in the visible region is critical for photosynthesis. UV radiation is present as well, and is damaging to life, especially to unprotected DNA close to the membrane's surface.

The amazing thing is how- effective microalgal antioxidant pigment solutions have proven to be! Paleobiologists. such as J. William Schop. describe how some blue-green algal species have changed little in the last few billion years Fossils of blue-green algae from central Australia, dating back more than 3.5 billion years, reveal early forms that are quite similar to living species today. It appears that blue-green algae achieved a sort of biological perfection—with perhaps little need to evolve—accompanied by strong protective mechanisms that minimized genetic mutations.[376]

A surprising variety of important biological mechanisms found in microalgae are characteristic throughout the entire animal kingdom. For example, the energy-producing mitochondria of animal cells are hypothesized by some scientists to share common characteristics with cyanobacteria. Furthermore, primitive molecular mechanisms for vision, movement, circadian rhythms, and even human polypeptide hormone-like substances, such as acetylcholine and melanin, can be found in microalgae species. As in all bacteria, the DNA of blue-green algae forms a simple loop, without chromosomes or a nuclear membrane. Thus, unlike nucleic acids in more advanced plant and animal species, cyanobacteria! Nucleic acids can constitute from 4 to 7% of the microalgae's dry weight and. without a nuclear membrane, are more orally available.[1]

The absorption and metabolism of dietary nucleic acids has received little attention compared to that of other organic nutrients, largely because of methodological challenges in tracking cellular utilization.
Conventional wisdom was that nucleosides played no useful nutritional role and were unable to survive the digestive tract. However, this old perspective is rapidly changing. [1]

Scientists at the University of Goettingen in Germany have found that significant quantities of orally ingested nucleic acids are capable of surviving the digestive process and are absorbed intact in specific cell tissues, as demonstrated by radioactive-tagging studies.[377] Most importantly, orally consumed nucleic acids displayed a strong tissue-regenerative effect. Might algal nucleic acids offer a sort of "bioregeneration effect" that operates at the genetic level?

Additional proof is found in hen and mouse experiments conducted at the Stable Isotope Laboratory in Baylor College of Medicine. Poultry and rodent food rations were supplemented with radioactively labeled blue-green algae Labeled isotopomers of algal dietary nucleosides, pyrimidine and purine, were detected in the experimental animals' isolated hepatic RNA. The researchers observed that large quantities of dietary pyrimidine nucleosides and minimal quantities of dietary purine nudeosides were incorporated into the animals' hepatic nucleic acids, without hydrolytic removal of the ribose moiety.[378] In other words, these laboratory results support the potential nutritional role for nucleosides and suggest that pyrimidines are "essential organic nutrients" that can be genetically incorporated at a cellular level.

Two studies by Devi and his team demonstrated the ability of algal diets to stimulate the regeneration of blood serum and liver proteins in rats.[379, 380] Because microalgal protein is composed of shorter and less complex polypeptide chains—with an abundance of all essential amino acids—it can be more readily utilized at the cellular level. One can think of it as supplying the foundational building blocks for cellular repair in easily usable form.

Might algal diets be able to confer to other cells some aspect of protection from genetic mutations? Researchers at the Institute of Molecular and Subcellular Biology in Slovakia found that freeze-dried AFA blue- green algae demonstrated anti-mutagenic effects on bacterial cells exposed to a mutagen [a substance that disrupts DNA/RNA transcription, causing mutations] using the standard Ames test. When the algae powder was added to the cell culture at the same time as the chemical mutagen, there was no benefit. However, if the algae

powder was added to the cell culture medium 2 to 24 hours before exposure to the mutagenic agent, a significant anti-mutagenic effect was evident.[297]

The most intense suppression of mutagenic activity was achieved when the algae powder was mixed in the cell culture medium 24 hours before the addition of the mutagen. This suggests that the algal phytochemicals were utilized by the cell culture as a protective cellular influence rather than neutralizing the chemical mutagen directly Beta-carotene derived from the microalga Dunaliella demonstrates anti-mutagenic effects on human lymphocytes, as shown in a Chinese study using in vitro micronucleus and chromosomal aberration tests.
The inhibitory effect of microalgae-extracted beta-carotene on mutagenesis induced by both gamma-rays and mitomycin, a known mutagenic agent, was demonstrated.[381]

New research provides evidence that dietary flavonoids (i.e.. pigments) may help repair a range of free radical damage in DNA and offer protection against strand breaks and base alterations in our cells' genetic material.

Scientists at the University of Auckland. New Zealand, demonstrated that antioxidant flavonoids can reduce the incidence of single-strand breaks in irradiated solutions of double-stranded DNA. in vitro. Using advanced pulse radiolysis measurements, scientists found that electron transfer from the flavonoids to free radical attack sites on DNA appears to result in a faster chemical repair, lessening the oxidative damage to DNA.[382]

Steve Gagne, a macrobiotic counselor and author of The Energetics of Food (1990). reports that "Algae are the masters of regeneration—they probably are the most highly regenerative foods on the planet.[383] In support of this empirical observation, it is noteworthy that microalgal extracts added to culture mediums dramatically increase human cell survival rates. In 1984. a U.S. Patent (no. 4.468.460) was granted to S. Kumamoto for "A Method of Human Cell Culture." described as follows: 'A method of culture of human cells is disclosed which comprises effecting the cultivation in a culture medium containing an extract of microalgae. . said method permitting the normal successive cultivation of human cells to be maintained efficiently without any morphological and generic mutations over a greater number of successive generations than has hitherto been possible .[384]

Dr. John Apsley states. "I have spent nearly 20years focusing on the places within biology where the Regeneration Effect appears the strongest. In my opinion, the most rewarding places to study surround undenatured chlorophyll and raw nucleoproteins Drawing from the Nobel Prize-winning work of Dr. Alexis Carrel. Apsley notes that regenerative food is best obtained from young, "embryonic" cell cultures. Microalgae can be considered an embryonic cell source, since in a suitable environment they may undergo rapid and massive cellular replication within a single day or two. Furthermore, according to Carrel, avoiding excessive heat is essential as most "regenerative effectors" are destroyed when heated above the body's physiological temperatures. Finally, optimal regenerative foods contain a wide and balanced spectrum of trace minerals, vitamins, all 10 essential amino acids, and essential fatty acids, as found in freeze-dried AFA microalgae.[385]

Chlorophyll is considered to be a cell regenerator because its central magnesium atom plays an important role in so many (325) different enzyme systems. Also, as an antioxidant, chlorophyll may help to protect our DNA during cell division, a very vulnerable time in thelife of any cell. Unfortunately, most of the published research on the regenerative properties of chlorophyll is found in older or foreign medical journals, which rely more upon natural therapies.[386-389]

Might a diet supplemented with algae increase life-span? 'In extensive traveling, beginning in 1927. Professor S. Kondo. of Tohoku University, discovered that geographic region and especially diet play a determining part in life-spans of the Japanese people.. On islands and in fishing villages, people eat less rice and salty food and more sea vegetables, with the result that they live longer. The village of Oki Island, in Shimane Prefecture, where the people eat plain food and soybeans and sea vegetables—of which they are very fond—has the highest incidence rate of longevity of any.. place in the nation.. ~ and one of the highest in the world.[390]

EFFECTS OF AFA ON CIRCULATION AND HEART FUNCTIONS

"The human heart so far surpasses all known motors in Junctional capacity that we can hardly hope to improve on it, even with the most ingenious machine produced by man.. .It beats 100.000 times per day. Approximately 40 million times in a year.. It pumps two gallons of blood per minute and 100 gallons per hour, through a vascular system of about 60.000 miles in length—2.5 times the circumference of the earth."

-Bircher-Benner. Nutrition Plan for High Blood Pressure Problems. 1973

Albert Sanchez. Ph.D.. a public health expert who examined obesity and heart disease risk factors in 249 high school students, reported. "We are appalled at the horrendous diet that 80 to 90% of our children are eating." to physicians at the 49th annual conference of the American College of Cardiology in Anaheim, California, in March. **2000**[472] More than 80% of the students ate diets that exceeded the recommended levels of total fat and saturated fat. with more than a third having elevated levels of LDL ("bad") cholesterol. Furthermore, the presence of an early buildup of fatty deposits in the carotid artery, as revealed by ultrasound images, was not uncommon. All these risk factors are predictive of heart disease and serious health problems only a few decades down the road.

Microalgae's potent range of antioxidants, in addition to its healthy balance of EFAs. offer top-quality cardio-vascular support. "A high consumption of fruit and vegetables, which are good sources of antioxidants, is associated with a lower coronary risk. More specifically,

there is evidence of a reduced coronary risk in populations with high blood levels of the antioxidant nutrients, vitamins C and E. Evidence is also accumulating that diabetes and microvascular complications associated with diabetes involve oxidative stress and compromised antioxidant status.. .evidence is sufficiently compelling to suggest that antioxidants are potential therapeutic agents in the above conditions.[437]

Dietary supplementation with algal beta-carotene may normalize the elevated LDL oxidation in patients with diabetes, and thus delay the onset and further development of atherosclerosis in these patients. Twenty patients with long-standing non-insulin-dependent diabetes mellitus were studied in comparison with age- and sex-matched control subjects. Diabetic patients showed overall greater LDL oxidative effects compared to the controls. An algae-derived beta-carotene supplement (60 mg daily dose) was given for 3 weeks. Upon supplementation, a marked reduction in oxidative effects was seen in the patients. Supplementation with algae naturally rich in beta-carotene appears to normalize the diabetic-enhanced LDL oxidation levels and consequently may be of importance in delaying the accelerated development of atherosclerosis in these patients. [473]

Researchers at the University of California - San Franciso found that children with an inherited tendency for high cholesterol levels benefited from antioxidant vitamins. Antioxidants seem to improve the condition of blood vessels by helping to neutralize the free radical molecules or "reactive oxygen species" (ROS) that may prevent endothelial cells lining the blood vessels from releasing nitric oxide. Nitric oxide is responsible for the dilation of blood vessels. [474]

One animal study suggests that a blue-green alga. "Spirulina maxima, may decrease vascular tone by increasing the synthesis and release of bah a vasodilating cyclo-oxygenase-dependent product of arachidonic acid and nitric oxide, as well as by decreasing the synthesis and release of a vasoconstricting eicosanoid from the endothelial cells.[475]

AFA algae has high concentrations of polyunsaturated fatty acids (PUFAs) which account for almost 10% of its dry weight. Even more important, it has a high percentage of the omega-3 fatty acids, comparing extremely favorably with most plants, seeds, nuts, and other microalgae The reason AFA has more concentrated amounts of valuable PUFAs than tropical algae like Spirulina is that it thrives in a much colder environment. AFA compensates for the cold by manufacturing more of the flexible omega-3 and cis-unsaturated fatty acids. (Cis-unsaturated forms of fatty acids are healthier than the trans-fatty acid forms, because their curved shape further contributes to cell membrane flexibility.) Tropical algae appear not to be as good a source of these particular nutrients.

While both omega-3 and omega-6 forms of EFAs are important. omega-3s are seriously lacking in the standard American diet. AFA algae contain an ideal ratio of essential omega-3 to omega-6 fatty acids and are especially high in the essential omega-3 fatty acid, alpha-linolenic acid (3-5% by weight). Greenland Eskimos, whose traditional diet was high in cold-water fish that eat blue-green algae, had little cardiovascular disease.[476]

"EFAs haw lubricating qualities and increase cell membrane flexibility. They are known to reduce blood cholesterol and thus help to

prevent cardiovascular disease. . . EFAs are especially useful because of the efficiency with which they increase the solubility of cholesterol deposits and wash these deposits away from our artery walls.. As the consumption of fish oils or essential fatty acids found in AFA increases, the tendency for blood platelets to aggregate decreases and blood pressure goes down. [477]

Algae-derived omega-3 fatty acids may support heart function, reduce blood viscosity, decrease arteriosclerosis (a disease of hardened arterial walls) and lower high blood pressure, according to research of **Zvi Cohen** at the Laboratory for Micro-algal Biotechnology in Israel and Helen Norman at the United States Department of Agriculture,[478] the flexibility of any cell membrane is directly proportional to the amount and type of polyunsaturated fatty acids (PUFAs) it contains. Research reveals that algae supplementation can significantly reduce high levels of arachidonic acid (AA) in the blood and liver lipids and cause a significant increase in the percentages of the omega-3 polyunsaturated fatty acids (PUFAs). [403]

Dr. Rafail Kushak[483] and colleagues demonstrated that AFA essential fatty acids are more easily assimilated than those of soybean oil and offer superior cardiovascular benefits. While both soybean oil and blue-green algae contain the essential omega-3 fatty acid, alpha-linolenic acid (LNA). the scientists found that rats required triple the amount of soybean oil in their diets to achieve the same level of circulating LNA as rats fed algae. Also, AFA significantly increased both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) blood plasma levels far more effectively than did soybean oil. [520] Both EPA and DHA are essential for optimal cardiovascular and brain function and can be synthesized in the body from LNA.

Other studies suggest not only that blue-green algae provide an excellent PUFA source, but also that algae oils might help the body to better utilize other vegetarian sources of PUFAs. R>r example, while flaxseed oil offers high levels of omega-3s. Many people have difficulty assimilating them from this source. Yet when microalgae is combined or taken with vegetable oils, like flaxseed oil, blood levels of omega-3s are significantly increased. Thus, microalgae's ability to raise PUFA levels might be a two-fold action: both acting as a superior source of PUFAs and helping to aid the assimilation of other dietary PUFAs.

Microalgae-derived DHA supplements markedly enhanced the DHA status levels in serum and platelets of healthy vegetarian subjects Researchers also found a substantial increase of EFA and a lowering of total and LDL-cholesterol: HDL-cholesterol ratios, suggestive of a decreased risk factor for heart disease in the DHA algal supplemented group.[479] Microalgae supplementation may be especially important for vegetarians who have a limited intake of fish and eggs.

Vegetarians, especially vegans who eat no animal products (i.e.. no fish. eggs, or dairy), tend to have lower serum and platelet phospholipid levels of DHA and EPA than omnivores. [480] DHA is found in high levels in the brain and retina, where it functions in mental performance and visual acuity, respectively [481] EPA and DHA may help to reduce the risk of developing cardiovascular diseases. Although vegetarians tend to have a lower risk for cardiovascular disease, due partly to their lower serum cholesterol levels, their thrombogenic (stroke) risk factors may be significant. [482]

The ability of AFA algae to lower blood cholesterol in animals was also reported by Kushaks Massachusetts General Hospital research group. Rats fed diets supplemented "with 10% and 15% algae decreased their blood cholesterol level to 54% and 25% of...[the levels of the] rats fed the standard diet." The researchers concluded that the cholesterol-reducing effect of AFA is probably influenced by something other than just its fatty acid content. because both experimental and control diets were rich in PUFAs They propose that microalgal pigments, such as chlorophyll, might contribute to this cholesterol-reducing effect. [483] Several prior studies had demonstrated lower blood cholesterol levels with the dietary intake of microalgae. [484-486]

Japanese White rabbits fed on a ten-week load of high-cholesterol diet and powdered Chlorella, showed a significant suppression of total and beta-lipoprotein cholesterol levels, along with less aortic atheromatous lesions. However, rabbits in the control, with no algae in their diet, showed a dramatic increase in serum total cholesterol and beta-lipoprotein cholesterol levels, with resulting symptoms of atherosclerosis.[487]

Chlorella. both in powder form and in glycolipid (GL) and phospholipid (PL) extracts suppressed the increase of serum cholesterol levels caused by the administration of a high-cholesterol diet to rats. There was no significant difference between using the algal powder form or the extracts in terms of effect on the serum lipid. But the algal extracts were more effective in increasing the fecal excretion of steroids (mostly cholesterol, deoxycholic. and lithocholic acid). It was concluded that algal extracts inhibited the absorption of exogenous steroids and promoted the turnover of bile acids in the liver, which suppressed the

increase of serum cholesterol levels caused by the high-cholesterol diet.[488]

Scenedesmus (a green microalga) powder fed to rats on a cholesterol-enriched diet prevented the excessive deposition of cholesterol in their livers. The algae-supplemented rat livers weighed about one third less than the control rats. Also the levels of plasma cholesterol and triglycerides were significantly improved in the algae-supplemented rats.[489]

The docosahexaenoic acid (DHA) and alpha-linolenic acid (LNA) in certain microalgae support heart function and may help prevent heart attacks.[490] Also, the carotenoids that are so plentiful in microalgae may reduce the risk of heart disease. [190,491]

Homocysteine blood levels are a significant predictor for risk of heart attack, the number one killer of adults in America.[392] Importantly, homocysteine can be transformed into the amino acid methionine—its beneficial alter form—with the help of B vitamins; especially folic acid in conjunction with B-6 and B-12. Microalgae contain a variety of B-vitamins and methionine.

Dietary hyperlipidemia caused by a high-fructose diet in rats was improved by blue-green algae supplementation. Male rats fed on a high-fructose diet (68%) showed heightened hyperlipidemia. while experimental, algae-supplemented rats had increased lipoprotein lipase enzyme activity, along with suppressed levels of hyperlipidemia.[492]

Hawaiian scientists have developed a way to grow and extract heart-healthy substances from microalgae. Currently a randomized, double-blind trial is underway in association with Michigan State University, to evaluate whether astaxanthin, a natural antioxidant from microalgae, reduces blood serum levels of C-reactive protein (CRP). CRP is an indicator of low-grade arterial inflammation and one of the single strongest predictors of risk of future heart problems in apparently healthy men and women.[493]

Dr. Krylov (University of Illinois) and colleagues concluded, after examining case histories of patients with hypertension who had consumed AFA blue-green algae, that this particular algae appears promising for the treatment of hypertension.[166]

Neurological aspects of AFA

"Blue-green algae and my new diet have helped me focus and concentrate better in school and on my homework. I am more relaxed and I don't have stomachaches anymore. I have more friends now and my mom is happier too."

—Chelsea, 8 years old

INTRODUCTION

The brain contains and uses one of the highest concentrations of nutrients of any organ in the body Oxygen consumption is the best indicator of "fuel use"—almost everyone recognizes how vital oxygen is to the brain. Unlike many organs (e.g., the liver) that have cellular fuel reserves, the brain is almost entirely dependent upon a continuous blood supply for fuel. Children's brains are even hungrier, more metabolically active, and proportionally larger than adults' brains. Per pound of body weight, children eat more food, drink more fluids, and breathe more air than adults, thereby increasing their potential exposure to toxins. Also, younger children's blood-brain barriers and intestinal linings are not as developed and are therefore less protective than those of most adults. This means that more incompletely digested foods and toxins can leak into a child's bloodstream and brain.[1]

All these factors contribute to children's heightened susceptibility to dietary imbalances. The increased susceptibility of children to neurotoxins from what they eat, breathe, and drink, is likely contributing to the epidemic of neurobehavioral problems sweeping our country today.[588] For example, many pesticides used on food crops are

specifically designed to attack the nervous system of pests. Vigorous industrialization and urbanization creates a greater discharge of hazardous environmental wastes. Given that our nervous systems are designed to be highly sensitive to environmental changes, the brain is especially vulnerable to the effects of pollution and stress.

The chemistry of the brain and nervous system is characterized by a heavy investment in lipid chemistry which accounts for up to 60% of its structural material.[589] Recent fossil evidence indicates that the rapid expansion of our species' ancestral archaic human brain took place in coastal areas, where aquatic food rich in long-chain polyunsaturated fatty acids (PUFAs), such as algae, mollusks, crustaceans, and fish, was abundant.

Some brain researchers have suggested that the development of the human brain—which requires up to 10 times as much energy as that of other land-based mammals—depended on a rich source of essential fatty acids, especially DHA (docosahexanoic acid). DHA is found in marine and coastal food chains, but is not so easily obtainable on land.[590,591] As the ultimate source of essential fatty acids in the food chain, algae may have significantly contributed to the evolution of the human brain.

In addition to providing an excellent source of PUFAs, microalgae potentially offer other neurologically active substances. Researchers in Spain have described the effects of several species of algae on the central nervous system. Aqueous extracts of two species of microalgae showed antidopaminergic effects and anticholinergic properties. [592,593] Extracts of two other microalgae species showed promise as a central nervous

system (CNS) depressant and a potential muscle relaxant.[594] Certainly the CNS effects of microalgal species may vary considerably, but biologically active constituents are likely.

AFA has high concentrations of the building blocks "neuropeptides" to help repair, rebuild and strengthen neurotransmitters in the brain, so that the brain's neurons, or nerve cells, can communicate at optimum effectiveness with the rest of the body, which is great for ADD/ADHD children. Interestingly, nearly 50% of its lipid content is the essential fatty acid alpha-linolenic acid (Omega-3). Omega-3 fatty acids support the immune system and build the white fatty myelin sheath on connective neural fibers in the brain. People who eat AFA on a regular basis report an overall increase in mental alertness, mental stamina, short and long term memory, problem solving, creativity and greater sense of well being.[632]

AFA has a full spectrum of naturally chelated minerals and trace minerals, giving the body the best access to these vital nutrients. There is also a wide range of vitamins found in AFA. One gram of Wild Blue Green Algae gives 48% of the recommended daily requirement of Vitamin B-1 (beta carotene), 133% of Vitamin B-12, and significant amounts of all the B Vitamins. B vitamins are required for optimum nerve function, something that ADD/ADHD children often lack. [632]

The cell protein wall of AFA is a source of glycogen, used by the liver for energy, which is one reason why people often report an increase

in energy once they start eating it. AFA gives the body many nutrients difficult to obtain from other sources, which could be why many people who eat it report that it has helped offset obesity, depression, autism, hypoglycemia, diabetes, ADD/ADHD, anemia, ulcers and many other symptoms of nutritional deficiency. [632]

In brief,[314] rats fed a PUFA-deficient diet (coconut oil) supplemented with AFA (which contains mostly alpha-linolenic acid) showed blood levels of alpha-linolenic acid (LNA; 18:3w3) eicosapentaenoic acid (EPA; 20:5w3) and docosahexaenoic acid (DHA; 22:6w3) greater than levels found in rats fed the control diet containing soybean oil, inspite of the fact that the amount of PUFA in the experimental diet was one fourth the amount present in the control diet.

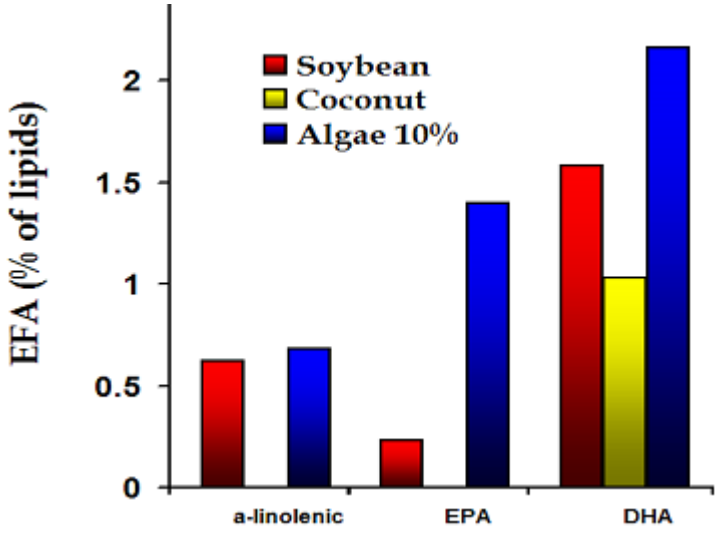


Fig. 61; Adding AFA (an exceptional source of Omega-3PUFA) to the diet was shown to significantly increase the level of blood PUFA.

Increased plasma levels of EPA and DHA have been associated with nervous membrane stability and cardiovascular health, while decreased levels have been associated with Attention Deficit Disorder, depression and cardiovascular diseases.[2]

Moreover Omega-3 fatty acids are an essential dietary factor in combating inflammation. Patients with the lowest levels of essential omega-3 fatty acids tend to be the most severely depressed, while healthy control subjects are more likely to have normal levels of omega-3s, as measured in red blood cell membranes.[620]

Alternative Doctor recommendations for lowering inflammation. Without doubt the most important anti-inflammatory supplements are omega-3 fatty acids EPA and DHA (eicosapentanoic acid and docosoheaenoic acid). In 2006, researchers analyzed results from six published studies on depression and omega-3 fatty acids. They found that omega-3 fatty acids can significantly reduce symptoms of depression among adults.[621] Omega-3s are best obtained from marine sources and true grass-fed beef. Vegetarians will need plant sources, such as flaxseed. Dose: 1000- 2000 mg daily. DHEA, an important hormone precursor, also has anti-inflammatory properties. Add nettle leaf extract and vitamin K. [622]

Note that omega-6 fatty acids, although "essential" by name, are far too prevalent in modern diets and considerably outweigh omega-3s. This creates a relative omega-3 deficiency.

According to a recent study, depressed people have lower levels of omega-3 fatty acids compared with the pro-inflammatory omega-6 fatty acids, and the severity of symptoms correlated with the ratio of omega-6 to omega-3 fatty acids. Moreover inflammatory markers — interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) — showed a direct correlation with the omega-6: omega-3 ratio.[623]

Studies among prison inmates by **Bernard Gesch** in the **UK** report that aggression and violence is reduced, as well as mood improved, by supplementation with omega-3 fatty acids. Apparently the chance of being murdered is 30 times greater in countries with a low fish consumption, according to **Joseph Hibbeln MD**, working at the **US National Institutes of Health (NIH).**[624]

To lower you omega-6 intake, cease eating manufactured food and rely instead of healthy whole foods, such as fruit, vegetables, salads and fish. Consider also enzyme formulas which contain bromelain and papain, possibly with pancreatic extract. These digestive enzymes dissolve and clean up pro-inflammatory chemical compounds.[625]

INTRODUCTION TO NEUROLOGIC DISORDERS

Chronic stress has long been held responsible as a primary contributor to neurotransmitter imbalance. Stress, both physical and emotional can lead to increased neurotransmitter excretion by the neurons in order to help the body cope with the situation. Acute stress is generally well tolerated by the body and normally does not lead to significant neurotransmitter imbalances. In contrast however, chronic stress will tax the nervous system and over time deplete neurotransmitter supplies. Poor dietary habits may also contribute to neurotransmitter imbalance, particularly when coupled with stress. The production of these brain chemicals is dependent upon sufficient levels of amino acid precursors. Diets which are low in protein may limit the supply of these amino acids.[626-631]

In the situation where the neurotransmitter concentrations are too low, they will be unable to simultaneously engage enough of the post synaptic receptor sites needed to continue a message. This disrupts signal transduction and important messages may not be sent. Another common situation is that the levels of certain neurotransmitters become too high and as such, the frequency of inappropriate signals that are relayed increases. This increased signaling can be described as “static” and make it difficult for the neurons to discern between incoming signals that are important against those background signals that should be ignored. [626-631]

Why choose nutrients over drugs?

