Natural Astaxanthin and the Immune Response

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Introduction

Cats & dogs, fish & mice, as well as human beings can all improve their immune response by supplementing with Natural Astaxanthin according to a great variety of medical research from around the world. This research started with a series of pre-clinical trials done in the 1990's at the University of South Florida and the University of Minnesota's School of Medicine by a medical doctor named Harumi Jyonouchi, MD. Dr. Jyonouchi established the positive effects on the immune system of rodents supplemented with Astaxanthin and also ran some very positive comparative in-vitro experiments pitting Astaxanthin against other carotenoids.

As Dr. Jyonouchi's pioneering research was winding down toward the end of the 1990's, a highly respected pair of carotenoid researchers at Washington State University were just getting going and taking up the mantle from Dr. Jyonouchi. The lead researcher is named Boon Chew, PhD and his associate is named Jean Soon Park, PhD. Dr. Chew has spent many years studying the potential health benefits of carotenoids. After overseeing studies on a variety of carotenoids and discovering that Natural Astaxanthin possesses superior properties to its carotenoid cousins, Dr. Chew embarked on a series of experiments in animals such as mice, cats and dogs to determine Natural Astaxanthin's ability to positively affect the immune response of mammals. The results in these animals were so positive that Drs. Chew and Park then conducted a landmark clinical trial which demonstrated that Natural Astaxanthin supplementation at only 2mg per day yields significant improvement in a variety of immune blood markers in humans. This study was published in 2010. Since then, additional human, animal and in-vitro research has corroborated the earlier work by Drs. Jyonouchi, Chew and Park.

In this review, we'll examine the studies referenced above in more detail as well as some of the other published studies indicating Astaxanthin's positive effect on immunity. There are dozens of studies in this area and in the related area of Astaxanthin's potential for cancer prevention and tumor reduction. In fact, with regards to research on Astaxanthin and cancer, there have already been approximately fifty studies completed. While we will touch on a few of these cancer-related studies, we want to unmistakably point out that to date, no human research has ever been conducted in this area. This volume of published cancer research has focused primarily on rodent studies and in-vitro work. While we can say with certainty that this pre-clinical research clearly indicates the potential for Astaxanthin as a prophylactic measure to prevent carcinogenesis and even a potential treatment for reduction of tumors, the fact that there has never been a study in humans means that it is impossible to draw conclusions that Astaxanthin would have any benefit in human subjects. The authors hope to see human trials in this area commence in the near future based on the overwhelming positive indications in animals.

Where it All Began: The Pioneering Work of Dr. Jyonouchi

Brief Overview of Astaxanthin Research

Dr. Harumi Jyonouchi was working at All Children's Hospital at the University of South Florida when she began exploring the effects of Astaxanthin on the immune response at the onset of the 1990's. Dr. Jyonouchi truly was a pioneer of Astaxanthin research; before she embarked on her immunity studies, there was a very limited body of research done on Astaxanthin. The earliest study was from 1946 when a French scientist exploring the antioxidant potential of carotenoids found Astaxanthin to be significantly more potent than beta carotene (Herisset, 1996). Since that first study, many other head-to-head experiments have verified that Natural Astaxanthin is the most potent antioxidant found in nature with approximately 10X the antioxidant strength compared to other carotenoids (Miki, et al, 1991) and 550X – 6000X greater antioxidant strength compared to other well-known antioxidants including CoQ10, green tea catechins and Vitamins C & E (Shimidzu, et al, 1996; Nishida, et al, 2007). Amazingly, it has also been determined that Natural Astaxanthin is a minimum 20X stronger than Synthetic Astaxanthin in antioxidant potential (Capelli, et al, 2013). We'll look at the vast differences between the Synthetic and Natural Astaxanthin molecules in more depth later as this is a very important topic with regards to today's commercial market for Astaxanthin.