The pharmaceutical industry has developed hundreds of drugs designed to treat a whole host of mood disorders. The vast majority of these drugs work directly upon the function of neurotransmission. A major drawback of these drugs however, is that they only affect the transportation or release of existing pools of neurotransmitters in the body. If the diet does not provide sufficient amounts of neurotransmitter precursors, then there may not be enough neurotransmitters to properly relay signals within the nervous system, even if the drugs are used. [626-631]

PHENLETHYLAMINE (PEA)

AFA is a Unique Source of PEA(Phenylethylamine) individuals have reported discontinuing their antidepressant medications after a few months of AFA consumption. More generally, people have been reporting an elevation of mood, an enhancement of mental energy and mental clarity, and an increase in quality of life.[1,2]

The type of effect reported by consumers was consistent with the presence in AFA of a neuroactive amino acid or a biogenic amine. An effort was then made to search for such compounds, and recent scientific analysis has revealed the presence of the biogenic amine phenylethylamine (PEA) in AFA. PEA (it is also found in chocolate) is well known to alleviate depression and elevate mood, and it plays an important role in the pathogenesis of learning disabilities and Attention Deficit Disorder. [1,2]

PEA, a compound naturally produced by the brain, is responsible for the feeling of experiences associated with pleasure and mental awareness. For example, when one is absorbed by an activity like painting, sculpting, or reading a fascinating book, when the world around seems suspended and nothing can disturb us, when worries vanish and hunger goes away, in such moments PEA is being produced by the brain. Likewise, PEA is released in the brain when one experiences the feelings of love and joy. For this reason, PEA has been coined "the molecule of love." When taken orally, PEA is known to readily cross the blood-brain barrier and become immediately available in the brain.[577-631]

In the brain, PEA acts by increasing the concentration of dopamine in the synaptic cleft, thereby enhancing dopamine transmission. Dopamine is a neurotransmitter associated with the sensation of pleasure. PEA has also been shown to enhance norepinephrine transmission in the brain. Norepinephrine is also involved in the experience of joy. Enhancing norepinephrine transmission in the brain increases the experience of joy and reduces appetite. For example, if an animal is implanted with an electrode in an area of the brain concentrated in norepinephrine, and this electrode is activated by a pedal that the animal has access to, the animal will disregard food and water and will press the pedal relentlessly until exhaustion to elicit an electrical impulse in this area of the brain. [577-631]

This ability to modulate dopamine and norepinephrine transmission provides PEA with interesting properties in alleviating depression and Attention Deficit Disorder, while increasing concentration and elevating mood. [577-631]

In many previous Nutritional News articles, we have addressed some of the major players involved in mood disorders– such as serotonin, dopamine, glutamate, gaba, norepinephrine and epinephrine. In this issue, we would like to focus on phenylethylamine (PEA) which is a stimulatory neurotransmitter that increases mental activity and alertness. PEA is classified as a minor neurotransmitter, this means that there is less of it; however it does not mean that it has any less significance than some of the other more well known and studied neurotransmitters. Early findings originally suggested that PEA was only a neuromodulator in that it altered the function or release of the major neurotransmitters, however in 2001 scientists discovered a receptor specific for PEA. This then meant that classification for PEA changed from a neuromodulator to neurotransmitter as PEA has the potential to relay messages on its own.[577-631]

Where does it come from?

PEA is synthesized from the amino acid phenylalanine via aromatic amino acid decarboxylase. It can be seen in the figure 62 that phenylalanine, tyrosine and L-DOPA are all amino acids which can be used to support catecholamine synthesis. Phenylalanine however, will also increase PEA levels while the other amino acids further downstream, will not affect PEA. Understanding of this biochemical pathway becomes very important when choosing appropriate nutritional interventional therapies.[626-631]

What role does PEA play in specific clinical conditions?

When taken orally, PEA is known to readily cross the blood-brain barrier and be immediately available in the brain. It is normally rapidly degraded by the enzyme monoamine oxydase (MAO), however AFA was

also shown to contain compounds that inhibit MAO activity, providing for a long-term modulation of brain activity. [626-631]

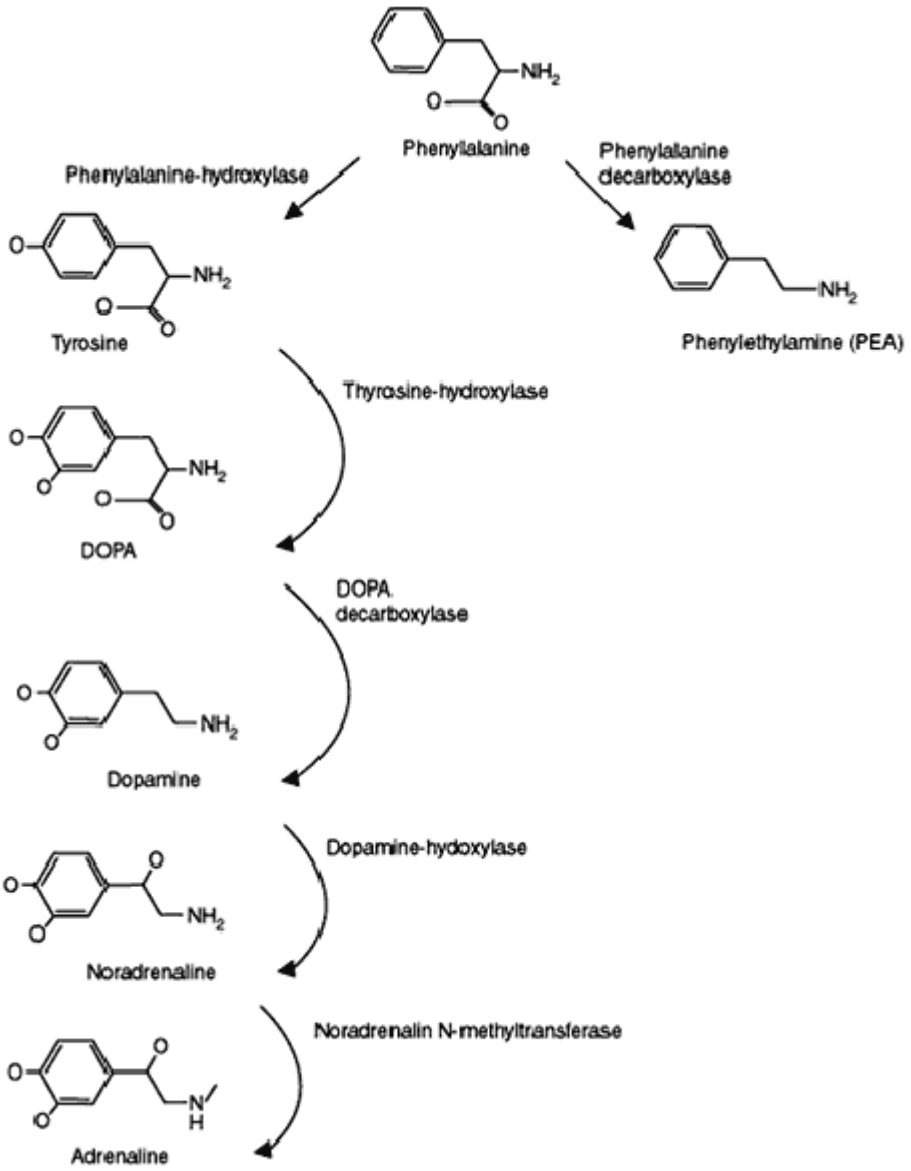


Fig. 62; PEA structure [631]

In the brain PEA acts by having a greater affinity for the re-uptake mechanism for dopamine in pre-synaptic vesicles. Therefore, when present in the brain, PEA is captured into the pre-synaptic vesicles and

occupies the space normally taken by dopamine. This leads to an increase in free circulating dopamine in the pre-synaptic terminal and a higher concentration of dopamine diffusing into the synaptic cleft, enhancing dopaminergic transmission. [577-631]

ROLE OF DOPAMINE TRANSMISSION IN PLEASURE

Dopamine is one of the main transmitters responsible for the sensation of pleasure. Many compounds known to alleviate depression are known to enhance dopamine transmission. Dopamine is also associated with attention and concentration. [633,634]

• The dopaminergic and opioidergic reward pathways of the brain are critical for survival since they provide the pleasure drives for eating, love and reproduction; these are called natural rewards' and involve the release of dopamine in the nucleus accumbens and frontal lobes. However, the same release of dopamine and production of sensations of pleasure can be produced by 'unnatural rewards' such as alcohol, cocaine, methamphetamine, heroin, nicotine, marijuana, and other drugs, and by compulsive activities such as gambling, eating, and sex, and by risk taking behaviors. [633,634]

However, serotonin, norepinephrine, GABA, opioid, and cannabinoid neurons all modify dopamine metabolism and dopamine neurons. We have proposed that defects in various combinations of the genes for these neurotransmitters result in a Reward Deficiency Syndrome (RDS) and that such individuals are at risk for abuse of the unnatural rewards. [633,634]

ROLE OF AFA IN DEPRESSION

It was discovered more than two decades ago that the amount of PEA in the brains of depressed patients was less than that of normal individuals and that PEA given orally to individuals suffering from depression was able to reverse the depressive condition.[577-579]

Oral intake of PEA may increase PEA levels in the brain and may alleviate subclinical symptoms of depression. A decrease in the brain levels and/or turnover of endogenous PEA may therefore play a major role in the etiology of certain forms of depression. In fact, it has been observed that many antidepressant drug treatments act by increasing the level of PEA in the brain.[580-583]

In one study, when taken orally (10 mg/day), PEA was shown to decrease the symptoms of depression in 60 percent of the patients tested. The patients did not develop tolerance, and PEA remained effective over time. None of the side effects associated with conventional antidepressant therapy was experienced (i.e., nausea, fatigue, decreased libido, cardiovascular problems). On average, patients did not gain weight, in fact many actually lost the weight they had gained on the previous conventional antidepressant therapy. [579]AFA contains on average 2 mg/g of PEA, and an AFA extract has been developed that contains up to 10 mg/g of PEA. Daily consumption of one gram a day of AFA extract could constitute an effective therapeutic approach in the treatment of depression and other affective disorders.[584]

In some studies, patients with depression have exhibited decreased PEA levels, whilst some schizophrenic patients and other psychopathic subjects were associated with increased levels. In other research, administration of the precursor amino acid phenylalanine has demonstrated the ability to improve depression on its own and even the therapeutic outcome when used as an adjunct to some antidepressants. PEA has also been associated with the antidepressant effects after exercise. This observation determined that the excretion of a PEA metabolite increased by 77% during a 24 hour period which included 30 minutes of moderate exercise compared to the previous 24 hour period in which subjects abstained from physical activity.[627]

Biochemically, PEA acts as an excitatory neurotransmitter and modulates neuron potentials to favour glutamate activity and neurotransmitter firing. As such, patients who have low PEA and related symptoms such as depression and fatigue are most likely to have the best outcome when their therapies include products like Orthoplex Inkephalin which contains 400mg of phenylalanine. In saying this, products which contain phenylalanine, may not be suitable for patients who present with anxiety or insomnia and potentially have elevated PEA levels.[627]

Phycocyanin, the blue pigment in AFA, is a natural selective COX-2 inhibitor with strong anti-inflammatory properties, which suggests it may also act directly against the inflammatory component of depression.[2]

ROLE OF AFA IN ATTENTION DEFICIT DISORDERS

PEA AND ATTENTION

Decrease in the concentration of PAA in urine has also been associated with attention deficit disorder (ADD), and oral intake of PEA was shown to alleviate ADD.

Baker et al. (1991)[628] examined Urinary excretion (24-hr) of beta-phenylethylamine (PEA), phenylacetic acid (PAA), phenylalanine (Phe), and p-tyrosine (Tyr), and plasma levels of PAA, Phe, and Tyr were in 18 normal children and 26 children diagnosed as having attention-deficit hyperactivity disorder (ADHD). The results indicated that urinary excretion (expressed per g of creatinine) of free and total PEA was significantly lower in the ADHD patients, and plasma levels of Phe and Tyr were also decreased in the ADHD subjects compared with the normal controls.

However, unlike amphetamines, PEA is endogenous to the brain and PEA may prove to be a safe and effective alternative for the treatment of ADD, does not develop tolerance or dependency, nor does it produce any side effects. Likewise, methylphenidate (Ritalin®), the most prescribed drug for the management of ADD, is believed to act by stimulating the release of endogenous norepinephrine and PEA. PEA may therefore prove to be a safe and effective alternative for the treatment of ADD. In fact, preliminary data indicates that AFA has been effective at

significantly improving concentration and mental performance shortly after consumption.[586-586]

Marnee Foldoe's Sonoma State University master's thesis documented that eating AFA improved Attention Deficit/Hyperactivity Disorder (AD/HD) symptoms in three elementary school students. She examined the children's, teachers', and parents' attributions as to what caused the noticeable improvements in these diagnosed children. Interestingly, none of the teachers attributed the improvements to the dietary intervention, whereas the parents were convinced that eating the AFA made the biggest difference. Only the children reported that multiple factors were involved—their own volition, eating AFA. and social factors. Unfortunately, teachers in this small sample appeared to be completely unaware that diet could have any strong influence on AD/HD.[611]

A team of medical researchers headed by Dr. Krylov of the University of Illinois concluded, after examining hundreds of well-documented case histories, that AFA appears promising for the treatment of depression and AD/HD as well as several other health challenges. [166]

A pilot study of several thousand AFA consumers indicated that symptoms of AD/HD, depression, and memory difficulties improved with AFA consumption. This study was based on medical questionnaires similar to those used in a wide-scale health survey by the National Center for Health Statistics. In addition to specific improvements in a number of areas, the study found that people consuming AFA reported significantly

better overall health than that reported by the general population in the larger survey. [296]

British biochemists at the Institute of Brain Chemistry and Human Nutrition in London report that any deficiency in essential fatty acids (EFAs) during early brain development in childhood can greatly increase the risk of learning disorders. Nervous tissue contains 50 to 60% lipids on a dry weight basis. Lipids play an essential role in the structure, fluidity, and function of brain membranes.[612] AFA blue-green algae is especially rich in PUFAs which are very important in maintaining membrane fluidity, comprising up to 10% of its dry weight.

Animal research at Massachusetts General Hospital and Harvard Medical School found that AFA algae dramatically raised blood levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are known to be important for optimal functioning of numerous organ systems, including the nervous system. They are extremely difficult to obtain in the modern American diet. The researchers found that AFA was far more effective than soybean oil, a good source of PUFAs, at raising blood levels of these important omega-3 fatty acids. [314,613]

Children with AD/HD are more likely to show deficiencies in DHA than children without attention problems, and EFA supplementation has shown initial promise in the treatment of AD/HD[614] Gamma-linolenic acid (GLA), a powerful fatty acid found in AFA. may be helpful as well.[615] GLA has been linked to the release of neurochemicals that improve mental attitude, increase alertness, and reduce depression.

Attention deficit/hyperactivity disorder is a leading mental health problem in children. Depression is one of the leading causes of disability in the world today. [616] Both of these brain-related problems are associated with difficulties in arousal. While AD/HD children appear hyperactive, many scientists believe this is actually a problem of under-
arousal, which increases symptoms of self-stimulation, risk-taking, and poor attention. This helps to explain why amphetamines (stimulant drugs) are prescribed to many children with AD/HD. Depression in children may sometimes even appear like AD/HD. Are there any links between the epidemic of hyperactivity in children (with acute arousal symptoms) and depression in adults (with chronic arousal symptoms)?

Although these two brain-related problems are usually diagnosed and treated as different "disorders," Larry Christensen in his survey of research on diet and behavior found evidence that depression and AD/HD are the two types of behavioral disorders most likely to respond to dietary intervention. [617] Interesting? Given that these two conditions appear to be increasing, what might this suggest about the standard American diet (SAD)?

Two expert opinions:

John Taylor, Ph.D., psychologist, author, and AD/HD expert states, I have been in a position to talk with thousands of parents and professionals very frankly about AD/HD. And blue-green algae is consistently mentioned to me by parents as being of help for children with AD/HD.[618]

Edward Hallowell, M.D., is the author of the New York Times best seller, Driven to Distraction, and a leading authority on learning disabilities, particularly AD/HD. In an appearance as a Keynote speaker at the 1998 Pacific Region Learning Disabilities Drug Treatment Conference in Honolulu, Hawaii, Dr. Hallowell reported that blue-green algae appears to offer a promising non-drug alternative for people with AD/HD. [619]

AFA ANTI-STRESS EFFECTS

Male rats exposed to water immersion-induced stress (i.e., experimental near-drowning) showed significantly fewer gastric mucosal lesions when fed Dunaliella bardawil (whole green microalgae) as compared to an isolated synthetic beta-carotene control group.[595] Additionally, oral administration of unicellular green algae, Chlorella vulgaris, was found to prevent stress-induced ulcers in rats, as well.[596] Researchers hypothesized that microalgae prevents ulcer formation primarily through the "immune-brain-gut" axis, and secondarily through possible gastric mucosa protective factors.

In the last decade or so, medical science has determined that most gastric ulcers involve a bacterial infection, and antibiotics are often the treatment of choice. Evidently, blue-green algae may be helping through a number of different possible mechanisms: an enhancement
of the immune response, direct antibacterial effects, general support for the healing process(enhancing tissue regeneration and modulating inflammation), or possibly by providing the trace mineral, bismuth, known to be effective in healing ulcers caused by the bacterium H. pylori. Anecdotal and experimental evidence from humans ingesting AFA blue-green algae is suggestive of an adaptogen, "anti-stress," effect. [151,599]

Beta-carotenes and antioxidants found abundantly in microalgae may contribute to protecting the central nervous system (CNS) from oxidative stress. Lipids, which comprise most of the brain tissue, are especially sensitive to oxidative damage. Researchers in Israel and Japan have demonstrated the protective effects of antioxidants in experimental

animal brain trauma models [600,601] Oxidative stress has been implicated in the pathogenesis of some disorders of the brain; hence antioxidants have become attractive therapeutic agents. [602] Furthermore, brain trauma and injury tends to increase whole-body oxidative stress.[603]

Scientists at the National Institute of Nutrition in Hyderabad, India, demonstrated that blue-green algae offers a cost-effective source of antioxidant carotenes for children[604] Microalgae is one of the richest natural food sources of carotenes, including beta-carotene.

Beta-carotene offers powerful anti-cancer, anti-aging, and antioxidant properties without the toxic risks of taking fat-soluble vitamin A.

EFFECT OF AFA ON LEARNING

At least six research studies have demonstrated the benefits of AFA on improving children's cognition, mood, behavior, and academic performance:

Sevilla and **Aguiree's** study of 1,567 students at the Monsenor Velez School in Nandaime, Nicaragua, demonstrated an 81% increase in the average standardized test scores among malnourished children eating only .5 to 1 gram of AFA a day over a six-month period. Subjects showed significantly increased classroom attendance and participation, as well as marked improvement in overall health. Academically, the Velez school went from having one of the lowest national scholastic test scores to achieving one of the best. [150]

Claudia Jarratt, family therapist at the Center for Family Wellness in Harvard, Massachusetts, studied 105 children given AFA and found a significant improvement in behavior as shown by both parent and teacher ratings. The children, who displayed a variety of behavioral problems, consumed between 0.5 and 1 gram of AFA daily and were observed over a ten-week period. Data from the Achenbach Child Behavior Checklists (parent and teacher versions) and extensive case histories were collected for all participants. Significant improvements were found on all 11 parent rating scales in pre- to post-test behavior. These findings were corroborated by teachers' ratings, which revealed significant improvements in seven of the ten behavioral problem areas measured. The use of an expectancy scale revealed little correlation between parents' initial expectations of treatment benefits and final outcomes.[609]

Subsequently, Claudia Jarratt has continued to work with an additional 250 children, using an AFA-based program, with similar positive results.[610]

Bruno J. (2011) and his research team studied 26 students with reading difficulties, who participated in a three-month AFA supplement study. All were enrolled in the Stilwell Learning Center, a reading tutorial program in Sierra Vista, Arizona. Participants included 18 boys and 14 girls, ranging from 6 to 17 years old, with a mean age of 11. The children were randomly assigned to one of two groups, (1) the low-AFA. 1.5 grams, treatment group or (2) the high-AFA. 3 grams, treatment group. There was also a non AFA comparison group.[151]

Both AFA treatment groups showed significant improvements on the following measures over the three- month trial period: attention and concentration indices, a sequential memory index, standardized academic testing, behavioral parent and teacher reports, health symptoms, tutorial attendance records, and decreased toxic levels of aluminum. Regardless of the assigned treatment group (i.e., high or low), both groups demonstrated significant improvements compared to pre-test baseline measures and a small non-supplemented comparison group.[151]

ROLE OF AFA IN CNS TRAUMA AND NEURODEGERATIVE DISEASES

Erythropoietin is a glycoprotein and together with its receptor (EPO-EPOR system) was initially documented to be produced by interstitial fibroblasts in the kidneys of the adult and in hepatocytes in the fetus. EPO is circulated towards the bone marrow where it regulates red cell production by preventing apoptosis.[635] Recently it was reported that EPO-EPOR system is expressed also in the brain[636,637] where EPO exerts its biological activity through binding to its cognate receptor EPOR, possibly activating the Janus kinase-signal transducer. It affects neurons and astrocytes [638,639] to possibly exert protective effect during neurodegenerative diseases[640,641] although long-term cognitive outcomes and optimal dosing regimens have not been clarifed. This has also been shown during acute illnesses such as traumatic brain injury, ischemia and stroke.[643,645]

In particular, it targets the vascular endothelium by stimulating neurovascular protection and angiogenesis[646], without enhancing vessel permeability,[647] unlike VEGF which may bring about bleeding phenomena and haemodynamic derangement. [648,649] It is known that oxidative stress is implicated in brain aging processes[650] thus, antioxidants play an intriguing role in preventing brain degeneration and may inhibit the age-related deficits in motor learning and memory ability.[651]

It has been shown that the age-dependent oxidative processes can be moderated by the application of various antioxidants, such to improve the brain morphology and the motor learning in aged rats.[652,653] De la Fuente

et al.[654], by using the T-maze test as a clear-cut parameter, have shown that some mice express overt features of premature aging with immunologic impairment and a shorter life span when compared to their age-matched fast-performing counterparts and this may represent a useful model to test pharmacological and nutraceutical intervention.

Among the latter, the microalgae of lake Klamath has always been appreciated not only for its peculiar nutritional properties, but also for its significant positive effects on mental attention, mood and anxiety.[655]

Sederiep S et al (2011) [655] examine the effect of Klamin®, a nutraceutical containing phenylethylamine, phycocyanins, mycosporine-like amino acids and aphanizomenon flos aquae-phytochrome on the learning and memory ability, the oxidative status and cerebral erythropoietin and its receptor EPO/EPOR system in prematurely senescent (PS) mice and concluded that this specific alga Klamath extract has considerable antioxidand and adaptogenig properties also through a stimulatory effect of cerebral EPO/EPOR system.

ROLE OF AFA IN TREATMENT OF ALZHEIMER'S DISEASE

Alzheimer' disease (AD) is a progressive neurodegenerative disorder that represents the major cause of dementia in the world today. Over 5 million persons in the US currently suffer from this fatal disease and that number is expected to quadruple by the year 2050, unless prevention efforts or disease modifying therapies are developed.[656,657]

The annual costs of care for persons with AD severely tax the health care system today, and are predicted to singlehandedly create a future health care crisis. AD is characterized clinically by the development of early amnestic and executive dysfunction, that eventually spreads across cognitive domains, that leads to the complete incapacity and development of end-stage dementia.[658] The major pathological hallmarks of AD are extracellular amyloid (Aβ) plaque deposition and intraneuronal neurofibrillary tangle formation.[659,660] Emerging evidence suggests that progressive inflammation and increased oxidative stress play a key role in the early development of such pathological features.[662-664]

Such mechanisms have also been clearly posited to play a key role in synaptic dysfunction and the loss of neuronal integrity that may precede the overt appearance of amyloid plaques and neurofibrillary tangles in the brains of affected individuals. Cellular pathways involved in the homeostasis and regulation of brain fatty acids are central players in both the inflammatory and oxidative stress cascades implicated in the pathogenesis of AD. [662-664]

Data from the cross-sectional, epidemiologic, and prospective cohort studies of the associations between dietary omega-3 PUFA intake and cognitive status have been largely positive. Dietary habits in these cohorts are likely longstanding rather than short-lived, as in a clinical trial paradigm. Docosahexanoic acid (DHA), the major omega-3 fatty acid found in neurons, has taken on a central role as a target for therapeutic intervention in Alzheimer's disease (AD). Several clinical trials investigating the effects of omega-3 fatty acid supplementation in AD have been completed and all failed to demonstrate its efficacy in the treatment of AD. However, these trials produced intriguing data suggesting that the beneficial effects of omega-3 fatty acid supplementation may depend on the stage of disease, other dietary mediators, and apolipoprotein E status.[664]

Other considerations include the effect of timing omega-3 PUFA supplementation over the lifespan. Several studies have convincingly demonstrated that omega-3 PUFA supplementation may be more beneficial if administered prior to or in the earliest stages of cognitive decline. It may be that increased omega-3 PUFA levels are protective against cognitive decline and AD, but later play no role in the pathogenic cascade of events that culminate in the clinical and biological expression of fulminate AD, even in the early stages of disease. Alternatively the beneficial effects of omega-3 PUFA augmentation may still exist, but simply be overwhelmed by the strength of the pathological processes responsible for AD once the disease has produced the clinical and biological manifestations of moderate or more severe disease. Lessons learned from the past might suggest a focus on earlier or even preclinical stages of AD as a target for omega-3 PUFA intervention. [664]

Researchers at Erasmus University Medical School in the Netherlands conducted a three-year study of 5,100 people between 55 and 95 years of age and found that beta-carotene molecules acted as "tiny molecular shields" and may provide dramatic protection against the ravages of aging, memory impairment, and general brain damage. These Netherlands findings suggest that beta-carotene foods, like microalgal foods, need to be further investigated with our aging population. Microalgae might also provide a cost-effective way to reduce brain-related risks of aging. As "baby boomers" turn into "senior boomers," the number of Americans with Alzheimer's disease is projected to increase by more than 300%.[663]

In **1985, Gabriel Cousens** published two case studies on the use of AFA blue-green algae in the improvement of Alzheimer's disease. He reported "some significant return of function" such as decreased hand tremors, better balance, and improved short term memory, attention span, judgment, and reasoning in one patient; in the second patient there was no significant return of previously lost function, but there was a halting of the typical "progressive degeneration associated with Alzheimer's" along with a corresponding improvement in the patient's marital relationship. [607]

ROLE OF AFA IN HEALING OF CNS TRAUMA

Andrew Valencia[413] and colleagues at the Neurolab Clinic associated with the University of New Mexico demonstrated that patients suffering from mild brain injury who ate AFA showed a 25% improvement in about half the time as patients who did not receive algae. According to

Valencia, in his study of more than 150 patients over two years, patients who ate AFA algae alone had improvements similar to those in a two-month hospital-based rehabilitation program. However, the best results were achieved when neuro-rehabilitation was combined with eating AFA algae, better than AFA alone or the hospital program alone. Valencia's research team hypothesized that AFA algae seems to promote reparative neuroplasticity—or, in lay terms, rewiring of the circuitry of the brain. [413]

Valencia also conducted electrophysiological studies of brain waves and found that the ingestion of AFA algae was linked with pronounced improvements in brain function, notably in the ability to focus and discriminate between various auditory signals. [413]

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a progressive disorder of the nervous system, where the lipid myelin sheath surrounding the nerves is gradually destroyed. Deficiencies of omega-3 oils may contribute to the syndrome of MS. Improved MS with AFA is multifactorial i.e through nutritional supplementation, stem cell mobilization and anti-inflammatory and antioxidant effects.

AFA AND CANCER

"At present we have overwhelming evidence... (that) none of the risk factors for cancer is. . .more significant than diet and nutrition.''

—B. Reddy. Committee on Diet. Nutrition and Cancer 1992.

"Today, treating cancer is a huge business. Every 30 seconds another American is diagnosed as having the disease. Typical cancer patients spend over $25,000.00 to try to treat their condition... Every 55 seconds, another American dies of cancer."

—J. Robbins. Diet for A New America. 1987.

America has poured about 200 billion dollars into cancer research since President Nixon declared war on cancer in 1971. How is the war going after three decades? Why do assessments vary as widely as "beating cancer" and "loosing the war on cancer?"

Sunny Y. Auyang, Cancer causes and cancer research on many levels of complexity2004.

The Underlying Causes of Cancer

Cancer, defined as cellular replication that has gone away, is the second leading cause of death in America. Cancerous cells are always being created in the body. It's an ongoing process so parts of your immune system are designed to seek out and destroy cancer cells.

Cancer has been around as long as mankind, but only in the second half of the 20th century did the number of cancer cases explode. Cancer tumors begin when more cancerous cells are being created than an overworked, depleted immune system can destroy. Constant exposure to tens of thousands of manmade chemicals from birth onward, chlorinated and fluoridated water, electromagnetic radiation, pesticides and other toxins, leads to the creation of too many free radicals and excessive numbers of cancerous cells. Alone this would be enough to raise cancer levels, but combined with an immune system weakened by a diet of refined and over processed food, mineral depleted soils, and too much exposure to artificial light at night, the immune system at some point no longer is able to keep cancer in check, and it starts to grow in your body.

Research shows that the immune system needs 9 1/2 hours of sleep in total darkness to recharge completely -- the authors of the book **Lights Out** explain. When was the last time you had this much sleep?

The Guardian, in Wednesday 7 December 2011[540] published the following data about underlying causes of cancer;

Cancers could be prevented by lifestyle changes in over 40% of cases: Drinking and smoking less and losing weight could save 134,000 cancer diagnoses each year, Cancer Research UK finds

It’s clear that around 40% of all cancers are caused by things we mostly have the power to change,' study author Professor **Max Parkin** said. Around 40% of cancers in women and 45% of those in men could

be prevented by a healthier lifestyle, including drinking less, smoking less and losing weight, according to the most comprehensive study of the risk factors to date.

Cancer Research UK, which funded the work, says that around 134,000 people could be spared a cancer diagnosis if they took more care of themselves. Although the biggest risk factor by far is smoking, which caused 23% of cancers in men and 15.6% in women, there were also some surprises. Putting on too much weight, for instance, was found to be a greater risk for breast cancer in women than drinking alcohol, and diet accounted for 9.2% of all cancers if a lack of fruit and vegetables, too much salt and red meat and too little fiber were all included.

Obesity and being overweight were a cause of 5.5% of cancers overall – but rose to 6.9% (10,800 cancer cases) in women compared to 4.1% in men (6,500 cases). The reason for the difference is mostly breast cancer – in post-menopausal breast cancer, being overweight is a factor in 80% of cases.

Alcohol was implicated in 4% of cancers overall (4.6% in men and 3.3% in women). Too much sunbathing and the use of sunbeds caused 3.5% of cancers. Men had a higher risk of cancer as a result of failing to eat the recommended five portions a day of fruit and vegetables – in men this was a risk factor in 6.1% of cancers, where in women it dropped to 3.4%.

The study is published in the **British Journal of Cancer. Prof Max Parkin**, a Cancer Research UK epidemiologist based at **Queen Mary, University of London,** said: "Many people believe cancer is down to fate or 'in the genes' and that it is the luck of the draw whether they get it. Looking at all the evidence, it's clear that around 40% of all cancers are caused by things we mostly have the power to change. [540]

"We didn't expect to find that eating fruit and vegetables would prove to be so important in protecting men against cancer. And among women we didn't expect being overweight to have a greater effect than alcohol."

The other risk factors the study examined were infections such as human papillomavirus (HPV) which causes cervical cancer, occupations such as working with asbestos, lack of physical exercise, radiation, lack of breast feeding and using hormone replacement therapy (HRT).

Ciarán Devane, chief executive at **Macmillan Cancer Support**,[540] said healthy lifestyle messages were not getting through. "No one chooses to have cancer and it would be wrong to blame people for making wrong lifestyle choices," he said.

"For a long time, people have been told that eating healthily, not smoking and exercising regularly can benefit them, and these figures show again the impact a healthy lifestyle can have. Yet these healthy

lifestyle messages are clearly not reaching enough people. They also need to be made more relatable to people's everyday lives.

"We know that being physically active reduces recurrence rates of cancer too, as well as your chance of getting it in the first place. There needs to be a cultural change, so that people see physical activity as an integral part of their lives, not just an optional add-on."

Dr Rachel Thompson, deputy head of science for the **World Cancer Research Fund**, said: "This adds to the now overwhelmingly strong evidence that our cancer risk is affected by our lifestyles. [540]

But why natural supplement are not getting popularity among doctors?

First is the nature of the medical/drug industry. Doctors, by and large, will just use and recommend drugs that are approved as drugs in a process that costs hundreds of millions of dollars. So the drug companies only make drugs that can be patented. That way they can sell it for huge mark-ups and have no competition. Natural substances must be modified in order to be patented.

Secondly, cancer is complex and tough. One single product is not often going to be enough on its own. So your doctor can dismiss as hype the take this supplement and it will cure your cancer approach. Overcoming cancer is a process of reversing the conditions that allowed

the cancer to develop, and going after and killing cancerous cells. Fifty years from now, the current conventional cancer treatments used by doctors will on the whole be viewed in the same light that we view the old medical practice of using leeches to cure illnesses.

Chemotherapy and other treatments damage cells and tear down and weaken the immune system. But the problem in the first place is that the patient's immune system is already weak, and that his cells are already damaged. Even if tumors do go into remission, these treatments will have damaged other cells, which are more likely to turn cancerous. The immune system, unless it is supported by supplements and diet to help it recover, will be in worse shape than ever. While it may have taken decades for cancer to develop the first time around, the second time usually takes a year or two.

It is not all gloom and doom with chemotherapy. For two decades **Dr. Perez Garcia** has been using a treatment he calls Insulin Potentiation Therapy (IPT). It consists of giving a patient a dose of insulin followed by a tiny dose of chemotherapy. Cancer cells have 15 times more insulin receptors than normal cells. The insulin dose helps to target chemotherapy into cancer cells because they have so many more insulin receptors. So small doses of chemotherapy can be used that cause little harm to normal cells. With Stage 1 or 2 cancer, IPT is about 80% successful, mixed results for more serious cancers. So after two decades of use, how many doctors were using IPT????

You don't have to avoid chemotherapy or radiation therapy to receive benefits from natural products and supplements. In fact, nutritional supplements are quite useful when used in conjunction with chemotherapy, radiation therapy and surgery. The best ones work to support the body so that radiation and chemo will actually work better. In addition, the immune system will be stronger, and better able to keep cancer from developing again.

Seeking other options after the doctors throw in the towel and say there is nothing else they can do, is necessary. It makes more sense to correct the underlying causes of cancer early on when the odds are much better. Especially with natural supplements that can do no harm.

In **1931 Dr. Warburg** won his first Nobel Prize for proving cancer is caused by a lack of oxygen respiration in cells. He stated in an article titled The Prime Cause and Prevention of Cancer that "the cause of cancer is no longer a mystery, we know it occurs whenever any cell is denied 60% of its oxygen requirements."

Decades ago, two researchers at the **National Cancer Institute, Dean Burn and Mark Woods**, (**Dean** translated some of **Warburg's** speeches) conducted a series of experiments where they measured the fermentation rate of cancers that grew at different speeds. What they found supported Dr. Warburg's theory. The cancers with the highest growth rates had the highest fermentation rates. The slower a cancer grew, the less it used fermentation to produce energy.

The AFA Blue Green Algae contains enzymes that are genetically related to the respiratory enzymes in the mitochondria. So when eaten, mitochondria with enzymes damaged by lack of oxygen may be able to make use of the algae enzymes to produce energy aerobically.

Acidic pH Levels Lead To Cancer

There is plenty of research showing that cancer thrives in an acidic environment, and doesn't survive in an normal, more alkaline environment. Cancer cells make your body even more acidic as they produce lactic acid.

Taking action to make your body more alkaline is vital in the battle against cancer. Unfortunately... The majority of the foods and drinks we consume are acidic, such as meat, grains and sugar, with colas and other soft drinks being highly acidic. So unless you have been eating a very healthy diet, full of fresh fruit and vegetables, your body is way too acidic. Creating a very good environment for cancer to grow in.

Actually, a too much acidity is an underlying factor in many degenerative diseases --diabetes, arthritis, fibromyalgia and more. A basic maxim of natural physicians is: Balance the bio terrain. Do this first, then everything can come back to normal.

CANCER IMMUNOLOGY [89,90]

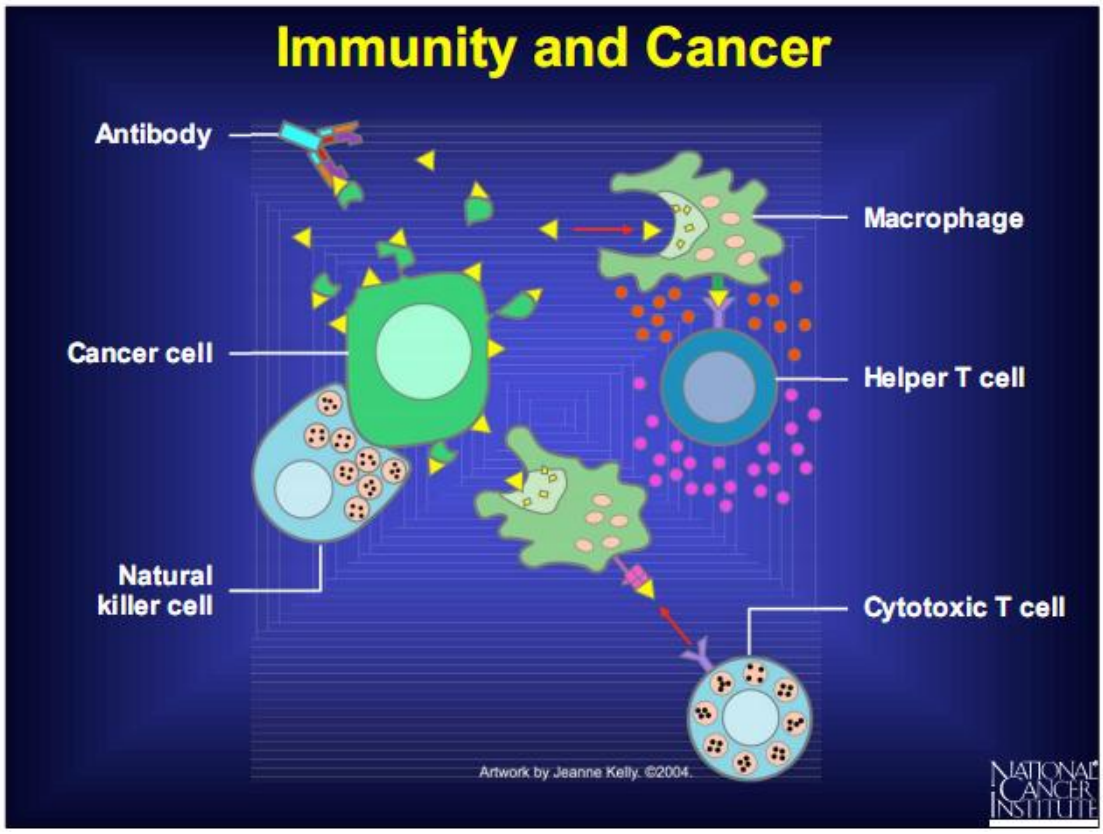


Fig. 58; Immunity and Cancer [89,90]

When normal cells turn into cancer cells, some of the antigens on their surface change. These cells, like many body cells, constantly shed bits of protein from their surface into the circulatory system. Often, tumor antigens are among the shed proteins. These shed antigens prompt action from immune defenders, including cytotoxic T cells, natural killer cells, and macrophages. According to one theory, patrolling cells of the immune system provide continuous body-wide surveillance, catching and eliminating cells that undergo malignant transformation. Tumors develop when this immune surveillance breaks down or is overwhelmed.

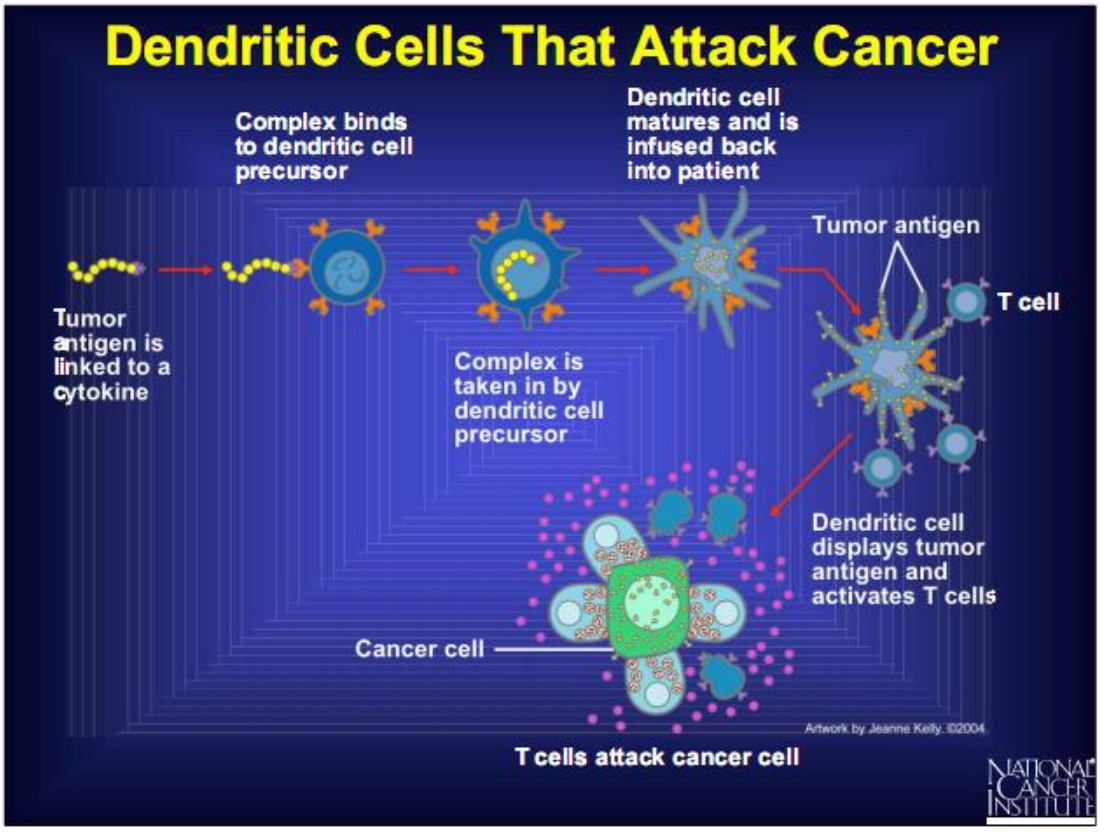


Fig. 59; Dendritic Cells That Attack Cancer [89,90]

Another approach to cancer therapy takes advantage of the normal role of the dendritic cell as an immune educator. Dendritic cells grab antigens from viruses, bacteria, or other organisms and wave them at T cells to recruit their help in an initial T cell immune response. This works well against foreign cells that enter the body, but cancer cells often evade the self/non-self detection system. By modifying dendritic cells, researchers are able to trigger a special kind of autoimmune response that includes a T cell attack of the cancer cells. Because a cancer antigen alone is not enough to rally the immune troops, scientists first fuse a cytokine to a tumor antigen with the hope that this will send a strong antigenic signal. Next, they grow a patient's dendritic cells in the incubator and let them take up this fused cytokine-tumor antigen. This enables the dendritic cells to mature and eventually display the same

tumor antigens as appear on the patient's cancer cells. When these special mature dendritic cells are given back to the patient, they wave their newly acquired tumor antigens at the patient's immune system, and those T cells that can respond mount an attack on the patient's cancer cells.

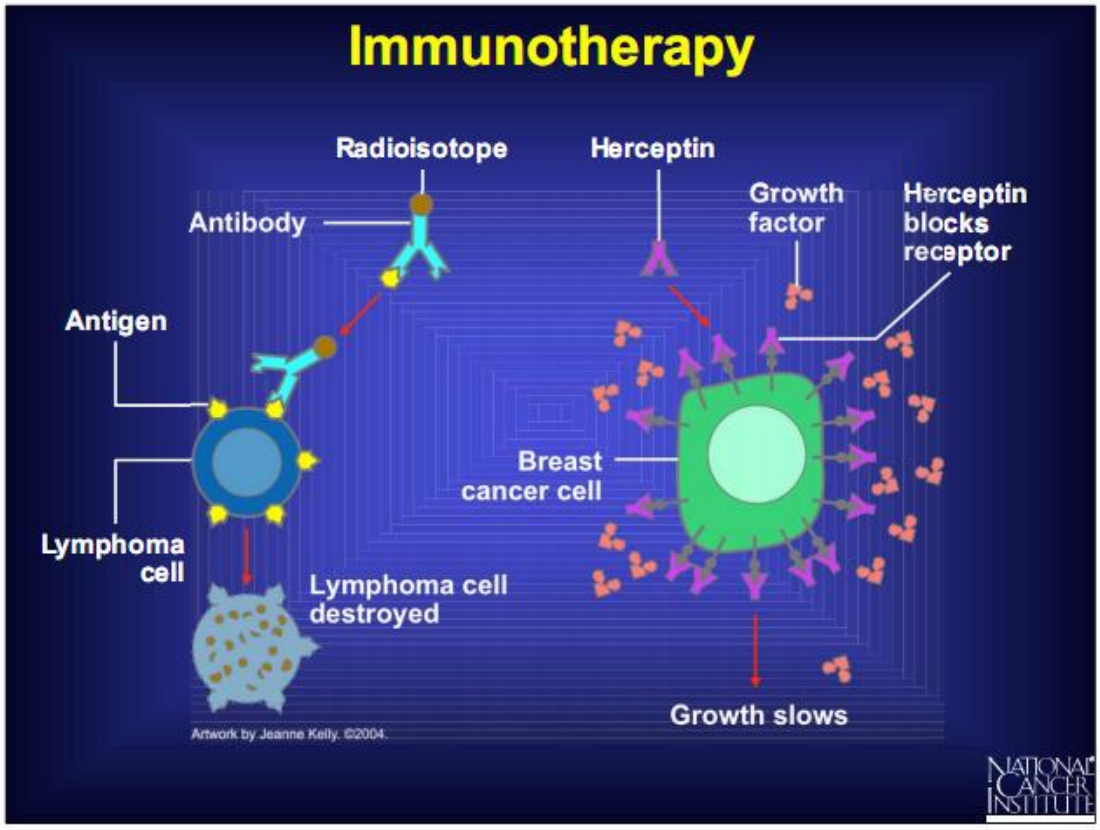


Fig. 60; Immunotherapy [89,90]

A new approach to cancer therapy uses antibodies that have been specially made to recognize specific cancers. When coupled with natural toxins, drugs, or radioactive substances, the antibodies seek out their target cancer cells and deliver their lethal load. Alternatively, toxins can be linked to a lymphokine and routed to cells equipped with receptors for the lymphokine.

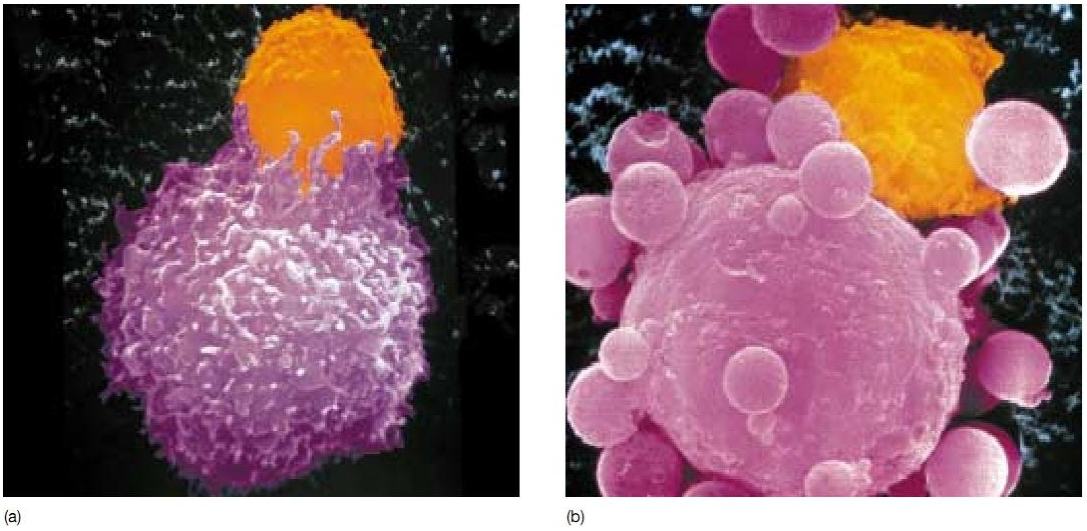


Fig. 26; Cytotoxic T cells destroy cancer cells. (a) The cytotoxic T cell (orange) comes into contact with a cancer cell (pink). (b) The T cell recognizes that the cancer cell is "nonself" and causes the destruction of the cancer.

HOW TO TREAT CANCER

Plants provide a valuable source of therapeutic agents and bases for synthetic drugs. Several plant species have been studied for anticancer properties, and it is estimated that approximately 40%–50% of the drugs on the market today are either derived from natural products or are natural products themselves.[573] Nonetheless, comprehensive and systematic evaluation of "natural" products is required to demonstrate efficacy and safety for clinical use.

The Center for Complementary Health Studies defines complementary and alternative medicine (CAM) as "any health-improving technique outside of the mainstream of conventional medicine".[574] The general public is very accepting of CAM, with recent reports stating that 34%-38% of the US adult population was using CAM,[575] and7.4%of the population had seen a CAM practitioner during the last 12months. [576]

The same factors within algae that optimize cellular regeneration and help to protect genetic replication and transcription may likewise offer cancer-protective effects. Despite the "war on cancer." involving decades of research and many billions of dollars spent, the incidence of a number of cancers has continued to increase Environmental factors and diet appear to play significant roles in certain forms of cancer. An important water-shed was an article by **Doll** and **Peto (1981)** estimating that 35% of cancer incidence was related to nutritional factors.[583]

Notwithstanding an ongoing debate—with an increasing recognition of multi-causal factors involved with the etiology of cancer—the role of

diet remains a strong one. In these last few decades researchers have increasingly begun to focus on foods, vitamins, and plant chemicals to provide protective factors that lower the risk of cancer.[495,496] Beta-carotene is one of the best-known anticancer substances found in food.

Dozens of large-scale studies have disclosed evidence that eating Vegetables rich in beta-carotene reduces the risks of cancer.[196,497-499] It is important to note, however, that isolated beta-carotene (sold as a supplement on its own or in multivitamin formulas) does not provide the same benefits. In fact, the large-scale study referred to as "CARET" (Carotenoid and Retinol Efficacy Trial) found that synthetic beta-carotene supplements were correlated with increased—not decreased—morbidity and mortality from cancer.[500]

Because microalgae are the foods richest in natural beta-carotene. several species have been extensively tested for anticancer effects and these effects have been well documented.[199,501-507]

Researchers at the Harvard University School of Dental Medicine demonstrated that algal extracts rich in beta-carotene applied to cancerous tumors in the mouths of hamsters reduced the number and size of tumors or caused them to disappear. [508] In a further study, when an algal extract was administered to 20 hamsters pre-treated to develop mouth cancer, none of the animals developed the disease. By comparison, two pretreated control groups that did not receive any algal extract (40 animals) all developed mouth cancer. Interestingly, when beta-carotene alone was

given (provided by Sigma Chemical Company) fully half the animals developed cancer. [509] This research team has continued to replicate these effects, repeatedly demonstrating the ability of blue-green algal extracts to inhibit and prevent tumor growth and cancer. [131,197,198]

Additionally, there is evidence that blue-green algae can make a significant contribution to the prevention of oral cancer in humans. Researchers at Maryland's Human Nutrition Center administered a single gram of algae a day to tobacco chewers in Kerala. India, who had pre-cancerous mouth lesions (oral leukoplakia). After one year, the sores had vanished or shrunk significantly in more than half of the people, with complete regression in 45% of the human subjects (N - 40). Lesions disappeared in only 7% of the people in the placebo group.