The other area of Astaxanthin research that preceded Dr. Jyonouchi's immunity studies was also done in France and also began in the 1940's. Two young scientists working toward their doctorates after World War II were clearly way ahead of their time: Before almost any scientist in the world had ever heard of "Astaxanthin," Renee Massonet and Rene Grangaud had already discovered how protective Astaxanthin is for the eyes. The work they started in the 1940's was completed and published in the 1950's as their doctoral theses, earning them their PhD's and great respect from their peers in France and establishing a preventative potential for Astaxanthin for eye health as well as the possibility of therapeutic benefits in treating eye diseases (Grangaud, 1951; Massonet, 1958).

The years between 1958 and 1991 when Dr. Jyonouchi published her first paper on Astaxanthin for immunity and a scientist in Japan discovered that Astaxanthin was approximately 10X stronger than other carotenoids as an antioxidant (Miki, et al, 1991) were extremely quiet in the world of Astaxanthin research. But since then, thanks to these pioneers from France, Japan and USA, the flood gates have opened and there have been hundreds of studies showing potential health benefits for Natural Astaxanthin. The areas of study have been extremely diverse; among others, potential benefits have been found for conditions including joint & tendon health, internal beauty & UV protection, cardiovascular health, brain health and a multitude of potential benefits for athletes and active people (Capelli and Cysewski, 2014).

Besides Astaxanthin's profound antioxidant strength, its broad-spectrum anti-inflammatory activity is generally regarded as one of its critical properties that leads to many of the aforementioned condition-specific health benefits in humans. Astaxanthin has been found active against six different inflammatory markers, but works in a gentle manner with no side effects as

compared to over-the-counter and prescription anti-inflammatories which are much more severe in their actions and can lead to a variety of serious side effects (Ohgami, et al, 2003; Lee, et al, 2003; Kishimoto, et al, 2010; Choi, et al, 2008; Yoshihisa, et al, 2014; Sakai, et al, 2009; Capelli and Cysewski, 2014).

The Jyonouchi Experiments

Let's look a little more in-depth at the pioneering immunity research spearheaded by Dr. Jyonouchi that we mentioned above. This is the base from which the dozens of studies in this area that followed emanated:

- **Astaxanthin superior to beta-carotene in immune modulation in-vitro.** Dr. Jyonouchi's first study was a cell study on mouse lymphocytes. (Lymphocytes are white blood cells that protect the body from infection and help it fight off disease.) She tested Astaxanthin against beta carotene in this test. In the 1980's and 1990's, a great deal of research was done on beta carotene as a potential immune system enhancer and for its preventative potential against cancer. This body of research clearly indicated benefits for beta carotene in both of these areas. However, when Dr. Jyonouchi pitted them against one another in a head-to-head experiment, she found Astaxanthin to be clearly superior in the four tests she performed. The conclusion stated: "These results indicate that immunomodulating actions of carotenoids are not necessarily related to pro-vitamin A activity, because Astaxanthin, which does not have pro-vitamin A activity, showed more significant effects in these bioassays" (Jyonouchi, et al, 1991).

- **Astaxanthin enhances in-vitro antibody production to T-dependent antigens.** The next in her series of experiments was done after she moved from the University of South Florida to the University of Minnesota's School of Medicine. Building on her first study, Dr. Jyonouchi attempted to examine the mechanisms of action in enhancing antibody production. This in-vitro experiment showed that Astaxanthin may be able to augment antibody production through affecting the initial stage of antigen presentation (Jyonouchi, et al, 1993).

- **Astaxanthin superior to beta-carotene in preventing formation of cancer in mice.** A related study that Dr. Jyonouchi took part in published the same year was her first animal study in this area. It again pitted Astaxanthin against beta carotene, and found Astaxanthin to be more effective than beta carotene in preventing carcinogenesis in autoimmune-prone mice (Tomita, et al, 1993).