Within one year of discontinuing algae supplements, about half of the experimental subjects developed recurrent lesions. There was no experimental intervention to change the carcinogenic behavior of their tobacco chewing. [510]

Beta-carotene is not the only cancer-protective substance to be found in microalgae Cancer researchers at the University of Hawaii isolated a blue-green algal pigment, called cryptophycin. That demonstrates powerful anticancer properties—especially useful in the chemotherapy of drug-resistant tumors.[511,512] Other new algal protein compounds have also exhibited "multidrug-resistance reversing activities" that may be useful in the treatment of difficult, drug-resistant tumors. [513-515]

Unicellular algae have some compounds in their cell walls that are similar to those found in bacteria and yeast. Scientists discovered that these algae protected mice against experimentally grafted sarcoma in much the same way as the other microorganisms.[516] Bacteria and yeasts are able to induce, when inoculated into laboratory rodents, a general stimulation of defenses through an immune process. Glycoproteins extracted from algae demonstrate potent anti-tumor properties in animal studies. [517-521] Initial human trials with these anti-tumor peptidoglycans from microalgae have shown promising effects.[522]

Several components of chlorophyll have shown tumor-suppressive activities.[523,524]A study published in the journal Cancer Research reported that chlorophyll, abundant in microalgae, is a "potent inhibitor of hepato-carcinogenesis (the development of liver cancer).. .and may have important implications in intervention and dietary management of human cancer risks.[179]

Much attention in recent years has been given to the antigenotoxicity of chlorophyll. Chlorophyll, however, is known to be converted into pheophytin, pyropheophytin, and pheophorbide in processed vegetable food and following ingestion by humans.

Studies conducted on the antimutagenic and tumoricidal potencies of these compounds by **Chernomorsky S et al (1999)**[539] concluded that all the chlorophyll derivatives tested exhibit identical antimutagenic effect towards 3-methylcholanthrene (3-MC), suggesting that the porphyrin nucleus may complex directly with the mutagen. It does not exclude,

however, another mechanism of activity involving inactivation the enzymatic transformation of 3-MC. In contrast, the action of N'-nitro-N'-nitrosoguanidine (MNNG) depends upon structural differences between the chlorophyll derivatives. It is significantly lower when the phytol-containing pheophytin and pyropheophytin are tested as to that of the phytol-lacking pheophorbide.

The higher concentrations of the chlorophyll derivatives were required to reduce the mutagenicity of MNNG than needed for 3-MC. The cytotoxicity of chlorophyll derivatives against tumor cells, also, was evaluated. The cellular uptake and inhibition of myeloma cell multiplicity were found to be greater for pheophorbide than for pheophytin. Calculated on the amount of cell associated chlorophyll derivative, however, pheophytin was more cytostatic/cytotoxic than pheophorbide.

The results presented in this report indicate that food sources that yield chlorophyll derivatives may play a significant role in cancer prevention.[539]

Numerous studies with unicellular green algae have demonstrated anti-tumor and cancer inhibitory effects in laboratory rodents. [525-531]These anti-tumor effects appear mainly to be mediated by an increased host immune response. In some cases the survival rates of algae-treated mice increased nearly 80% over control groups.[532] Such findings suggest that presurgical treatment with extracts of microalgae might decrease or prevent metastasis or tumor progression. [518]

Marine unicellular algae, diatoms, have displayed antiproliferative effects against solid human tumor cell cultures—lung carcinoma, kidney carcinoma and melanoma—and have been shown to inhibit non-small-cell bronchopulmonary carcinoma in in vitro cell studies.[533,534]

"In research in Japan, phycocyanin (the blue pigment of blue-green algae) was extracted and given orally to mice with liver cancer. The survival rate of the treatment group was significantly higher than the control group not given phycocyanin. After five weeks. 90% of the phycocyanin group survived, but only 25% of the control group were still alive. After eight weeks. 25% of the phycocyanin group still survived, yet none of the control group was alive This suggests eating phycocyanin may increase the survival rate of cancer stricken organisms. [535]

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. 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 findings that phycocyanin specifically interferes with this pathway holds promise.

Researchers at Dainippon Ink and Chemicals Company have a patent pending for using phycocyanin as an "anti-tumor agent ” They base their claim on a study demonstrating that after two weeks the levels of white blood cells of a cancer group treated with phycocyanin were significantly higher than those of the untreated cancer group and equal to or higher than those of the normal group without cancer. This suggests phycocyanin may raise lymphocyte activity[536]

Another patent claim filed by **Dainippon Ink** and Chemicals and Tokyo Kenkyukai is for “Anti-tumor agents containing phycobilin—also used to treat ulcers and hemorrhoid bleeding.[537] This patent application documents that taking a small dosage of algal extract daily maintains or accelerates normal cellular control functions that prevent generation of malignancy or inhibit its growth or recurrence. "Radiation and chemotherapeutic treatments of cancer often result in undesirable reduction of defensive white cells. "Whole body irradiation” animal studies suggest there may be a potential benefit for cancer patients given algal beta-carotene before and after radiation treatments to protect against cellular damage caused by free radicals induced from irradiation. [418]

Additionally. Japanese researchers using an animal model found that components of unicellular algae may be beneficial in the alleviation of cancer chemotherapy side effects (e.g.. immune suppression) while supporting the anti-tumor activity of the chemotherapeutic agents. [538]

Studies using a water soluble extract of blue-green algae have found a novel sulfated polysaccharide named calcium spirulan (Ca-SP) that acts as an antiviral agent. study, however, showed that the sulfated polysaccharide mentioned above, Ca-SP, appeared to inhibit tumor invasion and metastasis.[517] By examining both the in vitro and in vivo effects of Ca-SP, this study's results suggest that the intravenous administration of Ca-SP reduces the lung metastasis of melanoma cells by inhibiting invasion of the basement membrane. In vitro experiments show that this occurs through the prevention of the adhesion and migration of tumor cells to laminin substrate and of heparanase activity.

Chlorophyll (Chla) and chlorophyllin (CHL) were shown previously to reduce carcinogen bioavailability, biomarker damage, and tumorigenicity in trout and rats. Because aflatoxins, particularly aflatoxin B1 (AFB1),are potent carcinogens in some animals, there is interest in the effects of long-term exposure to low levels of these important mycotoxins on humans. Epidemiologic studies in Asia and Africa revealed a positive association between dietary aflatoxins and liver cancer.[541]

High levels of aflatoxins in combination with infection with hepatitis B seem to act synergistically to increase risk of hepatocellular carcinoma. [542] Intervention programs have been directed toward reducing hepatitis virus infection through immunization and screening of foods for aflatoxin levels. There is also interest in addition of dietary agents that may reduce the bioavailability of aflatoxins by binding in the intestine and limiting absorption, or by increasing metabolism and reducing systemic exposure. Intervention agents currently under investigation include apiginin clays, Oltipraz, and chlorophyll (Chla) or its derivative. [543-547] Chla and its derivatives have a long, well-known history of uses for medicinal and therapeutic preparations.[448] Both natural Chla and its water-soluble derivative sodium copper chlorophyllin (CHL) have been extensively studied for a variety of significant biological activities. [308,549-552]

Effects of Chlorophyll and Chlorophyllin on Low-Dose Aflatoxin B1 Pharmacokinetics in Human Volunteers were studied by **Carole Jubert, et al.2009** through a double-blinded, placebo-controlled intervention trial in Qidong, People's Republic of China showed that CHL

intervention can reduce aflatoxin-DNA adduct excretion among individuals in a population at high risk for liver cancer.[553]

This study clearly showed a decrease in a biomarker of AFB1-DNA damage in individuals chronically exposed to relatively high doses of aflatoxin through their diet i.e the animal findings were partially extended to humans, where CHL reduced excretion of the potent naturally occurring carcinogenic mycotoxin aflatoxin B1 (AFB1) -DNA repair products in Chinese unavoidably exposed to dietary AFB1. However, neither AFB1 pharmacokinetics nor Chla effects were examined. They conducted an unblended crossover study to establish AFB1 pharmacokinetic parameters among four human volunteers, and to explore possible effects of CHL or Chla cotreatment in three of those volunteers.

For protocol1, fasted subjects received an Institutional Review Board–approved dose of 14C-AFB1 (30ng, 5nCi) by capsule with 100mL water, followed by normal eating and drinking after 2hours. Blood and cumulative urine samples were collected over 72 hours, and 14C-AFB1 equivalents were determined by accelerator mass spectrometry. Protocols 2 and 3 were similar except capsules also contained 150 mg of purified Chla or CHL, respectively. Protocols were repeated thrice for each volunteer. The study revealed rapid human AFB1 uptake (plasma ka, 5.05±1.10h−1; T max, 1.0hour) and urinary elimination (95% complete by 24 hours) kinetics. Chla and CHL treatment each significantly impeded AFB1 absorption and reduced C max and AUCs (plasma and urine) in one or more subjects. These initial results provide AFB1

pharmacokinetic parameters previously unavailable for humans, and suggest that Chla or CHL co-consumption may limit the bioavailability of ingested aflatoxin in humans, as they do in animal models.[553]

The mechanisms that produce this effect in humans are undefined but may include limiting absorption in the intestine, systemic complexation with bioavailable forms of CHL, or induction of phase II enzymes leading to increased aflatoxin metabolism. [546,554,555]

Preclinical studies in rats and rainbow trout suggested that CHL acts primarily by binding certain aflatoxins, heterocyclic amines, and polycyclic aromatic hydrocarbons to reduce bioavailability, genomic damage, and tumor induction. [556-558] Responses to intervention with Chla and CHL were qualitatively similar among the three volunteers completing the study, but with some evidence for inter-individual variability in degree of response.

Many studies have characterized the molecular dosimetry of AFB1 as a measure of possible carcinogenic effects in the absence and presence of intervening agents. [559-561,566]

One such agent, CHL, has been characterized extensively for its cancer chemopreventive potential in animals and humans. [547,556,562-564] More recently, cancer chemopreventive effects of natural Chla itself have been reported in rodent and fish models, through mechanisms that

involve reduced systemic carcinogen uptake. [556,557] The previous study extends these findings by establishing the kinetics of low-dose aflatoxin absorption in each of four human volunteers, and the effects of CHL and Chla intervention on specific AFB1 pharmacokinetic parameters in three of those subjects.

The results showed that intervention by CHL and Chla produced similar, and frequently significant, effects on AFB1 uptake and distribution among all individuals. In all individuals, Chla intervention produced a significant 40% to 60% reduction in excretion of urinary aflatoxin equivalents. For CHL, the extent of protection in subject5 was identical to that provided by Chla. A similar but non-significant trend for CHL protection was seen for subject2 and, to a lesser extent, for subject1. We interpret these results as direct evidence for CHL- and Chla-mediated reductions in systemic uptake of aflatoxin in humans, as in preclinical models. [558,561,562]

An alternative interpretation could be that a single cotreatment with either Chla species alters AFB1 metabolism in a direction that would reduce urinary excretion, for instance, through rapid induction of phase II detoxication pathways.[563] This interpretation we believe is highly unlikely, especially since interference effects become apparent within 30 minutes of cotreatment, but is not formally ruled out by this study.

Based on present data, we conclude that the changes in pharmacokinetic parameters due to Chla and CHL cotreatment reflect an ability of these blocking agents to reduce the amount of aflatoxin absorbed in the intestine, in humans as well as animal models.[553]

Consequently, chemoprevention by dietary Chla may be limited by mechanisms restricted to the alimentary tract, whereas CHL may find broader chemopreventive application owing to a potential to reach many more target organs in the whole animal. As a practical matter, structural differences also render CHL considerably more stable than Chla against oxidation, and thus more readily available and affordable for large-scale experimentation and application in chemoprevention. [553]

Phycocyanin was shown to induce apoptosis in the chronic myeloid leukemia cell line, K562 [567,568] and other types of cancer[569-571] **Hart et al**. showed that the extract of (AFA) is a potent invitro activator of NK cells, which are capable of killing some tumor cells without prior sensitization to antigen. However, this effect appears to be dependent on accessory cells as activation was not observed on isolated NK cells.[567] **Pugh** and **Pasco** demonstrated that AFA extract activated the monocyte cell line THP-1.[572] Increased levels of both IL-1β and TNF-α in cells exposed to 0.5pg/ml of extract were detected. AFA extract alone increased apoptosis in all of the cancer cell lines we tested.

SUMMARY

AFA (Klamath Lake blue green algae) is an anciently used food with recently discovered amazing nutritional and therapeutic effects.

It is completely safe primitive unicellular plant naturally growing at volcanic lakes, present on the earth for more than 3.5 billion years to overcome the health problems couldn't be solved(or solved with horrible side effects) by modern scientific revolution.

The AFA has four possible explanations(AFA Square) that may summarize the possible mechanisms of combating diverse disease entities:

- Nutritional supplementation of the essential nutrients and trace elements in a perfect God Made Formula which may alone explain many of its effects(it is the most nutrient superfood ever known)
- Immune response modulation in a perfect way that overcomes disorders associated with low or abnormal immune response.
- Protection of the body from chemicals, toxins, radiation and oxidants, beside exerting powerful anti-inflammatory effects, it also helps at the same time to repair damaged cells and tissue regeneration by stimulating stem cells mobilization towards the site of injury.

- True therapeutic effects through biologic and biochemical modification of different pathologies(it has alkalizing effect combating acidity and dehydrating effect combating growth of cancer and harmful organisms).

The spectrum of diseases covered by AFA is so wide to be enumerated but it can be summarized as follows:

1. Enhancing Brain Function, Behavior, and Learning.
2. Management Of Depression, Schizophrenia, Alzheimer's Disease, Epilepsy, Myasthenia, Multiple sclerosis, Attention Deficit Hyperactive Disorders, anti-stress effects and mild traumatic brain lesions.
3. Improving Immune Function and Antibodies Production.
4. Antiviral, Antibacterial, Antifungal effects.
5. Allergy, Asthma Auto-immune diseases relief.
6. Improving cellular repair and stimulating stem cells mobility.
7. Radiation protective effects.
8. Cancer Protective effects.
9. Detoxification support effects.
10. Anti-inflammatory and antioxidant effects.
11. Improving circulation, high blood pressure and heart function.
12. Improving and supporting liver functions.
13. Hypocholesterolemic and antihyperlipidemic effects.
14. Anti-diabetes and anti-obesity effects.
15. Improving digestion and elimination.

16. Increased fertility
17. Help healing of external and internal lesions.
18. Mild diuretic and combating nephrotoxicity.

Dr. Gitte Jensen, an immunologist affiliated with McGill University, sought to establish a level at which uncontaminated AFA algae might exert a toxic effect on human blood cells. She created various dilutions of live blood mixed with AFA algae and observed the reactions. Even at extreme concentrations—the equivalent of a human ingesting 15.000 capsules of AFA per day—Dr. Jensen found no toxic effects on human blood cells.

The WHO recommended the daily dose by 1 gm/ 30kg.

REFERENCES

1. Bruno J. 2001 Edible microalgae A Review of the Health Research.

2. Drapeau C: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005.

3. Drapeau C: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, Rethinking Health.,p12.

4. **Drapeau C**: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, The Economics of Health, pp; 13:14.

5. Hoppe et al. (1979) Marine Algae in Pharmaceutical Science, Walter de Gruyter Eds., New York, 807 pp.

6. Richmond, A. (1990) Handbook of Microalgal Mass Culture. CRC Press: Boca Raton, FL, 528 pp.

7. Cannell, R.J.P. (1990) Algal biotechnology. In Applied Biochemistry and Biotechnology, H. Weetall Ed., pp 85-105.

8.Farrar, W.V. (1966) Tecuitlatl: A glimpse of Aztec food technology. Nature 5047: 341-342.

9.Ciferri, O. (1983) Spirulina, the edible microorganism. Microbiological Reviews 47: 551-578.

10- Ciferri, 0. and 0. Tiboni (1985). The Biochemistry and Industrial Potential of Spirulina." Annual Review of Microbiology 39: 503.

11- Gudin, C. (1988). Why Bother with Microalgae? Algal Biotechnology. T. Stadler, Mollion, J, Verdus, MC et al. Amsterdam, Elsevier Applied Science Publishers.

12-Kay, R. (1991). "Microalgae as Food and Supplement." Critical Reviews in Food Science and Nutrition 30: 555-573.

13- Federal Centers for Disease Control and Prevention. Atlanta. 1993.

14- Omenn G et al (1996). Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial, Journal of the National Cancer institute. November 1996; 88(21):1550-9.

15- Decava J. The Real Truth About vitamins & Antioxidants. Price-Pottenger Foundation.1997: 121.

16.**Hocman G**. Prevention of Cancer: vegetables and Plants Comparative Biochemistry and physiology. 1989; 93B(2): 201 -12.

17.United States Public Health Service. **The surgeon General's** Report on Nutrition and Health. Warner Books. 1989.

18.Kamath s. reported in American Medical News. May 24/31. 1985.

19.Serdula M et al. Fruit and Vegetable intake Among Adults in 16 States: Results of a Brief Telephone Survey. American journal of Public Health. 1995; 85(2):230-39.

20.Stitt P. Fighting the Food Giants. Natural Press.1980: 144.

21. Select Committee On Nutrition and Human Needs. United States Senate.Dietary Goals for the United States. Washington. DC: united states Printing office. 1977.

22. Physician's Desk Reference for Herbal Medicine. Medical Economics Company. 1999.

23. **Worback M**. Nutritional influences on illness: A Sourcebook of Clinical Research. Third Line Press. 1993.

24. Arasaki S. Arasaki T. Low Calorie. High Nutrition vegetables From the Sea: To Help you Look and Feel Better. japan Publishing. 1983: 59.

25.Hoppe H. Marine Algae in Pharmaceutical Science. De Gruyter and Co. 1975.

26.Abrams K. Algae to the Rescue. Logan House. 1990: 31.

27.Smith R. Where is the wisdom...I British radical journal. I991; 303:798-9.

28. Borowitzka, M. (1988). Fats, Oils, and Hydrocarbons. Micro-Algal Biotechnology. M. a. B. Borowitzka, LJ. New York, Cambridge University Press: 257**.**

29. Borowitzka, M. (1988). Vitamins and Fine Chemicals from Microalgae. Micro-Algal Biotechnology. M. a. B. Borowitzka, LJ. New York, Cambridge University Press: 153.

30. Becker, E. (1988). Microalgae for Human and Animal Consumption. Micro-Algal Biotechnology. M. a. B. Borowitzka, LJ. New York, Cambridge University Press: 222.

31. Lee w. Rosenbaum M. in Chlorella. Keats Publishing.1987:19.

32. Oswald W, Goluike C. The algatron.a novel microbial culture system. The Sun at Work.January 1966; n (l).

33.Oswald W. Goluike C. Man in space: he takes along his wastes problem. Wastes Engineering. September 1961; 32(9).

34.Tabibul Islam, wonder Cure for Malnutrition. MISAnet/inter Press Service. June 2. 1988.

35- Drapeau C: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, Prodigious Klamath Lake Ecology of Klamath Lake, pp;21:23.

36- Drapeau C: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, **Why AFA Flourishes** , pp;24, 25.

37- Drapeau C: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, **The Water of Klamath Lake**, pp;27:29.

38- Drapeau C: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, **Harvesting Today** , pp;18:19.

39- Drapeau C: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, **A Superior Drying Method**, pp;19:20.

40-Passwater R. Soloman N. eds Algae: The next generation of superfoods. The Experts' Optimal Health journal 1997; 1 :2.

41. Pirt S. Biochemical Society Trans. 1980; 8:479 cited by Aaronson and Dubinsky. Mass production of microalgae. Experientia. 1982; 43(suppl):42.

42.Abrams K. Algae to the Rescue. Logan House. 1990: 61.

43.Braverman R. The Healing Nutrients Within: Facts. Findings and Neu'Research on Amino Acids. Keats Publishing. 1987.

44.Shimizu Y. Microalgal metabolites: a new perspective. Annual Review of Microbiology.1996; 50:442.

45.Cohen A. Norman H. Heimer Y. Microalgae as a source of omega 3 fatty acids, world Review of Nutrition and Diet. 1995; 77:1-31.

46.Simopoulou A. Omega 3 fatty acids in health and disease in growth of development. American Journal of Clinical Nutrition.1991; 54:438-63.

47.Challem J. Spirulina. Keats Publishing.1981: 5.

48- Ginsberg D. 2000; Blue-Green Algae as a Nutritional Supplement: Evidence for effects on the circulation and function of immune cells in humans, MSc Thesis, **Department of Physiology, McGill University ,Montreal, Quebec .**

49-Hart AN, Zaske LA, Patterson KM, Drapeau C, Jensen GS, Natural killer cell activation and modulation of chemokine receptor profile in vitro by an extract from the cyanophyta Aphanizomenon flos-aquae. J Med Food. 2007 Sep; 10(3):435-41.

50- Benedetti S, Rinalducci S. et al; Purification and characterization of phycocyanin from the blue-green alga Aphanizomenon flos-aquae. J Chromatogr B Analyt Technol Biomed Life Sci. 2006 Mar 20;833(1):12-8. Epub 2005 Nov 2.

51- Benedetti S. et al; Antioxidant properties of a novel phycocyanin extract from the blue-green alga Aphanizomenon flos-aquae. Life Sci. 2004 Sep 24;75(19):2353-62.

52- Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS. Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from Spirulina platensis, aphanizomenon flos-aquae and Chlorella pyrenoidosa. Planta Med. 2001 Nov;67(8):737-42.

53- Effmert U, Mundt S, Teuscher E. [Investigations of the immunomodulatory effect of cyanobacterial extracts] Allerg Immunol (Leipz). 1991;37(2):97-102. German.

54. DATA SHEET GAL.E. Inc. dba E3Live For Horses Klamath Algae Products, Inc dba E3Live 2217 Mulberry Lane, Placerville CA95667, Last Updated: 02119/ 2010.

55. SCIENCE BASED NUTRACEUTICAL PRODUCTS 2008, Klamath Algae Nutratec s.r.l. 61029 Urbino (PU) Italy - via i Maggetti 14 - loc. Sasso

56. Drapeau C.'Why Blue-Green Algae Makes Me Tired': Perspective, 15 Years Later, Townsend Letters2010

57. Stoloff L, Van Egmond HP, Park DL. Rationales for the establishment of limits and regulations for mycotoxins. Food Addit Contam. 1991;8(2):213–221.

58. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 2001;56(1):5–51.

59. Wilson BJ, Yang DT, Boyd MR. Toxicity of mould-damaged sweet potatoes (Ipomoea batatas). Nature 1970;227(5257):521–522.

60. Barceloux DG. Potatoes, tomatoes, and solanine toxicity (Solanum tuberosum L., Solanum lycopersicum L.). Dis Mon. 2009;55(6):391–402.

61. Beier RC. Natural pesticides and bioactive components in foods. Rev Environ Contam Toxicol. 1990;113:47–137.

62. Kumar KP, Kumar SP, Nair GA. Risk assessment of the amnesic shellfsh poison, domoic acid, on animals and humans. J Environ Biol. 2009;30(3):319–325.

63. Carmichael, Wayne W.; Drapeau, Christian; Anderson, Donald M. (2000). "Harvesting of Aphanizomenon flos-aquae Ralfs ex Born. & Flah. var. flos-aquae (Cyanobacteria) from Klamath Lake for human dietary use". Journal of Applied Phycology 12: 585–595.

64.Carmichael, W.W., and P.R. Gorham. (1980) Fresh water cyanophyte toxins, In: Algae Biomass, Elsevier, New York, PP. 437-448.

65. Gearheart, R.A., J.K Anderson, M.G Forbes, M. Osburn, and D. Oros. (1995) Watershed strategies for improving water quality in Upper Klamath Lake, Oregon. Humboldt State University, Environmental Resources Engineering Department. 3 Volumes.

66. Carmichael, Wayne W.; Stukenberg, Mary; Betz, Joseph M. (2010). "Blue Green Algae (Cyanobacteria)". Encyclopedia of Dietary Supplements (2nd ed.). London, UK: Informa Healthcare. pp. 75–81.

67. Debella, HJ (2005). "Mass culture of Aphanizomenon flos-aquae Ralfs ex born. and Flah. var. flos-aquae (cyanobacteria) from Klamath Falls, Oregon, USA, in closed chamber bioreactors". Ethiopian Journal of Biological Science 4 (2): 135–145.

68. J Trace Elem Med Biol. 2011 Jul; 25(3):149-53.

69. Li, R., Carmichael, W.W., Liu, Y., and Watanabe, M.M. (2000) Taxonomic re-evaluation of Aphanizomenon flos-aquae NH-5 based on morphological and 16 rRNA gene sequences, Hydrobiologica, vol. 438, pp3 99-105.

70. Logan, D.J., and D.F. Markle (1993) Fish faunal survey of Agency Lake and northern Upper Klamath Lake, Oregon. In Environmental research in the Klamath Basin, Oregon – 1992 Annual Report. S.G. Campell (ed.) P. 341.

71. Gorham, P.R. (1964) Toxic Algae. In: Algae and Man, Plenum Press, New York, PP. 307-30

72.Gentile, J.H., and T.E. Maloney. (1969) Toxicity and environmental requirements of a strain of Aphanizomenon flos- aquae (L.) Ralfs, Can. J. Microbiol., Vol. 15 (2), PP. 165-173.

73.Gentile, J.H. (1971) Blue green and green algal toxins. In: Microbial Toxins, Vol. 7, Academic Press, New York, PP. 27-67.

74.Sawyer, P.J., Gentile J.H., and J.J. sanser. (1968) Demonstration of a toxin from Aphanizomenon flos-aquae (L.) Ralfs, Can. J. Microbiol., vol. 14, pp. 119-1204.

75.Rapala, J., Sivoneon, K., Luukkainen, R., and S.I. Niemela. (1993) Anatoxin-a concentration in Anabaena and Aphanizomenon under different environmental conditions and comparison of growth by toxic and non-toxic Anabaena strains – a laboratory study, J. Applied Phycol., vol. 5, pp. 581-591.

76. Alam M, Euler KL. Chemical studies on toxins from the blue-green alga Aphanizomenon flos-aquae. In: WW Carichael, ed. The Water Environment: Algal Toxins and Health. New York: Plenum Press; 1981:405–414.