- **Astaxanthin enhances humoral immune response in old mice better than lutein and beta-carotene.** The following year, Dr. Jyonouchi expanded the carotenoids she was testing and included both beta carotene and lutein in this test of immune enhancement in mice. In this trial, Astaxanthin performed better than both of its carotenoid cousins in relation to the immune response of older mice (Jyonouchi, et al, 1994).

- **Astaxanthin increases antibody response in-vitro.** An additional test-tube study verified earlier results showing that Astaxanthin has more profound effects on antibody response in animal cells than beta carotene (Jyonouchi, 1995a).

- **Astaxanthin but not beta-carotene enhances human immunoglobulin in culture.** Dr. Jyonouchi moved to human cells in this experiment and found that, again, Astaxanthin's effects in enhancing immunity in-vitro are far superior to beta carotene (Jyonouchi, et al, 1995b).

- **Astaxanthin effective in enhancing immunity in-vitro and was the sole carotenoid of several tested that performs as a T1-helper cell clone.** This time, Dr. Jyonouchi's group tested several carotenoids against Astaxanthin including lutein, lycopene, zeaxanthin and canthaxanthin. As expected, Astaxanthin's effects in immune enhancement in-vitro were the most potent. In fact, Astaxanthin was the only one of these antioxidant carotenoids that enhanced the number of antibody-secreting cells and performed as a T1-helper cell clone. Furthermore, both Astaxanthin and zeaxanthin, but none of the other carotenoids, successfully increased the number of immunoglobulin M antibody-secreting cells (Jyonouchi, et al, 1996).

- **Astaxanthin may exert anti-tumor activity through the enhancement of the immune response in mice.** The year 2000 marked the final study from Dr. Jyonouchi's decade of pioneering research on Astaxanthin. This study was done in mice to test Astaxanthin's ability to suppress fibrosarcoma tumor growth. The mice were fed a daily diet containing 0.02% Astaxanthin (which equates to 40mcg per kg of their body weight). The mice in the Astaxanthin group had significantly lower tumor size and tumor weight than the control group. Corresponding improvements in blood immune markers were also noted in the Astaxanthin group (Jyonouchi, et al, 2000).

The Prelinary Research of Drs. Chew & Park

In 1999, two early studies published by Drs. Chew and Park showed great promise for Astaxanthin's ability to positively affect the immune response of mice. Similar to some of the research by Dr. Jyonouchi, the first study compared Astaxanthin to other carotenoids, in this case beta carotene and canthaxanthin. This was a rodent study looking at anticancer activity, specifically how these carotenoids fare against the growth of mammary tumors in mice. They tested two different strengths of each carotenoid against placebo, starting the treatment feeds three weeks before the introduction of tumor cells. The authors stated: "Mammary tumor growth inhibition by Astaxanthin was dose-dependent and was higher than that of canthaxanthin and beta carotene." All three carotenoids showed some positive effects, with Astaxanthin being the clear winner. Additionally, lipid peroxidation activity in the tumors was lower in mice fed the stronger dose of Astaxanthin but not in mice fed beta carotene or canthaxanthin (Chew, et al, 1999a).

Later that year, another mouse trial showed that Astaxanthin and beta carotene both stimulate lymphocyte function while canthaxanthin had no effect (Chew, et al, 1999b). As we mentioned above, lymphocytes are white blood cells in the body that provide a baseline defense against infection and help to fight off disease. By stimulating the function of these disease fighters, Astaxanthin helps improve resistance to disease.

While more than a decade went by between these mouse studies and the next publication on Astaxanthin by Drs. Chew and Park, they were actively conducting experiments from which the next flurry of publications would flow. The first study to surface in the literature was another mouse study, this time looking again at tumor growth and immune response. Astaxanthin was again found to delay tumor growth, but only when the Astaxanthin feeding was started before tumor initiation. A corresponding modulation of the rodents' immune response was also found in the Astaxanthin treatment group (Nakao, et al, 2010).