77. Carmichael WA, Biggs DA, Peterson MA. (1979) Pharmacology of anatoxin-a, produced by the freshwater cyanophyte Anabaena flos-aquae. Toxicon 17:229–236.

78. Phinney HK, Peek CA. Klamath Lake, an instance of natural enrichment. In: Transcript of 1960 Seminar on Algae and Metropolitan Wastes. Robert A. Taft Sanitary Engineering Center, Cincinnati, OH; 1961.

79. Hunter PR. Cyanobacteria and human health. J Med Microb. 1992;36:301–302.

80. Tison DL, Pope DH, Cherry WB, Fliermans CB. Growth of Legionella pneumophila in association with blue-green algae (cyanobacteria). Appl Environ Microbiol. 1980;39:456–459.

81. Islam MS, Miah MA, Hasan MK, Sack RB, Albert MJ. Detection of non-culturable Vibrio cholerae O1 associated with a cyanobacterium from an aquatic environment in Bangladesh. Trans R Soc Trop Med Hyg. 1994;88:298–299.

82. Fawell JK. Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water. WRC Foundation for Water Research; 1994.

83. Falconer IR, Burch MD, Steffensen DA, Choice M, Coverdale OR. Toxicity of blue-green alga (cyanobacterium) Microcystis aeruginosa in drinking water to growing pigs, as an animal model for human injury and risk assessment. Environ Toxicol Water Qual. 1994;9:131–139.

84. Oregon Department of Agriculture, December1997.

85. Schaeffer DJ, Malpas PB, Barton LL. Risk assessment of microcystin in dietary Aphanizomenon flos-aquae. Ecotoxicol Environ Saf. 1999; 44(1):73–80.

86. Falconer IR, Smith, JV, Jackson ARB, Jones A, Runnegar, MTC. Oral toxicity of a bloom of the cyanobacterium Microcystis aeruginosa administered to mice over periods up to 1 year. J Toxicol Environ Health. 1988; 24:291–305.

87. Yoshida T, Makita Y, Nagata S, et al. Acute oral toxicity of microcystin-LR, a cyanobacterial hepatotoxin, in mice. Nat Toxins. 1997; 5(3):91–95.

88. Oregon Department of Agriculture. Statutory Reference 603-25-190.

89- U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH National Institute of Allergy and Infectious Diseases (NIAID) Understanding the Immune System How It Works.

90- Lydia Schindler, Donna Kerrigan, M.S., Jeanne Kelly and Brian Hollen; National Cancer Institute (NCI), Understanding Cancer and Related Topics, Understanding the Immune System**.**

91- Janeway, C. and P. Travers (1996). Immunobiology: The Immune System in Health and Disease. New York and London, Garland Publishing Inc.

92- Abbas, A., A. Lichtman, et al. (1994). Cellular and Molecular Immunology. Philadelphia, W.B. Saunders Co.

93-Carlos, T. and J. Harlan (1994). "Leukocyte-endothelial adhesion molecules." Blood 84(7): 2068-2101.

94- Imhof, B. and D. Dunon (1995). "Leukocyte migration and adhesion." Adv Immunol 58: 345-416.

95- Proost, P., A. Wuyts, et al. (1996). "The role of chemokines in inflammation." Int J Clin Lab Res 26(4): 211-223.

96- Giancarlo, B., S. Silvano, et al. (1996). "Migratory response of human natural killer cells to lymphotactin." Eur J Immunol 26(12): 3238-41.

97-Imai, T., K. Hieshima, et al. (1997). "Identification and molecualr characterization of fractaline receptor CX3CR1, which mediates both leukocyte migration and adhesion." Cell 91(4): 521-530.

98- Loetscer, P., M. Seitz, et al. (1996). "Activiation of NK cells by CC chemokines Chemotaxis, Ca2+ mobilization, and enzyme release." J immunol 156(1): 322-327.

99- Palm, S., E. Postler, et al. (1996). "Twenty-four hour analysis of lymphocyte subpopulations and cytokines in healthy subjects." Chronobiol Int 13(6): 423-434.

100- Zelazowska, E., A. Singh, et al. (1997). "Lymphocyte subpopulation expression in women: effect of exercise and circadian rhythm." Med Sci Sports Exerc 29(4): 467-473.

101- Rehrnan, J., P. Mills, et al. (1997). "Dynamic exercise leads to an increase in circulating ICAM-1: further evidence for adrenergic modulation of cell adhesion." Brain Behav Immun l(4): 343-351.

102- Mizobe, K., K. Kishihara, et al. (1997). "Restraint stress-induced elevation of endogenous glucocorticoid suppresses migration of granulocytes and macrophages to an inflammatory locus." J Neuroimmunol73(1-2): 81-89.

103- Espersen, G., A. Elbaek, et al. (1996). "Short-term changes in the immune system of elite swimmen under competition conditions. Different immunomodulation induced by various types of sport." Scaud J Med Sci Sports 6(3): 156- 163.

104-Singh, A., E. Zelazowska, et al. (1996). "Lyphocyte subset responses to exercise and glucocorticoid suppression in healthy men." Med Sci Sports Exerc 2%(7): 822-828.

105- Benschop, R., F. Oostveen, et al. (1993). "Beta-2-adrenergic stimulation causes detachment of natural killer cells from cultured endothelium." Eur J Immun01 23(12): 3242-7.

106-Schedlowski, M., W. Hosch, et al. (1996). "Catecholamines modulate human NK cell circulation and function via spleen-independent beta-2-adrenergic mechanisms." J Immunol 156(1): 93-99.

107 –Naliboff, B., G. Solomon, et al. (1995). "Rapid changes in cellular immunity following a confrontational role-play stressor." Brain Behav Immun 9: 207-2 19.

108- Kiekolt-Glaser, J., J. Cacioppo, et al. (1992). "Acute psychological stressors and short- term immune changes: What, why, for whom, and to what extent?'Psychosomatic Medicine 54: 680-685.

109-Brenner, G., S. Felten, et al. (1992). "Sympathetic nervous system modulation of tumor metastases and host defense mechanisms." J Neuroimmunol37: 191-202.

110-Madden, K., S. Felten, et al. (1994). "Sympathetic nervous system modulation of the immune system. Induction of lymphocyte proliferation and migration in vivo by chemical sympathectomy." J Neuroimmunol49: 67-75.

111- Felten, S. and D. Felten (1991). Innervation of Lymphoid Tissue. Psychoneuroimmunology. F. Ader, and Cohen, Academic Press: 27-69.

112-Rameshwar, P. and P. Gascon (1995). "Substance P mediates production of stem cell factor and IL1 in bone marrow stroma: potential autoregulatory role for these cytokines in Substance P receptor expression and induction." Blood 86(2): 482-490.

113-Rameshwar, P., A. Poddar, et al. (1997). "Hematopoietic regualtion mediated by interactions among neurokinins and cytokines." Leuk Lymphoma 28(1-2): 1-10

114-Sung, C.-P., A. Arleth, et al. (1991). "Neuropeptide Y upregulates the adhesiveness of human endothelial cells for leukocytes." Circul Res 68: 3 14-3 18.

115.Boon P.Chew and Jean Soon Park ; Carotenoid Action on the Immune Response, Functions and Actions of Retinoids and Carotenoids: Building on the Vision of James Allen Olson, J.Nutr.134: 257S–261S,2004.

116-Meydani, S. N.,Wu, D., Santos, M. S. &Hayek,M.G. (1995) Antioxidants and immune response in aged persons: overview of present evidence.Am.J.Clin.Nutr.62:1462S–1476S.

117.Thilsted, J. P., Shifrine, M& Wiger, N.(1979) Correlation of invite and invivo tests for cell mediated immunity in the dog. Am.J.Vet.Res.40: 1313–1318.

118. Bendich, A. (1993) Physiological role of antioxidants in the immune system. J.DairySci.76:2789–2794.

119.Green, H. N. &Mallanby E.(1930) Carotene and vitamin A: the anti-infective action of carotene. Br.J.Exp.Pathol.11:81–89.

120. Clausen, S. W.(1931) Carotinemia and resistance to infection.Trans. Am.Pediatr.Soc.43:27–30.

121. Chew, B.P.(1995) Antioxidant vitamins affect food animal immunity and health. Conference: Beyond deficiency: New views of vitamins in ruminant nutrition and health. J.Nutr.125:1804S–1808S.

122. Seifter, E., Rettura, G. & Levenson, S.M. (1981) Carotenoids and cell-mediated immune responses. In: The Quality of Foods and Beverages, Chemistry and Technology (Charalambois, G. &Inglett, G., eds.) Vol.2 ,pp; 335–347, Academic Press, New York, NY.

123. Bendich, A. & Shapiro, S.S. (1986) Effect of –carotene and canthaxanthin on the immune responses of the rat. J.Nutr.116:2254–2262.

124.Hoskinson, C.D., Chew, B.P. & Wong, T.S. (1992) Effects of injectable –carotene and vitamin A on lymphocyte proliferation and polymorphonuclear neutrophil function in piglets. Biol.Neonate62:325–336.

125. Daniel, L.R., Chew, B.P., Tanaka, T.S. & Tjoelker, L.W. (1990) Carotene and vitamin A effects on bovine phagocyte function in vitro during the peripartum period. J.DairySci.74:124–131.

126. Alexander, M., Newmark, H. & Miller, R.G. (1985) Oral beta-carotene can increase cells in human blood. Immunol.Lett.9:221–224.

127. Watson, R.R., Prabhala, R.H., Plzia, P.M. & Alberts, D.S. (1991) Effects of carotene on lymphocyte subpopulations in elderly humans: evidence for a dose-response relationship. Am.J.Clin.Nutr.53:90–94.

128.Garewal, H.S., Ampel, N.M., Watson, R.R., Prabhala, R.H. & Dols, C.L. (1992) A preliminary trial of beta carotene in subjects infected with the human immunodeficiency virus. J.Nutr.122:728–732.

129. Prabhala, R.H., Harinder, G.S., Hicks, M.J., Sampliner, R.E. & Watson, R.R. (1991) The effects of 13-cis-retinoic acid and beta-carotene on cellular immunity in humans. Cancer 67:1556–1560.

130.Santos, M.S., Meydani, S.N., Leka, L., Wu, D., Fotouhi, N., Meydani, M., Hennekens, C.H. & Gaziano, J.M. (1996) Natural killer cell activity in elderly men is enhanced by –carotene supplementation. Am.J.Clin.Nutr.64:772–777.

131. Shklar, G. & Schwartz, J. (1988) Tumor necrosis factor in experimental cancer regression with alpha-tocopherol, beta-carotene, canthaxanthin and algae extract. Eur. J Cancer.Clin.Oncol.24:839–850.

132.Verhasselt, V., Berghe, W.V., Vanderheyde, N., Willems, F., Haegeman, G. & Goldman, M. (1999) N-Acetyl-L-cysteine inhibits primary T cell responses at the dendritic cell level: association with NF B inhibition. J.Immunol.162:2569–2574.

133. Joshi, M.S., Ponthier, J.L. & Lancaster, J.R., Jr. (1999) Cellular antioxidant and prooxidant actions of nitric oxide. Free Radical Biol.Med.27:1357–1366.

134.Michal, J.J., Chew, B.P., Wong, T.S., Heirman, L.R. & Standaert, F.E. (1994) Modulatory effects of dietary –carotene on blood and mammary leukocyte function in peripartum dairy cows. J.Dairy Sci.77:1408–1422.

135.Tjoelker, L.W., Chew, B.P., Tanaka, T.S. & Daniel, L.R. (1988) Bovine vitamin A and carotene intake and lactational status.1.Responsiveness of peripheral blood polymorphonuclear leukocytes to vitamin A and –carotene challenge in vitro. J. Dairy Sci.71:3112–3119.

136.Prabhala, R.H., Maxey, V., Hicks, M.J., & Watson, R.R. (1989) Enhancement of the expression of activation markers of human peripheral blood mononuclear cells by in vitro culture with retinoids and carotenoids. J.Leuk.Biol.45:249–254.

137.Jyonouchi, H., Zhang, L., Gross, M. & Tomita, Y. (1994) Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. Nutr.Cancer21:47–58.

138. Schwartz, J.L., Flynn, E. & Shklar, G. (1990) The effect of carotenoids on the antitumor immune response in vivo and in vitro with hamster and mouse effectors. Micronutr.Immunol.Function587:92–109.

139.Chew, B.P., Park, J.S., Wong, T.S., Kim, H.W., Weng, B.C., Byrne, K.M., Hayek, M.G. & Reinhart, G.A. (2000) Dietary –carotene stimulates cell-mediated and humoral immune response in dogs. J.Nutr.130:1910–1913.

140. Kim, H.W., Chew, B.P., Wong, T.S., Park, J.S., Weng, B.C., Byrne, K.M., Hayek, M.G. & Reinhart, G.A.(2000) Dietary lutein stimulates immune response in the canine. Vet. Immunol. Immunopath. 74:315–327.

141.Kim, H.W., Chew, B.P., Wong, T.S., Park, J.S., Weng, B.C., Byrne, K.M., Hayek, M.G. & Reinhart, G.A. (2000) Modulation of humoral and cell-mediated immune responses by dietary lutein in cats.Vet.Immunol.Immu-nopath.73:331–341

142 Chandra, R. (1997). Nutrition and the immune system: an introduction." American Journal of Clinical Nutrition 66: 460s-3s.

143 Bendich, A. and R. Chancira, Eds. (1990). Micronutrients and immune hctions. New York Academy of Sciences. New York.

144-Munro, H. and G. Schlierf, Eds. (1992). Nutrition of the Elderly. New York, Raven

145 Chandra, R. (1996). "Nutrition, immunity, and infection: From basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival." PNAS 93: 14304-7

146 Chandra, R. (1992). Lancet 34: 1124-27.

147 Alexander, J. and M. Peck (1990). "Future prospects for adjunctive therapy: Pharmacologic and nutritional approaches to immune system modulation." Critical Care Medicine 18: S159-S164.

148. Beisel W. History of nutritional immunology: introduction and overview. journal of Nutrition. March 1992; 122(3 Supph:591-6.

149.Beisel W. Nutrition in pediatric HIV' infection: setting the research agenda. Nutrition and immune function: Overview, journal of Nutrition.1996; 126(10 Suppl):2611-15.

150. Sevilla l. Aguiree N. Study on the Effects of Super Blue-Green Algae on the Nutritional Status and School Performance of First-. Second-, and Third-Grade Children Attending the Monsehor Velez School in Nandaime. Nicaragua [doctoral dissertation, in Spanish], Unlversidad CentroAmericana. Nicaragua 1995.

151.Bruno J. Gittelman J. Tuchfeld B. Lowered aluminum, with better detoxification and improved cognitive, behavioral ratings by children eating Aphanizomenon flos-aquae. Submitted for publication. 2001.

152.Younghaung w et al. The study on curative effect of zinc-containing blue-green algae for zinc deficient children. Paper presented at the Fifth international Phycological Congress; Qingdao. China; June 1994.

153.Alexander M. Newmark H. Miller R. Oral betacarotene can increase the number of OKT4+ cells in human blood. immunology Letter. 1985; 9(4):221-4.

154. Abrams. K. Algae to the Rescue. Logan House. 1996:71.

155. Bendich. A. Carotenoids and the immune response, journal of Nutrition. 1989; 119:112-5.

156.okai Y. Higashi-Okai K. Possible immunomodulating activities of carotenoids in vitro cell culture experiments. international journal of immunopharmacology. December 1996; 18(12):753-8.

157.Qureshi M. Ali R. Spirulina platensis exposure enhances macrophage phagocytic function in cats. immunopharmacology and immunotoxlcology. 1996; 18(3):457-63.

158.Qureshi M et al. immune enhancement potential of Spirulina platensis in chickens. Poultry science.1994; 73:46.

159.Qureshi M. Garlich J. Kidd M. Dietary Spirulina platensis enhances humoral and cell-mediated immune functions in chickens, immunopharmacology and immunotoxlcology. 1996; 18(3):465-76.

160. Jensen G. Ginsberg D. Huerta P. Drapeau C. Consumption of Aphanizomenonflos-aquae has rapid effects on the circulation and function of immune cells in humans. A novel approach to nutritional mobilization of the immune system, yourna/ of the American Nutraceutical Association.January.2000; 2(3)50-8.

161.Drapeau C. increased macrophage activity observed in college basketball players eating AFA using live blood analysis. Presentation at Klamath Falls. Oregon; August 8. 1998.

162.Hayashi O. Katoh T. Okuwaki Y. Enhancement of antibody production in mice by dietary Spirulina platensis. Journal of Nutritional Science and vitaminology Tokyo). October 1994; 40(5):431-41.

163. Hayashi O. Hirahashi T. Katoh T. Mlyajima H. Hirano T. Okuwaki Y. Class-specific influence of dietary Spirulina platensis on antibody production in mice. Journal of Nutritional Science and Vitaminology (Tokyo).1998; 44(6):841 -51.

164. Terziev V. Planski B. Encheva I. Use of nonspecific agents and vaccination in bronchopneumonia prevention in cattle, Vit Med Nauki [Bulgarin], 1983; 20(1):36-9.

165 Manoukian R et al. Effects of the blue-green algae Aphanizomenon flos-aquae (L.) Ralphs on human natural killer cells, in Phytoceuticals: Examining the health benefits and pharmaceutical properties of natural antioxidants and phytochemicals.IBC Library Series I9ii.ch. 3.1. Boston. March 1998; 233-41.

166. Krylov V et al. Retrospective epidemiological study using medical records to determine which diseases are improved by Aphanizomenon flos-aquae supplements. Submitted for publication. 2000.

167. Horrobin E et al. The nutritional regulation of T-lymphocyte function. Medical Hypotheses 1979: 5:969.

168. Tornabene T et al. Lipid and lipopolysaccharide constitutents of cyanobacterium Spirulina platensis. Ecol Prog Serv. March 1985; 22:121.

169 Cheng-Wu Z. Chao-Tsi T. Zhen Z. The effects of polysaccharide and phycocyanin from Spirulina platensis on peripheral blood and hematopoietic system of bone marrow in mice. Proceedings of the Second Asia-Pacific Conference on Algal Biotechnology'. National University of Singapore; 1994:58.

170. Kovats E. Potentiation of HIV envelope glycoprotein and other immunogens by endotoxin and Its molecular fragments. international journal of immunopharmacology. March 1996; 43(3):248-56.

171. Besednova N. Smolina T. Mlkheiskaia L. Ovodova R. immunostlmulating activity of the lipopolysaccharides of blue-green algae. ZhurnalMikrobiiolgii. Epidemiologii I immunobiologii (Russian). December 1979; (12):75-9.

172. Abrams K. Algae to the Rescue. Logan House.1996:75.

173 Kim, H., E. Lee, et al. (1998). "Inhibitory effect of mast cell-mediated immediate-type allergic reactions in rats by Spirulina." Biochem Pharmacol SS(7): 107 1-6.

174 Yang, H., E. Lee, et al. (1997). "Spirulina platensis inhibits anaphylactic reaction." Life Sci 61(l3): 1237-44.

175-Lenczowski, M., E. Schmidt, et al. (1998). "Individual variation in hypothalamus- pituitary-adrenal responsiveness of rats to endotoxin and interleukin-1 beta." Ann NY Acad Sci 856: 139-147.

176 Goeler, L., R. Gaykema, et al. (1999). "Interleukin-1 beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems?" J Neurosci 19(7): 2799-2806.

177- Romay, C., N. Ledon, et al. (1998). "Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation." inflammation Research 47(8): 334-8.

178 Gonzalez, R., S. Rodriguez, et al. (1999). Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats." Pharmacological Research 39(1): 55-9.

179.Breinholt et al. (1995) Dietary chlorophyllin is a potent inhibitor of aflatoxin B1 hepatocarcino-genesis in Rainbow trout. J. Cancer Res.55: 57-62.

180.Negishi et al. (1997) Antigenotoxic activity of natural chlorophylls. Mutat Res 376: 97-100.

181.Hernaez et al. (1997) Effects of tea and chlorophyllin on the mutagenicity of N-hydroxy-IQ: studies of enzyme inhibition, molecular complex formation, and degradation/scavenging of the active metabolites. Environ Mol Mutagen 30(4): 468-74.

182.Park and Surh. (1996) Chemopreventive activity of chlorophyllin against mouse skin carcinogenesis by benzo[a]pyrene and benzo [a] pyrene-7,8-dihydrodiol-9,10-epoxide. Cancer Lett 102: 143-9.

183.Hughes and Latner. (1936) Chlorophyll and hemoglobin regeneration after hemorrhage. J. Physiol., 86: 388.

184. Larry Cook (2003) Attentional Priority Disorder Not Attention Deficit Disorder; GreenerMagazine.com • © 2003 p;12

185.Goldberg, S.L. (1943) The use of water-soluble chlorophyll in oral sepsis. American Journal of Surgery (October): 117-123.

186.Gruskin, B. (1940) Chlorophyll—its therapeutic place in acute and suppurative disease. Am J Surg 49:49-55.

187.Gahan et al. (1943) Chlorophyll in the treatment of ulcers. Arch of Dermatology and Syphilology 49: 848-851.

188.Gaziano and Hennekens. (1993) The role of beta-carotene in the prevention of cardiovascular disease. In Carotenoids in Human Health. L.M. Canfield, N.I. Krinsky, J.A. Olson, Eds. New York Academy of Sciences, New York. 148-155.

189.Street et al. (1994) Serum antioxidants and myocardial infarction. Are low levels of carotenoids and alpha-tocopherol risk factors for myocardial infarction? Circulation 90(3): 1154-1161.

190.Shaish et al. (1995) Beta-carotene inhibits atherosclerosis in hypercholesterolemic rabbits. J Clin Invest 96: 2075-2082.

191.Morris et al. (1994) Serum carotenoids and coronary heart disease. The lipid research clinics coronary primary prevention trial and follow-up study. JAMA 272(18): 1439-1441.

192.Kazi et al. (1997) Immunomodulatory Effect of B-Carotene on T Lymphocyte Subsets in Patients with Resected Colonic Polyps and Cancer. Nutrition and Cancer 28(2): 140-145.

193.Moriguchi et al. (1996) Beta-carotene supplementation enhances lymphocyte proliferation with mitogens in human peripheral blood lymphocytes. Nutr Res 16(2): 211-218.

194.Murata et al. (1994) Effect of long-term administration of beta-carotene on lymphocyte subsets in humans. Am J Clin Nutr 60: 597-602.

195.Santamaria et al. (1988) Chemoprevention of indirect and direct chemical carcinogenesis by carotenoids as oxygen radical quenchers. Ann New York Acad Sci 534: 584-596.

196.Garewal et al. (1995) Emerging role of beta-carotene and antioxidant nutrients in prevention of oral cancer. Archives of Otolaryngology 121(2): 141-144.

197.Schwartz et al. (1987) Regression of experimental hamster cancer by beta-carotene and algae extracts. J Oral Maxillofac Surg 45: 510-515.

198.Schwartz et al. (1989) Prevention and inhibition of oral cancer in the hamster buccal pouch model associated with carotenoid immune enhancement. Tumor Biol 10: 297-309.

199.Nagasawa et al. (1989) Inhibition by beta-carotene-rich algae dunaliella of spontaneous mammary tumourigenesis in mice. Anticancer Res 9:71-76.

200.Di Mascio et al. (1991) Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. Am J Clin Nutr 53: 194S-200S.

201. Franceschi et al. (1994) Tomatoes and risk of digestive-tract cancers. International Journal of Cancer 59: 181-184.

202.Simopoulos, A.P. (1989) Summary of the NATO-advanced research workshop on dietary omega-3 and omega-6 fatty acids: biological effects and nutritional essentiality. Am Inst Nutr 119(4): 521-527.

203.Spielman et al. (1989) Biochemical and bioclinical aspects of blackcurrant seed oil: omega-3/omega-6 balanced oil. In: Dietary Omega-3 and Omega-6 Fatty Acids: Biological Effects and Nutritional Effects and Nutritional Essentiality, Galli, C. & Simopoulos, A. P., Eds., Plenum Publishing, New York, pp 309-322.

204.Kromhout, D. (1989) Fish (oil) consumption and coronary heart disease. In: Dietary Omega-3 and Omega-6 Fatty Acids: Biological Effects and Nutritional Effects and Nutritional Essentiality, Galli, C. & Simopoulos, A. P., Eds., Plenum Publishing, New York, pp 273-282.

205.Renaud et al. (1989) Influence of long-term diet modification on platelet function and composition in Moselle farmers. Am J Clin Nutr 43: 136-150.

206.Wood et al. (1987) Linoleic and eicosapentaenoic acids in adipose tissue and platelets and risk of coronary heart disease. Lancet (Jan 24): 177-183.

207.DeWille et al. (1979) Effects of essential fatty acid deficiency, and various levels of dietary polyunsaturated fatty acids, on humoral immunity in mice. J Nutr 109(6): 1018-1027.

208.Kremer et al. (1989) Different doses of fish-oil fatty acid ingestion in active rheumatoid arthritis: a prospective study of clinical and immunological parameters. In: Dietary Omega-3 and Omega-6 Fatty Acids: Biological Effects and Nutritional Effects and Nutritional Essentiality, Galli, C. & Simopoulos, A. P., Eds., Plenum Publishing, New York, pp 343-350;

209.Kremer et al. (1987) Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blind controlled, crossover study. Ann. Intern. Med. 106: 497-503.

210.Hibblen and Salem (1995) Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. Am J Clin Nutr 62:1-9.

211.Stevens et al. (1995) Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 62:761-768.

212.Bierve, K. S. , Btekke, O. L. , Fougner, K. J. & Midthiell, K. (1989) Omega-3 and omega-6 fatty acids in serum lipids and their relationship to human disease In: Dietary w3 and w6 Fatty Acids: Biological Effects and Nutritional Essentially (Galli, C & Simopoulos, A. P., eds.), pp. 241-252, Plenum Publishing, New York.

213.Catalan J, Moriguchi T, Slotnick B, Murthy M, Greiner RS, Salem N Jr. (2002) Cognitive deficits in docosahexaenoic acid-deficient rats. Behav Neurosci 116(6):1022-31.

214.Wright, S. and Burton, J.L. (1982) Oral evening primrose seed oil improves atopic eczema. Lancet 2(8308):1120-1122.

215.Nordoy, A. and Simonsen, T. (1987) Dietary n-3 fatty acids, experimental thrombosis and coronary heart disease in man. In: Proceedins of the AOCS short course on polyunsaturatted fatty acids and eicosanoids, edited by Lands, W.E.M.Biloxi, Mississippi:American Oil Chemists Society, p. 25-34.

216.Lagarde, M., Crpset, M. & Hariarine, M. (1989) In vitro studies on docosahexaenoic acid in human plateelets. In: Dietary w3 amd w6 Fatty Acids: Biological Effects and Nutritional Effects and Nutritional Essentiality (Galli, C & Simopoulos, A. P., eds.), pp 91-96, Plenum Publishing, New York.

217.Siess, W., Scherer, B., Bohlig, B., Roth, P., Kurzmann, I., and Weber, P.C.(1980) Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. Lancet 1(8166):441-444.

218.Sugano M, Ide T, Ishida T, Yoshida K (1986) Hypocholesterolemic effect of gamma-linolenic acid as evening primrose oil in rats. Ann Nutr Metab 30(5):289-99

219.Karmali, R.A. (1989) Dietary w3 and w6 fatty acids in cancer. In: Dietary w3amd w6 Fatty Acids: Biological Effects and Nutritional Effects and Nutritional Essentiality (Galli, C & Simopoulos, A. P., eds.), pp351-360, Plenum Publishing, New York.

220.Wargovich, M.J. (1997) Experimental evidence for cancer preventive elements in foods. Cancer Letters 114:11-17.

221.Jamal, G.A., Carmichael, H., and Weir, A.I. (1986) Gamma-linolenic acid in diabetic neuropathy. Lancet 1(8489): 1098.

222.Houtsmuller, A.J., van Hal-Ferwerda, J., Zahn, K. J., and Henkes, H. E. (1981) Favorable influences of Linoleic acid on the progression of diabetic micro- and macro-angiopathy in adult onset diabetes mellitus. Prog, Lip. Res. 20: 377

223.Vymazal, J. (1984) Short-term uptake of heavy metals by periphyton algae. Hydrobiolgia 119: 171-179.

224.Vymazal, J. (1984) Short-term uptake of heavy metals by periphyton algae. Hydrobiolgia 119: 171-179.

225.Volesky and Holan (1995) Biosorption of heavy metals. Biotechnology 11: 235-250.

226.Fukino et al. (1990) Effect of Spirulina (S.platensis) on the Renal Toxicity Induced by Inorganic Mercury and Cisplatin. Symposium on Environmental Pollutants and Toxicology, November 9-10, Eisei Kagaku, 36.

227.Pore, R.S. (1984) Detoxification of chlordecone poisoned rats with chlorella and chlorella-derived sporopollenin. Drug and Chem Toxicol 7(1): 57-71.

228- Stewart Cooper MD (2008); Caring Ambassador Hepatitis C Choises: 4th choices, pp; 81:99

229. Orland JR. Wright TL, Cooper SL Acute hepatitis C. Hepotology. 2001:33:321.

230. Choo Q-L, Kuo 6, Weiner AJ, Overby LR, Bradley DW, and Houghton M. Isolation of a cDNA clone derived from blood-borne non-A, non-8 viral hepatitis genome. Science. 1989; 244:359.

231. Bartenschlager R, Lohmann V. Replication of hepatitis C virus. J Gen Virol. 2000; 81:1631.

232. Simmonds P. Generic diversity and evolution of hepatitis C virus-15 years on. J Gen Virol. 2004;85:3173.

233. Martell M. Esteban Jl, Quer J et al. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. J Virol. 1992,-66:3225.

234. Weiner A, Erickson A, Kansopon J et of. Persistent hepatitis C virus infection in a chimpanzee is associated with emergence of a cytotoxic T lymphocyte escape variant. Proc Notl Acod Sci USA. 1995;92:2755.

235. Erickson AL. Kimura Y, Igarashi S et of. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. Immunity. 2001;15:883.

236. Hepburn, MJ, Hepburn LM, Cantu NS, Lapeer MG, Lawitz EJ. Differences in treatment outcome for hepatitis C among ethnic groups. Am J Med 2004; 117:163.

237. Large MK, Kittlesen DJ, Hahn YS. Suppression of host immune response by the core protein of hepatitis C virus: possible implications for hepatitis C virus persistence. J Immunol 1999:162:931.

238. Gale M. Kwiedszewski B.Jr, Dossett M, Nakao H, Katze MG. Antiapoptotic and oncogenic potentials of hepatitis C virus are linked to interferon resistance by viral repression of the PKR protein kinase. J Virol 1999:73:6506.

239. Chen CM, You LR, Hwang LH, Lee YH. Direct interaction of hepatitis C virus core protein with the cellular lymphotoxin-beta receptor modulates the signal pathway of the lymphotoxin-beta receptor. Journal of Virology 1997;71:9417.

240. Taylor DR, Shi ST, Romano PR, Barber GN, Lai MM. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. Science 1999; 285:107.

241. Bartenschlager R, Frese M, Pietschmann T. Novel insights into hepatitis C virus replication and persistence. Adv Virus Res 2004;63:71.

242. Melen K, Fagerlund R, Nyqvist M, Keskinen P. Julkunen I. Expression of hepatitis C virus core protein inhibits interferon-induced nuclear import of STATs. J Med Virol 2004:73:536.

243. Ok polder H, Zachoval R, Hoffmann R, et al. Possible mechanism invoking T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection, lancet 1995;346:1006.

244. Cucchiarini M, Kammer AR, Grabscheid B, et al. Vigorous peripheral blood cytotoxic T cell response during the acute phase of hepatitis C virus infection. Cell Immunol 2000:203:111.

245. Belkaid Y. Piccirillo CA, Mendez S. Shevach EM. Sacks DL C04»CD25« regulatory T cells control Leishmania major persistence and immunity. Nature 2002;420:502.

246. Guidotti LG, Rochford R. Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999,284:825.

247. Lawlor D A Warren E, Ward FE, Parham P. Comparison of MHC Alleles in Humans and Apes. Immunologicol Reviews 1990;113:147.

248. Bontrop RE. Otting N. Slierendregt BL. Lanchbury JS. Evolution of Major Histocompatibility Complex Polymorphisms and T-Cell Receptor Diversity in Primates. Immunologicol Reviews 1995;143:33.

249. Jaeger EE, Bontrop RE, Lanchbury JS. Structure, diversity, and evolution of the T-cell receptor VB gene repertoire in primates. Anmunogenecici 1994;40:184.

250. Houghton M. Choo Q-L. Kuo G, et al. Prospects for prophylactic and therapeutic hepatitis C virus vaccines. Princess Takamatsu Symp 1995:25:237.

251. Qian S. Lu L. Fu F. et al. Apoptosis Within Spontaneously Accepted Mouse Liver Allografts. J Immunol 1997;158:4654.

252. Nuti S, Rosa D, Valiante NM, et al. Dynamics of intra-hepatic lymphocytes in chronic hepatitis C: enrichment for Valpha24* T cells and rapid elimination of effector cells by apoptosis. EurJ Immunol 1998:28:3448.

253. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution.) Exp Med 1973;137:1142.

254. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cels in immunity. Nat Immunol 2004;5:1219.

255. Goutagny N, Fatmi A, De Ledinghen V, et al. Evidence of viral replication in circulating dendritic cells during hepatitis C virus infection. J Infect Dis 2003;187:1951.

256. Lauer GM, Walker BD (2001) Hepatitis C virus infection. N Engl J Med 345: 41-52.

257. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, et al. (2009) Genetic history of hepatitis C virus in East Asia. J virol 83: 1071-1082.

258. Pybus OG, Charleston MA, Gupta S, Rambaut A, Holmes EC, et al. (2001) The epidemic behavior of the hepatitis C virus. Science 292: 2323-2325.

259. Simmonds P (2004) Genetic diversity of the hepatitis C virus- 15 years on. J Gen Virol 85: 3173-3188.

260. WHO (2002) WHO Traditional Medicine Strategy 2002-2005.

261. Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, et al. (2011) A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int 31: 61-80.

262. Camma C, Di Bona D, Schepis F, Heathcote EJ, Zeuzem S, et al. (2004) Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. Hepatology 39: 333-342.

263. Kamal SM, Nasser IA (2008) Hepatitis C genotype 4: what we know and what we don't yet know. Hepatology 47: 1371-1383.

264. Boyd MR, Gustafson KR, McMahon JB, Shoemaker RH, O'Keefe BR, et al. (1997) Discovery of cyanovirin-N, a novel human immunodefciency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob Agents Chemother 41: 1521-1530.

265. Helle F, Wychowski C, Vu-Dac N, Gustafson K, Voisset C, et al. (2006) Cyanovirin-N Inhibits Hepatitis C Virus Entry by Binding to Envelope Protein Glycans. J Biol Chem 281: 25177-25183.

266. Hayashi K, Hayashi T, Kojima I (1996) A natural sulfated polysaccharide, calcium spirulan, isolated from Spirulina platensis: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodefciency virus activities. AIDS Res Hum Retroviruses 12: 1463-1471.

267. Ayehunie S, Belay A, Baba TW, Ruprecht RM (1998) Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthrospira platensis). J Acquir Immune Defc Syndr Hum Retrovirol 18: 7-12.

268. Schaeffer DJ, Krylov VS (2000) Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicol Environ Saf 45: 208-227.

269. Jensen GS, Hart AN, Zaske LA, Drapeau C, Gupta N, et al. (2007) Mobilization of human CD34+ CD133+ and CD34+ CD133(-) stem cells in vivo by consumption of an extract from Aphanizomenon flos-aquae-related to modulation of CXCR4 expression by an L-selectin ligand? Cardiovasc Revasc Med 8: 189-202.

270. Takeshita M, Ishida Y, Akamatsu E, Ohmori Y, Sudoh M, et al. (2009) Proanthocyanidin from blueberry leaves suppresses expression of subgenomic hepatitis C virus RNA. J Biol Chem 284: 21165-21176.

271. Eisenbrand G; Senate Commission on Food Safety (SKLM), German Research (2008) Microcystins in algae products used as food supplements. Mol Nutr Food Res 52: 735-736.

272. Baicus C, Tanasescu C (2002) Chronic viral hepatitis, the treatment with spirulina for one month has no effect on the aminotransferases. Rom J Intern Med 40: 89-94.

273. Yano M, Ikeda M, Abe K, Dansako H, Ohkoshi S, et al. (2007) Comprehensive Analysis of the Effects of Ordinary Nutrients on Hepatitis C Virus RNA Replication in Cell Culture. Antimicrob Agents Chemother 51: 2016-2027.

274.Sheir Z, Badra G, Salama O, Gomaa AI, Saber W (2013) Effect of Combination of Some Natural Products and Chloroquine on HCV Infection in Egyptian Patients: Pilot Study. J Liver 2: 116.

275. Woodruff H. Natural products from microorganisms. Science. June 1980; 208(449): 1225-9.

276. Wltvrouw M. De Clercq E. Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. General Pharmacology. October 1997; 29(4):497-5ii.

277. Bewick P et al. Chlorella: The Emerald Food. Ronin Publishing. 1984.

278. Schmidt R. Treatment for Leprosy. Price-Pottenger Nutrition Foundation Health journal.1997.21(3).

279. Martlnez-Nadal F. Antimicrobial activity of Sptrultna maxima. Paper presented at the Tenth international Congress of Microbiology. Mexico City; August 1970.

280. jorjani G, Amirani P. Antibacterial activities of Spirulina platensis. Maj Limy PuzDaniskpndtshap (Danish). 1978; 1:14-18.

281. Ostensvlk O. Skulberg O. Underdal B. Hormazabal V. Antibacterial properties of extracts from selected planktonic freshwater cyanobacteria - a comparative study of bacterial bioassays, journal of Applied Microbiology. 1998;84(6):111 7-24.

282. Kreitlow S. Mundt S. Lindequist U. Cyanobacteria — a potential source of new biologically active substances, journal of Biotechnology. April 1999; 70(1-3):61-3.

283. Frankmolle w et al. Antifungal cyclic peptides from the terrestrial blue-green alga Anabaena laxa. I. isolation and biological properties. yournafo/Antf&iotfcs (Tokyo). September 1992; 45(9):1451-7.

284. Aaronson A. Dublnsky Z. Mass production of microalgae. Experientia. 1982; 43(Suppl.):44.

285. Gustafson K et al. AIDS antiviral sulfolipids from cyanobacteria, journal of the National Cancer institute. 1989; 81:1254-8.

286. Cardellina J et al. A chemical screening strategy for the dereplication and prioritization of HIV-inhibitory aqueous natural products extracts. Journal of Natural Products. July 1993; 56(7): 1123-9.

287. Lau A et al. inhibition of reverse transcriptase activity by extracts of cultured blue-green algae (cyanophyta). Planta Medica. April 1993; 59(2): 148-51.

288. Ayehunie S. Belay A. Baba T. Ruprecht R. inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthrospiraplatensis). journal of Acquired immune Deficiency syndrome and Human Retrovtrotogy. May 1998; 18(1):7-I2.

389. Hayashi et al. Calcium spirulan. an inhibitor of enveloped virus replication, from a blue-green algae Spirulina platensis. journal of Natural Products. 1996; 59:83-7.

290. Loya S. Reshef V. Mlzrachl E et al. The inhibition of the reverse transcriptase of HlV-1 by the natural sulfoglycolipids from cyanobacteria: contribution of different modalities to their high potency journal of Natural Products. 1998;61 (7):891 -5.

291. Gandhi M. Boyd M et al. Properties of cyanovirin-N (CV-N): inactivation of HlV-1 by sessile cyanovirin-N. Developments in Biological Standardization. 2000; 102:14i -8.

292. Larsen L. Moore R. Patterson G. Beta-carotenes from the blue-green alga Dichothrixbaueriana. journal of Natural Products. March 1994; 57(3):419-21.

293. Hayashi K et al. An extract from Spirulina platensis is a selective inhibitor of Herpes simplex virus type 1 penetration into HeLa cells. Phytotherapy Research. 1993; 7:76-80.

294. Guzman-Murillo M. Ascencio F. Anti-adhesive activity of sulphated exopolysaccharides of microalgae on attachment of red sore disease-associated bacteria and Helicobacter pylori to tissue culture cells. Letters of Applied Microbiology. June 2000; 30(6):473-8.

295. Fabregas J et al. in vitro inhibition of the replication of Haemorrhagic septicaemia virus and African swine fever virus by extracts from marine microalgae. Antiviral Research. November 1999; 44(1):67-73.

296.Drapeau C. Results of Survey of AFA Consumers. Presentation at Klamath Falls. Oregon. August 8. 1998.

297.Lahitova N. Doupovcova M, Zvonar J. Chandoga J. Hocman G. Antimutagenic properties of fresh-water blue-green algae. Folia Microbiologica 1994; 39(4):301-3.

298.Hasegawa T et al. Augmentation of the resistance against Listeria monocytogenes by oral administration of a hot water extract of Chlorella vulgaris in mice, immunopharmacology and immunotoxicology. 1994: 16(2): 191 -202.

299.Haegawa T et al. Hot water extracts of Chlorellavulgaris reduce opportunistic infection with Listeria monocytogenes in C57BL/6 mice infected with LP-BM5 murine leukemia viruses. International journal of immunopharmacology.1995; 1 7(6):505-l 2.

300.Tanaka K et al. Augmentation of host-defense by a unicellular green algae. Chlorella vulgaris, to Escherichia coli infection, infection and immunity.1986; 53:267.

301. Ibusuki K Minamishima Y. Effect of Chlorella vulgaris extracts on murine cytomegalovirus infections. Natural immunity and Cell Growth Regulation.1990; 9:121.

302.Konishi F et al. Enhanced resistance against Escherichia coli infection by subcutaneous administration of the hot-water extract of Chlorella vulgaris in cyclophosphamide-treated mice. Cancer immunology and immunotherapy.1990; 32(1):1.

303.Hasegawa T et al. Accelerated restoration of the leukocyte number and augmented resistance against Escherichia coli in cyclophosphamide-treated rats orally administered with a hot water extract of Chlorella vulgaris. international journal of immunopharmacology. 1990; 12:883.

304.Bock K. sabin N. The Road to immunity. Pocket Books.1997:177.

305.Sommer A et al. increased risks of respiratory disease and diarrhea in children with pre-existing mild vitamin A deficiency. American journal of Clinical Nutrition.1984; 40:1090-95.

306.Pinnock C et al. Vitamin A status in children who are prone to respiratory tract infections.Australian Pediatric journal. 1986; 22(2):95-9.

307.Nikitin A. Simvolokov S. The efficacy of the natural antibacterial preparation chlorophyll in the combined treatment of acute lung abscesses. TerArkh (Russian). 1989; 61 (8):113-6.

308.Bowers w. Chlorophyll in wound healing and suppurative disease. American journal of Surgery.1947; 73:37.

309. Boyd, M. (1997). "Protein lsolated from Blue-Green Algae Inactivates HIV." Antimicrob Agents Chemother 41 : 152 1 - 1530.

310. Helle F, Wychowski C, Vu-Dac N, Gustafson K, Voisset C, et al. (2006) Cyanovirin-N Inhibits Hepatitis C Virus Entry by Binding to Envelope Protein Glycans. J Biol Chem 281: 25177-25183.

311.Schmidt M. Smith L. Sehnert K. Beyond Antibiotics: Healthier Options for Families. North Atlantic Books.1993 : 74.

312. Cited in**: Challem J.** Spirulina. Keats Publishing. 1981: 17-8.

313.Yang H. Lee E. Kim H. spirulina platensis inhibits anaphylactic reaction. Life Sciences. 1997; 61(13):1237-44.

314.Kushak R et al. Favorable effects of blue-green algae Aphanizomenon flos-aquae on rat plasma lipids, journal of the American Nutraceutlcal Association. January 2000; 2(3):59-65.

315.Bisgaard H. Leukotrienes and prostaglandins in asthma. Allergy 1984; 69:413-20.

316. Neuman I. Nahum H. Ben-Amotz A. Prevention of exercise-induced asthma by a natural isomer mixture of beta-carotene. Annals of Allergy and asthma immunology. June 1999; 82(6):549-53.

317. Cited in: **Challem J.** Spirulina. Keats Publishing. I981:17.

318. Abrams K. Attention Deficit Hyperactivity Disorder: A Nutritional Approach. Book Crafter. 1998: 48-9.

319Stephen Holt Stem Cell support ; The Nutraceutical Induction of Adult Stem Cell Recruitment (IASCR)pp;1:11

320. Scott CR, Stem Cell Now: A Brief Introduction to the Coming Medical Revolution. First Plume Printing, Penguin Group, New York etc, 2006.

321. Mattei JF, The ethical question of the embryo, Bull Acad Natl Med; 2000, 184: 1227-1235.

322.Jensen and Drapeau (2002) The use of in situ bone-marrow stem cells for the treatment of various degenerative diseases. Med Hypotheses 59(4): 422-428.

323. Parker SM, Bringing the 'gospel of life' to American jurisprudence: a religious, ethical, and philosophical critique of federal funding for embryonic stem cell research, J Contemp Health Law Policy, 2001; 17: 771–808.

324. Ferrari G., Cusella-De-Angelis G., Coletta M. et al. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998; 279(5356): 1528–1530.

325. Orlic D, Kajstura J, Chimenti S et al, Bone marrow cells regenerate infracted myocardium. Nature, 2001, 410(6829): 701–705.

326. Kocher AA, Schuster MD, Szabolcs MJ et al, Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling,and improves cardiac function, Nat Med, 2001; 7: 430–436.

327. Carmeliet P, Luttun A, The emerging role of the bone marrow-derived stem cells in (therapeutic) angiogenesis, Thromb Haemost, 2001; 86: 289–297.

328. Petersen BE, Bowen WC, Patrene KD et al, Bone marrow as a potential source of hepatic oval cells, Science, 1999; 284(5417): 1168–1170.

329. Lagasse E, Connors H, Al-Dhalimy M et al, Purified hematopoietic stem cells can differentiate into hepatocytes in vivo, Nat Med, 2000; 6: 1229–1234.

330. Pereira R.F, O'Hara MD, Laptev AV et al, Marrow stromal cells as a source of progenitor cells for non-hematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta, Proc Natl Acad Sci USA,1998; 95: 1142–1147.

331. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ, Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats similarities to astrocyte grafts, Proc Natl Acad Sci USA, 1998; 95: 3908–3913.

332. Prockop DJ, Azizi SA, Colter D, Digirolamo C, Kopen G, Phinney DG, Potential use of stem cells from bone marrow to repair the extracellular matrix and the central nervous system, Biochem Soc Trans, 2000; 28: 341–345.

333. Kopen GC, Prockop DJ, Phinney DG, Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains, Proc Natl Acad Sci USA, 1999; 96: 10711–10716.

334 Polli EE, Transplanting bone-marrow stem cells in the central nervous system, Haematologica, 2000; 85: 1009–1010.

335. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR, Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow, Science, 2000; 290: 1779–1782.

336. Brazelton TR, Rossi FM, Keshet GI, Blau HM, From marrow to brain: expression of neuronal phenotypes in adult mice, Science, 2000; 290: 1775–1779.

337. Eglitis MA, Mezey E, Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice, Proc Natl Acad Sci USA, 1997; 94: 4080–4085.

338. Lu D, Li Y, Wang L, Chen J, Mahmood A, Chopp M, Intraarterial administration of marrow stromal cells in a rat model of traumatic brain injury, J Neurotrauma, 2001; 18: 813–819.

339. Korbling M, Kutz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, Chamblin RE, Estrov Z, Hepatocytes and epithelial cells of donor origin in recipients of peripheral blood stem cells, N Engl J Med, 2002; 346: 738–746.

340. Ogawa M, Differentiation and proliferation of hematopoietic stem cells, Blood, 1993: 81: 28442853.

341. Socolovsky M, Constantinescu SN, Bergelson S, Sirotkin A and Lodish HF, Cytokines in hematopoiesis: specificity and redundancy in receptor function, Adv Protein Chem, 1998; 52: 141–198.

342. Henschler R, Brugger W, Luft T, Frey T, Mertelsmann R and Kanz L, Maintenance of transplantation potential in ex vivo expanded CD34(+)-selected human peripheral blood progenitor cells, 1994; Blood, 84: 2898–2903.

343. Bickford PC, Tan J, Shytle RD, Sanberg CD, El-Badri N, Sanberg PR, Nutraceuticals synergistically promote proliferation of human stem cells, Stem Cell Dev, 2006; 15(1): 118-123.

344. Holt S, Proceedings at Stem Cell Summit, American Academy of Anti Aging Medicine, September 11, 2009

345.Goodell et al. (2001) Stem cell plasticity in muscle and bone marrow.Ann N Y Acad Sci 938: 208-218.

346.Mattson, M.P. (2000) Emerging neuroprotective strategies for Alzheimer's disease: dietary restriction, telomerase activation, and stem cell therapy. Exp Gerontol 35: 489-502.

347.Williams and Hickey (1995) Traffic of hematogenous cells through the central nervous system. Curr Top Microbiol Immunol 202: 221-245.

348.Knopf et al. (1998) Antigen-Dependent Intrathecal Antibody Synthesis in the Normal Rat Brain: Tissue Entry and Local Retention of Antigen-Specific B Cells. J Immunol 161: 692-701.

349.Hickey, W.F. (1999) Leukocyte traffic in the central nervous system: the participants and their roles. Semin Immunol 11: 125-37.

350. Deb A, Wang S, Skelding KA, et al. Bone marrow-derived cardiomyocytes are present in adult human heart: A study of gender-mismatched bone marrow transplantation patients. Circulation. 2003;107(9):1247–1249.

351. Körbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl. J Med. 2002;346(10):738–746.

352. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest. 2003;111:843–850.

353. Hasegawa Y, Ogihara T, Yamada T, et al. Bone marrow (BM) transplantation promotes beta-cell regeneration after acute injury through BM cell mobilization. Endocrinology 2007;148(5):2006–2015.

354. Cantürk NZ, Vural B, Esen N, et al. Effects of granulocyte-macrophage colony-stimulating factor on incisional wound healing in an experimental diabetic rat model. Endocr Res. 1999;25(1):105–116.

355. Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD, Crain B. Transplanted bone marrow generates new neurons in human brains. Proc Natl Acad Sci USA. 2003;100, 1364–1369.

356. Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med. 2005;353(10):999–1007.

357. Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res. 2001;89(1):E1–7.

358. Marchesi C, Belicchi M, Meregalli M, et al. Correlation of circulating CD133+ progenitor subclasses with a mild phenotype in Duchenne muscular dystrophy patients. PLoS ONE. 2008;3(5):e2218.

359. Diller GP, van Eijl S, Okonko DO, et al. Circulating endothelial progenitor cells in patients with Eisenmenger syndrome and idiopathic pulmonary arterial hypertension. Circulation. 2008;117(23):3020–3030.

360. Junhui Z, Xingxiang W, Guosheng F, Yunpeng S, Furong Z, Junzhu C. Reduced number and activity of circulating endothelial progenitor cells in patients with idiopathic pulmonary arterial hypertension. Respir Med. 2008;102(7):1073–1079.

361. Herbrig K, Haensel S, Oelschlaegel U, Pistrosch F, Foerster S, Passauer J. Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells. Ann Rheum Dis. 2006;65(2):157–163.

362. Grisar J, Aletaha D, Steiner CW, et al. Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. Circulation. 2005;111(2):204–211.

363. Zhu J, Wang X, Chen J, Sun J, Zhang F. Reduced number and activity of circulating endothelial progenitor cells from patients with hyperhomocysteinemia. Arch Med Res. 2006;37(4):484–489.

364. Westerweel PE, Luijten RK, Hoefer IE, Koomans HA, Derksen RH, Verhaar MC. Hematopoietic and endothelial progenitor cells are deficient in quiescent systemic lupus erythematosus. Ann Rheum Dis. 2007;66(7):865–870.

365. Moonen JR, de Leeuw K, van Seijen XJ, et al. Reduced number and impaired function of circulating progenitor cells in patients with systemic lupus erythematosus. Arthritis Res Ther. 2007;9(4):R84.

366. Choi JH, Kim KL, Huh W, et al. Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. Arterioscler Thromb Vasc Biol. 2004;24(7):1246–1252.

367. Drapeau C, Antarr D, Ma H, et al. Mobilization of bone marrow stem cells with StemEnhance® improves muscle regeneration in cardiotoxin-induced muscle injury. Cell Cycle. 2010;May;9(9):1819–1823.

368. Drapeau C, The Discovery of StemEnhance; HEALTHSPAN CANADA winter 2009/2010, volume2 number2 pp; 4:5

369. Drapeau C, Stem Tech, H EALTH L INK VOLUME 1, ISSUE 2, AUGUST, 2006,p2

370. Tamoda H,Aoki. Bone marrow stimulation and left ventricular function in acute myocardial infarction. Clin Cardiol 2003;26(10) 455-7

371. Werner N, Koisol S et al, Circulating endothelial progenitor cells and cardiovascular outcomes, N Eng J Med2005;353(10):999-1007

372. R. Douglas Shytle et al, Effects of blue green algae extracts on the proliferation of adult stem cells in vitro A preliminary study,Med Sci Monit, 2010; 16(1): BR-5

373.Aposhyan S. Natural Intelligence: Mind-Body integration and Human Development. William and Wilkins.1999:150.

374.Rothschild L. Cockell C. Radiation: microbial evolution, ecology, and relevance to Mars missions. Murafiorc Research. 1990; 430:281.

375.Tomitani A et al. Chlorophyll b and phycobilins in the common ancestor of cyanobacteria and chloroplasts. Nature. July 1999; 400(6740): 159-62.

376.Pennisi E. Science News. March 12. 1994.

377.Grelfe H. Molnar S. Studies of nucleic acid metabolism in rats by use of carbon-14-labeled purine and pyrimidine bases and nucleic acids: Anabolic pathways of nucleic acid derivatives. Tlerphystologte (German). 1978: 40(5):248-56.

378.Berthold H et al. Evidence for incorporation of intact dietary pyrimidine (but not purine) nucleosides into hepatic RNA.Proceedings of the National Academy of science USA. October 1995: 92(22):I 0123-7.

379.Devi M. The effect of algal protein diets on the regeneration of serum and liver proteins in protein depleted rats. Plant Foods for Human Nutrition.1983; 33:287.

380. Devi M et al. Serum protein regeneration studies on rats on algal diets. Nutrition Research international.1979; 19:785.

381.Ma G. Xue K. Wu J. Yuan S. Qin H. Antimutagenic effects of beta-carotene from Dunaliellasalina (Chinese). Chung Kuo Yao Li HsuehPao. May 1998: 19(3):282-4.

382.Anderson R et al. Reduction in free-radical-induced DNA strand breaks and base damage through fast chemical repair by flavonoids. Free Radical Research. July 2000; 33(1):91-103.

383.Gagne S. Energetics of Food Spiral Sciences inc..1990: 2 73.

384.Kumamoto S. (1984. August 28) U.S. Patent * 4.468.460 for Method of Human Cell Culture.

385.Apsley J. The Regeneration Effect.Genesis Communications. 1996.

386.Krasnikova N. Proliferation of the epithelium surrounding a skin wound in hairless mice exposed to sodium chlorophyllin. StullEkspBiol .Vied (Russian). October 1973; 75(10):99-102.

387.Krasnikova N. Experimental use of an aqueous solution of sodium chlorophyllin to speed the healing of skin wounds. EkspKhtrAnesteztol (Russian). Sep-Oct 1972; 17(5): 19-22.

388.Hughes J. Lamer L. Chlorophyll and hemoglobin regeneration after hemorrhage, journal of Physiology. 1936;86:388.

389.Patek A. Chlorophyll and regeneration of blood. Archives of internal Medicine.1936; 57:76.

390.Arasaki S. Arasaki T. Low Calorie, High Nutrition vegetables From the Sea: To Help you Look and Feel Better. Japan Publications. 1983: 60.

391.Abrams K. Algae to the Rescue. Logan House.1996:26.

392.Arenesen E. Serum total homocysteine and coronary heart disease, international journal of Epidemiology. August 1995; 24(4):704-9.

393.Bakers. Detoxification and Healing: The Key to Optimal Health. Keats Publishing. 1997.

394.Tel-Or E. Huflejt M. Packer L. The role of glutathione and ascorbate in hydroperoxide removal in cyanobacteria. Biochemical and Biophysical Research Communications. October 1985; 132(2):533-9.

395.Cui J. wakabayashl s et al. isolation and sequence studies of cysteinyl peptides from Spirulina glutathione reductase: comparison of active site cysteine peptides with those of other flavoprotein disulfide oxidoreductases.journal of Biochemistry (Tokyo). March 1989: 105(3):390-4.

396.Sundquist A. Fahey R. Evolution of antioxidant mechanisms: thiol-dependent peroxidases and thioltransferase among prokaryotes, journal of Molecular Evolution. November 1989; 29(5):429-35.

397.Torres-Duran P et al. Studies on the preventive effect of Spirulina maxima on fatty liver development induced by carbon tetrachloride, in the rat. journal of Ethnopharmacology. February 1999; 64(2): 141 -7.

398. Torres-Duran P et al. Spirulina maxima prevents induction of fatty liver by carbon tetrachloride in the rat. Biochemistry and Molecular Biology international. April 1998; 44(4):787-93.

399.Vadiraja B. Gaikv/ad N. Madyastha K. Hepatoprotective effect of C-phycocyanin: Protection for carbon tetrachlorideand R (+)-pulegone-mediated hepatotoxicity in rats. Biochemical and Biophysical Research Communications. 1998;249(2):428-3l.

400.Gonzalez de Rivera C et al. Preventative effect of Spirulina maxima on the fatty liver induced by a fructose-rich diet in the rat. a preliminary report. Life Science. 1993; 53(1):57-61.

401.Dashwood R. Guo D. Protective properties of chlorophylls against the covalent binding of heterocyclic amines to DNA in vitro and in vivo. Paper presented at the Princess Takamatsu Symposium. 1995; 23:181-9.

402.Challemj. Spirulina.Keats Publishing.1981: 14.

403.Sukenik A. Takahasi H. and Modady S. Dietary lipids from marine unicellular algae enhance the amount of liver and blood omega-3 fatty acids in rats. Annals of Nutrition and Metabolism. 1994; 38(2):85-90.

404.Levin, G. Yeshurun M. Mokady S. in vivo antiperoxidative effect of 9-cis beta-carotene compared with that of the all-trans isomer. Nutrition and Cancer. 1997; 27(3):293-7.

405.Gagnes. Energetics of Food. Spiral Science inc. 1990:274.

406.Challemj. Spirulina. Keats Publishing.1981: 18.

407.Yamane Y The effect of Spirulina on nephrotoxicity in rats. Paper presented at Annual Symposium of the Pharmaceutical Society of Japan. April 15. 1988.

408.Lee w. Rosenbaum M. Chlorella. Keats Publishing.1987: 17.

409.Burnatowska-Hledin M, Kaiser L. Mayor G. Aluminum, parathyroid hormone, and osteomalacia. Special Topics in Endocrinology and Metabolism.1983; 5:201 -26.

410.Shastri D. Kumar M. Kumar A. Modulation of lead toxicity by Spirulina fusiformis. Phytotherapy Research. May 1999; 13(3) :2 58-60.

411.Rimland B and Larson G. Hair mineral analysis and behavior: an analysis of 51 studies, journal of Learning Disabilities. 1983; 16:279-85.

412.Phil R. and Parkes M. Hair element content in learning disabled children. Science.1977; 198:204-6.

413.Valencia A. walker J. A multi-axial treatment paradigm for mild traumatic brain injury to achieve reparative functional metaplasticity. Paper presented at the Third world Congress on Brain injury. Quebec City. Canada. June 1999.

414.Golubkina N et al. The selenium haemostasis during experimental anaphylaxis reaction in rats treated with reduced glutathione and selenium enriched Spirulina. voprosyMedltsinskolKhlmlt (Russian). jan-Feb 2000; 46(1):22-7.

415.Young R. Bergei J. Use of chlorophyllin in the care of geriatric patients, journal of the American Geriatric Society. January 1980; 28(1):46-7.

416.Needleman H. Landrlgan P. Raising Children Toxin Free. Avon Books.1994: 138.

417.Weiss J. Landauer M. Radioprotection by antioxidants. Annals of the New York Academy of Science.2000; 899:44-60.

418.Ben-Amotz A et al. Natural beta-carotene and whole body irradiation in rats. Radiation and Environmental Biophysics. November 1996; 35(4):285-8.

419.Umegaki K. Takeuchi N. Ikegami S. Ichikawa T. Effect of beta-carotene on spontaneous and X-ray-induced chromosomal damage in bone marrow cells of mice. Nutrition and Cancer. 1994; 22(3):277-84.

420.Kumar S et al. inhibition of radiation-induced DNA damage in plasmid pBR322 by chlorophyllin and possible mechanism(s) of action. Mutation Research. March 1999; 425(1):71 -9.

421.Karpov L et al. The postradiation use of vitamin-containing complexes and a phycocyanin extract in a radiation lesion in rats. RadtatsBiolRadloecol (Russian). May-Jun 2000; 40(3):310-4.

422.Sarma L. Tiku A. Kesavan P. Ogaki M. Evaluation of radioprotective action of a mutant (E25) form of Chlorella vulgaris in mice, journal of Radiation Research (Tokyo). December 1993; 34(4):277-84.

423. Vacek A. Rotkovska D. Bartonickova A. Radioprotection of hemopoiesis conferred by aqueous extract from chlorococcal algae (A-astimul) administered to mice before irradiation. Experimental Hematology.1990; 18:234-37.

424.Rotkovska D. vacek A. Bartonickova A. The radioprotective effects of aqueous extract from chlorococcal freshwater algae (Chlorella kessleri) in mice and rats. Sirahlentheronkologle. November 1989; 165(11):813-6.

425.Rotkovska D. vatsek A. Bartonichikova A. increase in the radiation resistance of mice using ivastimul. Radloblologlla(Russian). Sep-Oct 1989; 29(5):652-4.

426.Qlshen P. Guo B. Kolman A. Radioprotective effect of extract from Spirulina platensis in mouse bone marrow cells studied by using the micronucleus test. Toxicology Letters. August 1989; 48(2): 165-9.

427.Singh S. Tiku A. Kesavan P. Post-exposure radioprotection by Chlorella vulgaris (E-25) in mice. Indian journal of Experimental Biology. August 1995; 33(8):612-5.

428.Apsleyj. The Regeneration Effect.Genesis Communications.1996:75.

429.Abrams K. Algae to the Rescue. Logan House.1996:32.

430.Ben-Amotz A et al. Effect of natural beta-carotene supplementation in children exposed to radiation from the Chernobyl accident. Radiation Environmental Biophysics. October 1988; 37(3): 187-93.

431.Christian Drapeau (former Cell Tech research director). Personal communication. August 1998.

432. **Christian Drapeau, MSc**: Primordial Food; Aphanizomenon Flos-Aquae, Latin for: "Invisible Living Flower of the Water" a Wild Blue Green Alga with Unique Health Properties; Second Edition: January 2005, p40.

433. Johnson E. Schroeder w. Microbial carotenoids. Advances in Biochemical Engineering and Biotechnology. 1996;53:119-78.

434. Ben-Amotz A. Mokady S. **Edelstein S. Avron M.** Bioavailability of a natural isomer mixture as compared with synthetic all-trans-beta-carotene in rats and chicks, journal of Nutrition. July 1989; n 9(7): 1013-9.

435. Ben-Amotz A. Levy Y. Bioavailability of a natural isomer mixture compared with synthetic all-trans beta-carotene in human serum. American journal of Clinical Nutrition. May 1996; 63(5):729-34.

436. Levin G. Mokady S. Antioxidant activity of 9-cis compared to all-trans beta-carotene in vitro. Free Radical Biological Medicine. July 1994; 17(1):77-82.

437. Kelly F. Use of antioxidants in the prevention and treatment of disease, journal of The international Federation of Clinical Chemistry. March 1998; 10(1):21-3.

438. Frei B. Reactive oxygen species and antioxidant vitamins: mechanism of action. American journal of Medicine. September 1994; 97(3A):5S-i 3S:22S-28S.

439. Apsleyj. The Regeneration Effect.Genesis Communications.1996:85.

440. Balz Frei cited in: The Wall Street journal - interactive Edition. Green and slimy and two billion years old? Eat it. January 9. 2000.

441. Romay C, Gonzalez R. Phyoocyanin is an antioxidant protector of human erythrocytes against lysis by peroxyl radicals, journal of Pharmacy and Pharmacology. April 2000; 52(4):367-8.

442. Romay C. Ledon N. Gonzalez R. Phycocyanin extract reduces leukotriene B4 levels in arachidonic acid-induced mouse-ear inflammation test, journal of Pharmacy and Pharmacology. May 1999; 51(5):641-2.

443. Romay C et al. Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae, inflammation Research. 1998; 47(1):36-41.

444. Baker J. in: Natural Products and Drug Development. Krogsgaard-Larsen.Brogger Christensen. Kofod.eds. Muksgaard. Copenhagen. 145-63.

445. Okai Y. Higashi-Okai K. Potent anti-inflammatory activity of pheophytin A derived from edible green algae. Enteromorphaprofilera (Sujiao-nori).international journal of Immunopharmacology. June 1997; 19(6):355-8.

446. Jensen G. written communication. February 2000.

447.Tolomio C et al. Colonization by diatoms and antirheumatic activity of thermal mud.Cell Biochemistry and Function. 1999; 17:29-33.

448.Coccettin M. Bellometti S et al. Serum interleukin-1 changes in arthroslc patients after mud-pack treatment. Phys Rehab Kur Med. 1995; 5:92-3.

449.Sukenik A. Takahashi H. Mokady S. Dietary lipids from marine unicellular algae enhance the amount of liver and blood omega-3 fatty acids in rats. Annals of Nutrition and Metabolism. 1994; 38(2):85-96.

450.Krylov v. Drapeau C. et al. Retrospective epidemiological study using medical records to determine which diseases are improved by Aphanizomenon flos-aquae supplements. Submitted for publication 2001.

451. Reddy et al., 2000. Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from Spirulina platensis. Biochem Biophys Res Commun 2 ;27 7 (3):599 -603 .

452. Remirez et al.,1999. Effect of phycocyanin in zymosan-induced arthritis in mice - Phycocyanin as an antiarthritic compound. Drug Dev Res 48:70-75.

453.Yoshida, A., Takagaki, Y., Nishimune, T., 1996. Enzyme immunoassay for phycocyanin as the main component of spirulina color in foods. Bioscience Biotechnology and Biochemistry60(1),57–60.

454.Cohen, Z., 1986. Products from microalgae. In: Richmond, A. (Ed.), Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, pp.421–454.

455.Stocker, R., McDonagh, A.F., Glazer, A.N., Ames, B.N., 1990. Antioxidant activities of bile pigments: biliverdin and bilirubin. Methods in Enzymology186,301–309.

456.Bhat, V.B., Madyastha, K.M., 2001. Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from Spirulina platensis: protection against oxidative damage to DNA. Biochemical and Biophysical Research Communications285(2), 262–266.

457-Galli, F., Canestrari, F., Bellomo, G., 1999. Pathophysiology of oxidative stress and its implication in uremia and dialysis. Contributions to Nephrology127,1–31.

458-Parthasarathy, S., Khan-Merchant, N., Penumetcha, M., Santanam, N., 2001. Oxidative stress in cardiovascular disease.Journal of Nuclear Cardiology 8(3),379–389.

459-Cooke, M.S., Evans, M.D., Dizdaroglu, M., Lunec, J., 2003. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB Journal17(10),1195–1214.

460-Gey, K.F., 1998. Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. Biofactors7(1–2),113–174.

461-Spillert CR, Pelosi MA, Parmer LP, Lazaro EJ.A peroxide-induced inflammation model for drug testing. Agents Actions 1987;21:297–8.

462. Glazer, A.N., 1988. Phycobiliproteins. Methods in Enzymology 167, 291–303.

463-Weber GF. The measurement of oxygen-derived free radicals and related substances in medicine. J Clin Chem Clin Biochem1990; 28:569–603.

464-Hodgson EK, Fridovich I. The role of superoxide in the chemiluminescence of luminol.PhotochemPhotobiol1973;18: 451–5.

465-Halliwell B. How to characterize a biological antioxidant. Free Rad Res Comm1990;9:1–32.

466-Parij N, Nagy AN, Neve J. Linear and non-linear competition plots in the deoxyribose assay for determination of rate constants for reaction of nonsteroidal anti-inflammatory drugs with hydroxyl radicals. Free Rad Res 1995;23:571–9.

467-Lindena J, Burkhardt H, Dwenger A. Mechanisms of non-opsonizedzymosan-induced and luminol-enhanced chemiluminescence in whole blood and isolated phagocytes. J Clin Chem Clin Biochem 1987;25:765–78.

468 Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G. (2005) Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med. 8;353(10):999-1007.

469 Bozlar M, Aslan B, Kalaci A, Baktiroglu L, Yanat AN, Tasci A. (2005) Effects of human granulocyte-colony stimulating factor on fracture healing in rats. Saudi Med J. 26(8):1250-4.

470 Kong D, Melo LG, Gnecchi M, Zhang L, Mostoslavsky G, Liew CC, Pratt RE, Dzau VJ. (2004) Cytokine-induced mobilization of circulating endothelial progenitor cells enhances repair of injured arteries. Circulation. 110(14):2039-46.

471 Eroglu E, Agalar F, Altuntas I, Eroglu F. (2004) Effects of granulocyte-colony stimulating factor on wound healing in a mouse model of burn trauma. Tohoku J Exp Med. 204(1):11-6.

472.Sanchez A. Cited in: Heartlnfo. March 13. 2000.

473.Levy Y et al. Dietary supplementation of a natural isomer mixture of beta-carotene inhibits oxidation of LDL derived from patients with Diabetes mellitus. Annals of Nutrition and Metabolism. March 2000; 44(2):54-60.

474.Mietus-Snyder. M and Malloy. M. Endothelial dysfunction occurs in children with two genetic hyperlipidemias: improvement with antioxidant vitamin therapy journal of Pediatrics. 1998; 133(1):35-40.

475.Paredes-Carbajal M et al. Effects of dietary Spirulina maxima on endothelium dependent vasomotor response of rat aortic rings. Life Science. 1997; 61(l5):211-9.

476.Smith D et al. Eskimo plasma constituents, dihomo-linolenlc acid, eicosapentaenolc acid and docosahexaenoic acid inhibit the release of atherogenic mitogens. Lipids.1989; 24:70-5.

477.Abrams K. Algae to the Rescue. Logan House. 1996:39-40.

478.Cohen Z. Norman H. Helmer Y. Microalgae as a source of omega three fatty acids. Plants in Human Nutrition. 1995; 77:1-31.

479 Conquer J. Holub B. Supplementation with an algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects, journal of Nutrition. 1996; 126:3032-9.

480.Reddy S. Sanders. T Obeid O. The influence of maternal vegetarian diet on essential fatty acid status of the newborn. European journal of Clinical Nutrition.1994; 48:358-68.

481.Neuringer M et al. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annual Review of Nutrition.1988; 8:517-41.

482.Pan w et al. Hemostatic factors and blood lipids in young Buddhist vegetarians and omnivores. American journal of Clinical Nutrition.1993; 58:354-9.

483.Kushak R. Drapeau C. van Cott E. Winter H. Blue-green alga Aphanizomenon flos-aquae as a source of dietary polyunsaturated fatty acids and a hypocholesterolemic agent. Paper presented at the Annual Meeting of the American Chemical Society Anaheim. California. March 21-25. 1999.

484.Rolle l. Pabst w. The cholesterol-lowering effect of the unicellular green alga - Scenedesmusacutus. Nutrition and Metabolism. 1980: 24(5):291-301.

485.Hori K. ishibashi G. Okita T. Hypo-cholesterolemic effect of blue-green alga, ishikurage (Nostoc commune) in rats fed atherogenic diet. Plant Foods Human Nutrition. 1994; 45(1):63-70.

486.de Caire G. de Cano M. de Mule C. Steyerthal N. Piantanida M. Effect of Spirulina platensis on glucose, uric acid and cholesterol levels in the blood of rodents, international journal Experimental Botany. 1995; 57:93-6.

487. sano T. Tankaka Y. Effect of dried, powdered Chlorella vulgaris on experimental atherosclerosis and alimentary hypercholesterolemia in cholesterol-fed rabbits. Artery. 1987; 14(2):76-84.

488.Santo T et al. Effect of lipophilic extract of Chlorella vulgaris on alimentary hvperlipidemia in cholesterol-fed rats. Artery. 1988; 15(4):2l 7-24.

489.Rolle l. Pabst w. uber die cholesterinsenkendeW'irkung der einzelligenGrunalgeScenedesmusacutus. Nutritional Metabolism (English abstract). 1980: 24:291-301.

490.Conquer J. Holub B. Effects of supplementation with an algae source of docosahexaenoic acid on risk factors for heart disease in vegetarians. Abstract of the 64th congress of the European Arteriosclerosis Society. Utrecht. Netherlands. June. 1995: 10-13.

491.Morris D et al. Serum carotenoids and coronary heart disease.The Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study, journal of the American Medical Association.1994; 272:1439-41.

492.iwata K. inayama T. Kato T. Effects of Spirulina platensis on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats, journal of Nutritional Science and Vitaminology (Tokyo). April 1990; 36(2): 165-71.

493.Delio M. Blue Goo and the Healthy Heart. Wired News. Aug. 30. 2000

494. Cited by **Linet M.** Evolution of cancer epidemiology. Epidemiology Review. 2000; 22(1):35.

495.Murakami A. Ohigashi H. Koshlmizu K. Anti-tumor promotion with food phytochemicals: a strategy for cancer chemoprevention. Blosdence. Biotechnology, and Biochemistry. January 1996; 60(1):1-8.

496.Hocman G. Prevention of Cancer: vegetables and plants. CBP: Comparative Biochemistry and Physiology B.1989; 93(2):201 -12.

497.Sarkar A et al. Beta-carotene prevents lipid peroxidation and red blood cell damage in experimental hepatocarcinogenesis. Cancer Biochemistry and Biophysics. 1995; 15(2): in-25.

498.Tinkler J et al. Dietary carotenoids protect human cells from damage, journal of Photochemistry and Photobiology. 1994; 26(3):282-5.

499.Le Marchand L et al. intake of specific carotenoids and lung cancer risk. Cancer Epidemiology.1993; 2:183-7.

500 Omenn G et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial, journal of the National Cancer institute. November 1996; 88(21):1 550-9.

501.Johnson E. Schroeder w. Microbial cartenoids. advanced Biochemistry Engineering and Biotechnology. 1996; 53:119-178.

502.Mittal A et al. Modulatory potential of Spirulina fusiformis on carcinogen metabolizing enzymes in Swiss albino mice. Phytotherapy Research. March 1999; 13(2):111-4.

503.Kozlenko R. Henson. R. The study of Spirulina. effects on the AIDS virus, cancer and the immune system. Healthy & Natural. 1996; 3(5):66-7.

504.Fujii Y. Sakamoto S. Ben-Amotz A. Nagasawa H. Effects of beta-carotene-rich algae Dunaliellabardawil on the dynamic changes of normal and neoplastic mammary cells and general metabolism in mice. Anticancer Research. Mar-Apr 199; 13(2) :38 9-93.

505. Xue L. Experimental study on extract of Dunaliellasalina in preventing NSAR-induced cancer of proventriculus in mice. Chung Hua Yu Fang I Hsueh 7$a Chlh (Chinese). November 1993; 27(6):350-3.

506.Nagasawa H et al. Suppression by beta-carotene-rich algae Dunaliellabardawil of the progression, but not the development, of spontaneous mammary tumcurs in SHN virgin mice.Anticancer Research. Mar-Apr 1991; 11(2):713-7.

507.Combs w et al. in vivo and in vitro effects of B-carotene and algae extracts in murine tumor models. Nutrition and Cancer. 1989; 12(4):371-9.

508.Schwartz J. Shklar G. Suda D. inhibition of experimental oral carcinogenesis by topical beta carotene. Carcinogenesis. 1986; 7(5):711 -5.

509.Schwartz J. Shklar G. Reid S. Trickier D. Prevention of experimental oral cancer by extracts of Sptrulina-Dunalteila algae. Nutrition and Cancer. 1988; 11 (2):1 27-34.

510. Mathew B et al. Evaluation of chemoprevention of oral cancer with Spirulinafusiformis.Nutrition and Cancer. 1995; 24(2): l 97-202.

511.Smith C. Zhang X. Mechanism of action of cryptophycin.yourna/ of Biological Chemistry. 1996; 271 (11):6192-8.

512.Smith C. Zhang X et al. Cryptophycin: a new antimicrotubule agent active against drug-resistant cells. Cancer Research. July 1994; 54(14):3779-84.

513.Ogino J et al. Dendrcamides. new cyclic hexapeptides from a blue-green alga. Multidrug-resistance reversing activity of dendroamide A. journal of Natural Products. June 1996; 59(6):581-6.

514.Smith C et al. Reversal of multiple drug resistance by tolyporphin. a novel cyanobacterial natural product. Oncology Research. 1994; 6(4-5):211-8.

515.Smith C. Carmeli S. Moore R. Patterson G. Scytophycins. novel microfilament-depolymerizing agents which circumvent P-glycoprotein-mediated multidrug resistance. Cancer Research. March 1993; 53(6): 1343-7.

516.vermeil C. Morin O. Experimental role of the unicellular algae Prototheca and Chlorella (chlorellacease) in anti-cancer immunogenesis. ComptesRendus Des Seances De La Societe De BlologieEt De SesFlllales. October 1976; 170(3):646-9.

517.Mishima T et al. inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP). a novel sulfated polysaccharide derived from blue-green algae. Spirulinaplatensis.Clinical and Experimental Metastasis. 1998; 16(6):541 -50.

518. Tanaka K et al. A novel glycoprotein obtained from Chlorella vulgaris strain CK22 shows antimetastatic immuno-potentiation. Cancer immunology and immunotherapy. February 1998; 45(6):313-20.

519.Hayakawa Y. Hayashi T et al. Calcium spirulan as an inducer of tissue-type plasminogen activator in human fetal lung fibroblasts. BiochimicaEtBiophysicaActa. March 1997; 1355(3):241-7.

520. Noda K et al. A water-soluble antitumor glycoprotein from Chlorella vulgaris.PlantaMedica. October 196;62(5) :42 3-6.

521. Tokuda H et al. inhibition of 12-O-TPA promoted mouse skin papilloma by digalactosyldiacylglycerols from the freshwater cyanobacteriumPhormtdiumtenue. Cancer Letters. 1996; 104(1):91-5.

522. Vermeil C. Morin O. Le Bodic L. The stimulation of tumoricidal peritoneal macrophages can be directly induced by peritoneal implantation of unicellar algae in humans. Archives De L institut Pasteur De Tunis (French). Mar-Jun 1985; 62(l-2):91-4.

523.Higashi-Okai K. Okai Y. Potent suppressive activity of chlorophyll a and b from green tea (camellia sinensis) against tumor promotion in mouse skin. journal of UOEH (Japanese). September 1998; 20(3): 181 -8.

524. Okai Y et al. Suppressive effects of chlorophyllin on mutagen-induced umu C gene expression in Salmonella typhlmurlum (TA 1535/pSK 1002) and tumor promoter dependent ornithine decarboxylase induction in BALB-'c 3T3 fibroblast cells. Mutation Research. August 1996; 370(l):11-7.

525. Singh et al. inhibitory potential of Chlorella vulgaris (E-2S) on mouse skin papillomagenesis and xenobiotic detoxification system. Anticancer Research.1999; 19:1887-92.

526. Tanaka K et al. Oral administration of Chlorella vulgaris augments concomitant antitumor immunity. immunopharmacology and immunotoxlcology. 1990; 12(2):277-91.

527.Konlshi F et al. Antitumor effect induced by a hot water extract of Chlorella vulgaris: resistance to meth-A tumor growth mediated by CE-induced polymorphonuclear leukocytes. Cancer immunology and immunotherapy. 1985;19(2):73-8.

528. Tanaka K et al. Augmentation of antitumor resistance by a strain of unicellular green algae. Chlorella vulgaris. Cancer immunology and immunotherapy. 1984; 17(2):90-4.

529. Nomoto K. Yokokura T. Satoh H. Mutai M. Antitumor activity of Chlorella extract. PCM-4.by oral administration. Can To Kagaku Ryohoijapanese). March 1983; 10(3):781-5.

530. Morin O. Guilard R. Guihard D. vermeil C. New approach to the study of the experimental inhibitory effect of the
unicellular alga Chlorella pyrenoldosa against the murine sarcomas BP8 and L1210.ComptesRendus Des Seances De La Soclete De BiologieEt De Ses Finales (French). 1980; 174(1):74-81.

531. Neveu P et al. Modulation of antibody synthesis by an antitumor algae. Expenentla. December 1978; 34(12): 1644-5.

532. Miyazawa Y et al. immunomodulation by a unicellular green algae (Chlorella pyrenoldosa) in tumor-bearing mice. journal of Ethnopharmacology. December 1988; 24(2): 135-46.

533. Carbonnelle D et al. Antitumor and antiproliferative effects of an aqueous extract from the marine diatom Haslea ostrearta (simonsen;. against solid tumors: lung carcinoma (NSCLC-No). kidney carcinoma (E39) and melanoma (M96) cell lines. Anticancer Research.1999; 19:621-4.

534. Berge J et al. Antiproliferative effects of an organic extract from the marine diatom Skeletonemacostatum (grev.) against a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). Anticancer Research.1997; 17:2115-20.

535. Henrikson R. Earth Food Spirulina. Ronore Enterprises.1989: 69.

536. Lijjima N et al. Anti-tumor agent and method of treatment there with. US patent pending, ref. PH50-726-A82679, Application filed 15Sep. 1982.

537. Patent claim filed by **Dainippon ink** and Chemicals and Tokyo Kenkyukai for "Anti-tumor agents containing phycobilin — also used to treat ulcers and hemorrhoid bleeding" (1983, JP58065216-A830418).

538.Konlshi F et al. Protective effect of an acidic glycoprotein obtained from culture of Chlorella wlgarts against myelosuppression by 5-fluorouracil. Cancer immunology and immunotherapy. June 1996; 42(5):268-74.

539. Chernomorsky S, Segelman A, Poretz RD. (1999), Effect of dietary chlorophyll derivatives on mutagenesis and tumor cell growth: Teratog Carcinog Mutagen.19(5):313-22

540.The Guardian, in Wednesday 7 December 2011

541. Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ HealthPersp2003;111:217–20.

542. Liaw Y-F, Sollano JD. Factors influencing liver disease progression in chronic hepatitis B. Liver Int 2006; 26:23–9.

543. Phillips TD. Dietary clay in the chemoprevention of aflatoxin-induced disease. Toxicol Sci1999; 52:118–26.

544. Phillips TD, Lemke SL, Grant PG. Characterization of clay-based enterosorbents for the prevention of aflatoxicosis. Adv Exp Med Biol 2002; 504:157–71.

545. Kensler TW, He X, Otieno M, etal. Oltipraz chemo-prevention trial in Qidong, People's Republic of China: modulation of serum aflatoxin albumin adduct biomarkers. Cancer Epidemiol Biomarkers Prev1998; 7:127–34.

546. Egner PA, Wang JB, Zhu YR, et al. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. ProcNatlAcadSciUSA2001;98:14601–6.

547. Egner PA, Munoz A, Kensler TW. Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. MutatRes2003; 523–524:209–16.

548. Kephart JC. Chlorophyll derivatives-their chemistry, commercial preparation, and uses. EconBotany1955;9:3–38.

549. Edwards BJ. Treatment of chronic leg ulcers with ointment containing soluble chlorophyll. Physiotherapy1954;40:177–9.

550. Larato DC, Pfau FR. Effects of a water-soluble chlorophyll in ointment on gingival inflammation. NYStateDentJ1970;36:291–3.

551. Tawashi R, Cousineau M, Sharkawi M. Effect of sodium copper chlorophyllin on the formation of calcium oxalate crystals in rat kidney. InvestUrol1980;18:90–2.

552.Young RW, Beregi JS, Jr. Use of chlorophyllin in the care of geriatric patients. JAmGeriatrSoc1980;28:46–7.

553. Jubert C, Mata J et al; Effects of Chlorophyll and Chlorophyllin on Low-Dose Aflatoxin B1 Pharmacokinetics in Human Volunteers, CancerPrevRes2009; 2(12) December2009

554. Egner PA, Stansbury KH, Snyder EP, Rogers ME, Hintz PA, Kensler TW. Identification and characterization of chlorine (4) ethylester in sera of individuals participating in the chlorophyllin chemoprevention trial. ChemResToxicol2000; 13:900–6.

555. Fahey JW, Stephenson KK, Dinkova-Kostova AT, Egner PA, Kensler TW, Talalay P. Chlorophyll, chlorophyllin and related tetrapyrroles are significant inducers of mammalian phase 2 cytoprotective genes. Carcinogenesis2005; 26:1247–55.

556. Simonich MT, Egner PA, Roebuck BD, et al. Natural chlorophyll inhibits aflatoxin B1-induced multi-organ carcinogenesis in the rat. Carcinogenesis 2007; 28:1294–302.

557. Simonich MT, Mc Quistan T, Jubert C, et al. Low- dose dietary chlorophyll inhibits multi-organ carcinogenesis in the rainbow trout. FoodChemToxicol 2008;46:1014–24.

558. Hayashi T, Schimerlik M, Bailey G. Mechanisms of chlorophyll in anticarcinogenesis: dose-responsive inhibition of aflatoxin uptake and biodistribution following oral co-administration in rainbow trout. ToxicolApplPharmacol1999; 158:132–40.

559. Bailey GS et al, Quantitative carcinogenesis and dosimetry in rainbow trout for aflatoxin B1and aflatoxicol, two aflatoxins that form the same DNA adduct. MutatRes1994; 313:25–38.

560. Gorelick NJ. Risk assessment for aflatoxin: I. Metabolism of aflatoxin B1 by different species. Risk Anal 1990; 10:539–59.

561-Kensler TW, Gange SJ, Egner PA, et al. Predictive value of molecular dosimetry: individual versus group effects of oltiprazon aflatoxin-albumin adducts and risk of liver cancer. Cancer Epidemiol BiomarkersPrev1997;6:603–10.

562-Dashwood R, Liew C. Chlorophyllin-enhanced excretion of urinary and fecal mutagens in rats given 2-amino-3-methylimidazo [4,5-f] quinoline. EnvironMolMutagen1992;20:199–205.

563-Mata JE et al, Effects of chlorophyllin on transport of dibenzo(al) pyrene,2-amino-1-methyl-6-phenylimi-dazo-[4,5-b] pyridine, and aflatoxin B1 across Caco-2 cell monolayers. Toxicology2004;196:117–25.

564-Dashwood RH. The importance of using pure chemicals in (anti) mutagenicity studies: chlorophyllin as a case in point. Mutat Res FundMolM1997;381:283–6.

565. Breinholt V, Arbogast D, Loveland P, et al. Chlorophyllin chemoprevention in trout initiated by aflatoxin B1 Bath treatment: an evaluation of reduced bioavailability vs. target organ protective mechanisms. ToxicolApplPharmacol1999;158:141–51.

566. Wong ZA, Hsieh DPH. The comparative metabolism and toxicokinetics of aflatoxinB1 in the monkey, rat, and mouse. ToxicolApplPharm1980;55:115–25

567-A.N. Hart et al, Natural killer cell activation and modulation of chemokines receptor profile in vitro by an extract from the cyanophyta Aphanizomenon flos-aquae," Journal of Medicinal Food, vol.10, no.3, pp.435–441,2007.

568-J. Subhashini et al, Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562," Biochemical Pharmacology,vol.68, no.3,pp.453–462,2004.

569-B. Li, X. Chu, M. Gao, and W. Li, "Apoptotic mechanism of MCF-7 breast cells in vivo and in vitro induced by photodynamic therapy with C-phycocyanin," Acta Biochimica et Biophysica Sinica, vol.42, no.1, pp.80–89,2010.

570-O.M. Basha et al, C-Phycocyanin inhibits cell proliferation and may induce apoptosis in human HepG2 cells," The Egyptian Journal of Immunology,vol.15,no.2,pp.161–167,2008.

571-B.V. Pardhasaradhi, A.M. Ali, A.L. Kumari, P. Reddanna and A. Khar, "Phycocyanin-mediated apoptosis in AK-5 tumor cells involves down-regulation of Bcl-2 and generation of ROS," Molecular Cancer Therapeutics, vol.2,no.11,pp.1165–1170,2003.

572-N. Pughand D.S. Pasco,"Characterization of human monocyte activation by a water soluble preparation of Aphanizomenon flos-aquae," Phytomedicine,vol.8,no.6,pp.445–453,2001.

573-T. Molinski, "Marine natural products," Clinical Advances in Hematology and Oncology,vol.7,no.6,pp.383–385,2009.

574-D.Tant,"Urinary tract infection in children," The British Journal of General Practice,vol.45,no.398,p.506,1995.

575-A. Lorenc, Y. Ilan-Clarke, N. Robinson, and M. Blair, "How parents choose to use CAM: a systematic review of theoretical models," BMC Complementary and Alternative Medicine, vol.9, article9,2009.

576-A. Steinsbekk, M.B. Rise, and M. Aickin, "Cross-cultural comparison of visitors to CAM practitioners in the United States and Norway," Journal of Alternative and Complementary Medicine, vol.15, no.11,pp.1201–1207,2009.

577-Sandler et al. (1979) Decreased cerebrospinal fluid concentration of free phenylacetic acid in depressive illness. Clin Chim Acta 93(1): 169-71.

578-Sabelli et al. (1983) Urinary phenyl acetate: a diagnostic test for depression? Science 220(4602): 1187-8.

579.Sabelli et al. (1996) Sustained antidepressant effect of PEA replacement. J Neuropsychiatry Clin Neurosci 8(2): 168-71.

580.Finberg et al. (2000) Modification of dopamine release by selective inhibitors of MAO-B. Neurobiology (Bp) 8(2): 137-42. 75.

581-Mosnaim et al. (1973) Ultraviolet spectrophotometric determination of 2-phenylethylamine in biological samples and its possible correlation with depression. Biol Psychiatry 6(3): 235-57.

582-Mosnaim et al. (1974) The influence of psychotropic drugs on the levels of endogenous 2-phenylethylamine in rabbit brain. Biol Psychiatry 8(2): 227-34.

583-Sabelli and Mosnaim (1974) Phenylethylamine hypothesis of affective behavior. Am J Psychiat 131: 695-699.

584.Drapeau et al. (2002). Antidepressant properties of the blue-green alga Aphanizomenon flos-aquae. Annual meeting of the American Holistic Medicine Association, Toronto, May 2002.

585.Baker et al. (1991) Phenylethylaminergic mechanisms in attention-deficit disorder. Biol Psychiatry 29(1): 15-22.

586.Parker and Cubeddu (1988) Comparative effects of amphetamine, phenylethylamine and related drugs on dopamine efflux, dopamine uptake and mazindol binding. J Pharmacol Exp Ther 245(1): 199-210.

587.Sabelli and Javaid (1995) Phenylethylamine modulation of affect: therapeutic and diagnostic implications. J Neuropsychiatry Clin Neurosci 7(1): 6-14.

588. May M. Disturbing Behavior. Environmental Health Perspectives. June 2000; 108(6):A262-67.

589. Crawford M. The early development and evolution of the human brain.Upsala journal of Medical sciences.Supplement. 1990;48:43-78.

590. Crawford M et al. Evidence for the unique function of docosahexaenoic acid during the evolution of the hominid brain. Lipids. 1999; 34<suppl):S39-S47.

591.Broahurst C. Cunnane S. Crawford M. Rift valley lake fish and shellfish provided brain-specific nutrition for early Homo. British Journal of Nutrition.January 1998; 79(l):3-21.

592.Laguna M. Villar R. Calleja J. Cadavid I. Effects of Chlorella stigmatophora extract on the central nervous system. PlantaMedica. April 1993; 59(2): 125-30.

593 Villar R. Laguna R. Calleja J. Cadavid I. Effects of Sketettonemacostatum extracts on the central nervous system. PlantaMedica. October 1992; 58(5)398-404.

594 Villar R. Laguna M. Calleja J. Cadavid I. Effects of Dunaliellatertiolecta extracts on the central nervous system. PlantaMedica. October 1992; 58(5):404-9.

595.Takenaka H et al. Protective effect of Dunaliellabardairtl on water-immersion-induced stress in rats.PlantaMedica. October 1993; 59(5):421-4.

596Tanaka K et al. Oral administration of a unicellular green algae. Chlorella vulgaris, prevents stress-induced ulcer. PlantaMedica. October 1997; 63(5):465-6.

597 Challemj. Spirulina.Keats Publishing. 1981:10.

598.Shinpo K et al. Paper presented at the 36th Conference of Japanese Society of Nutrition and Food Science; 1982: 102.

599. jarratt C. Jewett M, Peters S. Tragash E. The Children and Algae Report.The Center for Family wellness. Harvard. Mass.. 1995.

600.Bitterman N. Melamed Y. Ben-Amotz A. Beta-carotene and CNS oxygen toxicity in rats, journal of Applied Physiology. March 1994; 76(3): 1073-6.

601. Komatsu M. Hiramatsu M. The efficacy' of an antioxidant cocktail on lipid peroxide level and superoxide dismutase activity in aged rat brain and DNA damage in iron-induced epileptogenic foci. Toxicology. August 2000; 148(2-3): 143-8.

602. Vatassery G. Vitamin E and other endogenous antioxidants in the central nervous system. Geriatrics.September 1998; 53(Suppl 1); S25-S7.

603. Shohami E et al. Closed head injury in the rat induces whole body oxidative stress: overall reducing antioxidant profile, journal of Neurotrauma. May 1999; 16(5):365-76.

604. Annapurna A. et al. Bioavailability of blue-green algae carotenes in preschool children, journal of Clinical Biochemistry and Nutrition. 1991; l6: 145-51.

605. jama J et al. Dietary antioxidants and cognitive function in a population-based sample of older persons. The Rotterdam Study. American journal of Epidemiology. 1996; 144(3):275-80.

606. Brunett L. New ways to explain the brain. Wisconsin Alumini Journal. Summer 2000:36.

607. Cousens G. Report of treatment of Alzheimer's disease with Alphanae Klamathonmenon flos-aqua [sic]. Orthomedicine. Winter/Spring, 1985; 8(1):2.

608. Cousens G. Microalgae: First and finest superfood. Body Mind Spirit. April 1995; 14(3): 12-18.

609. jarratt C. Jewett M, Peters S. Tragash E. The Children and Algae Report.The Center for Family wellness. Harvard Mass.. 1995.

610. jarratt C. Personal communication. December 1998.

611. Foldoe. M. A Case Study of The Perceived Effects of Eating Aphanizomenon Flos-Aquae, a Blue-Green Algae On the Attention, Academic, and Social Behavior of Three Elementary School-Aged Children diagnosed with Attention Deficit/Hyperactivity Disorder (AD/HD) [thesis], Sonoma State University. 1999.

612. BuletsaB. Ihnatovych I. Lupyrh P. Pulvk O. The prevalence, structure and clinical problems of multiple sclerosis in the Transcarpathian area based on epidemiological study data. LikSprava. Oct-Dec 1996; (10-12):163-5.

613.Bourre J et al. Function of dietary polyunsaturated fatty acids in the nervous system. Prostaglandins Leukotrtenes and Essential Fatty Adds. 1993; 48:5-15.

614.Colquhoun I. Bunday S.A lack of essential fatty acids as a possible cause of hyperactivity in children. Medical Hypotheses. May 1981; 7(5):673-9.

615.Duvner T. Gamma-linolenlc acid as a treatment in AD/HD. Paper presented at the Sixth European Conference of Neurodevelopmental Delay. Stockholm, reported in Hyperactive Children Support Croup journal. 1994:48.

616-Murray C. Lopez A. Alternative projections of mortality and disability by cause 1990 to 2020: Global Burden of
Disease Study. Lancet. May 1997; 349(9064): 1498-504.

617.Christensen L. Dtet-Behartor Relationships. American Psychological Association Publications.1996:168.

618.Taylor J. The algae AD/HD connection: Can blue-green algae be of help with attention deficit/hyperactivity disorder? Network of Hope Newsletter. February 1998.

619.Cited in: **Carroll L. Tober j**. The indigo Children: The New Kids Have Arrived. Hay House inc. 1999:188.

620-Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. J Affect Disord 1998 Mar; 48(2-3):149-55.

621-Williams AL et al 2006. Do essential fatty acids have a role in the treatment of depression? Journal of Affective Disorders 93(2006)117–123

622-LE Magazine December 2003: Omega-3 Fatty Acids Vital to a longer, healthier life for information on omega-3s and brain function health.

623-Psychosom Med. 2007;69:217-224.

624-Feed Your Brain**, Ode Magazine, Jurriaan Kamp,** vol 5 issuie 7, pp 40- 45.

625 -LE Magazine January 2002: Protecting Against Inflammatory-Related Disease for a comprehensive overview of anti-inflammatory supplements.

626-Zucchi R, et al., Trace amine-associated receptors and their lignands. British Journal of Pharmacology, 2006. 149(8): p. 967.

627-Szabo A, Billett E, and Turner J, Phenylethylamine, a possible link to the antidepressant effects of exercise? Br J Sports Med, 2001. 35: p. 342-343.

628-Janssen PAJ, et al., Does phenylethylamine act as an endogenous amphetamine in some patients? Int J of Neuropsychopharm, 1999. 2: p. 229-240.

629-Kusaga A, Decreased beta-phenylethylamine in urine of children with attention deficit hyperactivity disorder and autistic disorder. No To Hattatsu, 2002. 34(3): p. 243-248.

630-Kusanga A, et al., Increased urinary phenylethylamine after methylphenidate treatment in children with ADHD. Annals of Neurology, 2002. 52(3): p. 372-374.

631-Gillis M; Phenylethylamine: More Than Just A Pea-Sized Neurochemical, biococepts Nutritional News

632-Cook L., Attentional Priority Disorder Not Attention Deficit Disorder, potent supplements for ADD/ADHD, GreenerMagazine. Com 2003

633-Comings DE, Blum K. (2000) Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog Brain Res 126:325-41**.**

634-Naranjo et al. (2001) The role of the brain reward system in depression. Prog Neuropsychopharmacol Biol Psychiatry (4):781-823.

635- Wojchowski DM, Menon MP, Sathyanarayana P, Fang J, Karur V, Houde E, Kapelle W, Bogachev O. Erythropoietin-dependent erythropoiesis: New insights and questions. Blood Cells Mol Dis 2006; 36:232-38.

636-Sola A, Rogido M, Lee BH, Genetta T, Wen TC. Erythropoietin after focal cerebral ischemia activates the Janus Kinase–Signal transducer and activator of transcription signaling pathway and improves brain injury in postnatal day 7 Rats. Pediatr Res 2005; 57:481-87.

637-Chen ZY, Asavaritikrai P, Prchal JT, Noguchi CT. Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. J Biol Chem 2007; 282:25875-83.

638-Wallach I, Zhang J, Hartmann A, van Landeghem FK, Ivanova A, Klar M, Dame C. Erythropoietin-receptor gene regulation in neuronal cells. Pediatr Res 2009; 65:619-24.

639- Park MH, Lee SM, Lee JW, et al. ERK-mediated production of neurotrophic factors by astrocytes promotes neuronal stem cell differentiation by erythropoietin. Biochem Biophys Res Commun 2006; 339:1021-28.

640-Assaraf MI, Diaz Z, Liberman A, Miller WH Jr, Arvanitakis Z, Li Y, Bennett DA, Schipper HM. Brain erythropoietin receptor expression in Alzheimer disease and mild cognitive impairment. J Neuropathol Exp Neurol 2007; 66:389-98.

641-Jun Y, JiangTao X, YuanGui H, et al. Erythropoietin pre-treatment prevents cognitive impairments following status epilepticus in rats. Brain Res 2009; 1282:57-66.

642-Sättler MB, Merkler D, Maier K, Stadelmann C, Ehrenreich H, Bähr M, Diem R. Neuroprotective effects and intracellular signaling pathways of erythropoietin in a rat model of multiple sclerosis. Cell Death Differ 2004; 11:181-92.

643-Ehrenreich H, Weissenborn K, Prange H, et al. EPO Stroke Trial Group. Recombinant human erythropoietin in the treatment of acute ischemic stroke. Stroke 2009; 40:647-56.

644-Shein NA, Grigoriadis N, Alexandrovich AG, Simeonidou C, Spandou E, Tsenter J, Yatsiv I, Horowitz M, Shohami E. Differential neuroprotective properties of endogenous and exogenous erythropoietin in a mouse model of traumatic brain injury. J Neurotrauma 2008; 25:112-23.

645 -Grasso G, Graziano F, Sfacteria A, et al. Neuroprotective effect of erythropoietin and darbepoetin alfa after experimental intracerebral hemorrhage. Neurosurgery 2009; 65:763-69.

646. Li Y, Lu Z, Keogh CL, Yu SP, Wei L. Erythropoietin-induced neurovascular protection, angiogenesis, and cerebral blood fow restoration after focal ischemia in mice. J Cereb Blood Flow Metab 2007; 27:1043-54.

647-Chi OZ, Hunter C, Liu X, Weiss HR. Effects of erythropoietin on blood-brain barrier disruption in focal cerebral ischemia. Pharmacology 2008; 82:38-42.

648-Martinez-Estrada OM, Rodriguez-Millan E, Gonzalez-De Vicente E, Reina M, Vilaró S, Fabre M. Erythropoietin protects the in vitro blood-brain barrier against VEGF-induced permeability. Eur J Neurosci 2003; 18:2538-44.

649-Wang Y, Kilic E, Kilic U, et al. VEGF overexpression induces post-ischaemic neuroprotection, but facilitates haemodynamic steal phenomena. Brain 2005; 128:52-63.

650-Glade MJ. Oxidative stress and cognitive longevityNutrition 2010; 26:595-603.

651-Shih PH, Chan YC, Liao JW, Wang MF, Yen GC Antioxidant and cognitive promotion effects of anthocyanin-rich mulberry (Morus atropurpurea L.) on senescence-accelerated mice and prevention of Alzheimer's disease. J Nutr Biochem 2009; 21:598-605.

652- Ishrat T, Parveen K, Khan MM, Khuwaja G, Khan MB, Yousuf S, Ahmad A, Shrivastav P, Islam F. Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. Brain Res 2009; 1281:117-27.

653-Chan YC, Hosoda K, Tsai CJ, Yamamoto S, Wang MF. Favorable effects of tea on reducing the cognitive defcits and brain morphological changes in senescence-accelerated mice. J Nutr Sci Vitaminol (Tokyo) 2006; 52:266-73.

654- De La Fuente M, Mi ˜Nano M, Victor VM. Relation between exploratory activity and immune function in aged mice: a preliminary study. Mech Ageing Dev 1998; 102:263-77.

655-S. Sedriep et al, Benefecial Nutraceutical Modulation of Cerebral Erythropoietin Expression and Oxidative Stress, an experimental study, Journal of Biological Modulators and Haemostatic Agents Vol. 25, no. 2, 0-0 (2011)

656-Jicha GA, Carr SA. Conceptual Evolution in Alzheimer's Disease: Implications for Understanding the Clinical Phenotype of Progressive Neurodegenerative Disease. J Alzheimers Dis. 2010;19(1):253–272.

657-Alzheimer's disease facts and figures. Alzheimers Dement. 2009;5(3):234–270.

658-McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984;34(7):939–944.

659-Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Neurobiol Aging. 1997;18 (4 Suppl):S1–S2.

660-Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol (Berl). 1991;82(4):239–259.

661-Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology. 1991;41(4):479–486.

662-Cole GM, Ma QL, Frautschy SA. Omega-3 fatty acids and dementia. Prostaglandins Leukot Essent Fatty Acids. 2009;81(2–3):213–221.

663-Cunnane SC, Plourde M, Pifferi F, Begin M, Feart C, Barberger-Gateau P. Fish, docosahexaenoic acid and Alzheimer's disease. Prog Lipid Res. 2009;48(5):239–256.

664-Jicha Gregory A. ET AL 2010 Omega-3 fatty acids: potential role in the management of early Alzheimer's disease; Clinical Interventions in Aging 2010:5 45–61

665-Takai, Y., Y. Hosoyamada, et al. (1991). "Effect of water soluble and water insoluble fractions of Sprulina over serum lipids and glucose resistance of rats." J. Jap. Soc. Nutr. Food Science 44: 273-277.

666-Becker, E., B. Jakover, et al. (1986). "Clinical and biochemical evaluations of the alga Spirulina with regard to its application in the treatment of obesity: a double blind cross- over study." Nutr. Rep. Int. 33: 565-574.

667-Belay, A., O. Yoshimichi, et al. (1993). "Current Knowledge on Potential Health Benefits of Spirulina." Journal of Applied Phycology 5: 235-241.

668-Jensen G. personal communications1999.