Moving up the mammal chain, Drs. Park and Chew did their next two experiments on cats and dogs. Both studies were done using similar methods, and both studies yielded similar results. The cat study showed that Astaxanthin improved a variety of immune markers such as increasing T helper cells, increasing concentrations of plasma immunoglobulin G and immunoglobulin M, and heightening NK cell cytotoxic activity. The results showed increases in both the cell-mediated and humoral immune response in cats fed Astaxanthin (Park, et al, 2011).

The dog study was even more interesting than the cat study. Again, several immune markers increased in the dogs that were fed Astaxanthin. But additional positive findings in the Astaxanthin-fed animal group surfaced. For one, DNA damage was reduced in the dogs that were fed Astaxanthin, indicating a potential for cancer prevention. Additionally, C-reactive protein (CRP) was also measured in both the control and treatment groups. C-reactive protein is the principal blood marker for systemic inflammation, a chronic condition that can lead to a plethora of life-threatening diseases in humans. In addition to all of the immunity benefits in the dogs fed Astaxanthin, they benefited with reduced CRP levels as well (Chew, et al, 2011).

Landmark Human Clinical Trial Shows Immune Response Benefit from Natural Astaxanthin Supplementation

The logical endpoint of the extremely promising pre-clinical immune research by Dr. Jyonouchi and the steady progression of mammal studies by Drs. Chew and Park manifested as a double-blind, placebo-controlled human clinical trial. The study was done in healthy, young women averaging just over 20 years old. And of course, the study was state-of-the-art: Randomized, double-blind and placebo-controlled. The study lasted for eight weeks. The women were separated into three different groups: The control group took placebos every day, while the two treatment groups took either 2mg of Natural Astaxanthin per day or 8mg per day. The researchers assessed immune response at the beginning of the trial as a baseline, then halfway through the trial after four weeks, and finally at the end of the trial after eight weeks of supplementation.

The results of this landmark clinical trial were excellent. Natural Astaxanthin proved to be a strong immune system stimulator in healthy humans. Results showed that Astaxanthin:

- Increases the total number of antibody-producing B-cells
- Amplifies natural killer cell cytotoxic activity
- Leads to increased number of T-cells
- Stimulates lymphocyte (white blood cell) counts
- Significantly increases delayed-type hypersensitivity response
- Dramatically decreases DNA damage
- And additionally, similar to the pre-clinical trial in dogs, Astaxanthin had the extra benefit of reducing C-reactive protein (CRP), the key marker for systemic inflammation in human blood (Park, et al, 2010)

The rationale for considering this clinical trial to be a "landmark" study is the variety of positive results. Should Astaxanthin have only worked on one or two immune markers, the conclusion would have been that some potential exists for immune-boosting abilities. But in this case, the experiment certainly couldn't be considered a landmark clinical study. However, results showed five distinct benefits related to immunity, with the reduction in CRP and the decrease in DNA damage as two additional possible avenues toward preventing life-threatening diseases as topping on the cake.

One very interesting finding of this study was that the group supplementing with 2mg per day of Natural Astaxanthin experienced slightly better results than the group taking the higher 8mg per day dose. This was unexpected, particularly since research on the many other health benefits of Natural Astaxanthin have generally found optimal results in the 4mg per day to 12mg per day range. One possible explanation is that humans' bodies have a vastly different ability to absorb carotenoids. The range is extreme: Some people may only absorb 5% of the carotenoids in their diet, while other people's bodies may be capable of absorbing over 90%. For this reason, we generally recommend that people take at least 4mg per day of Natural Astaxanthin to be on the safe side, even if their primary goal is immune system enhancement.

This landmark clinical trial not only established clarity for Natural Astaxanthin's potential immune modulating effects in humans, it also corroborated some previous human research showing that Natural Astaxanthin can reduce CRP levels. Researchers and doctors are paying close attention to CRP recently after finding substantial evidence that systemic inflammation is a leading cause of heart disease, cancer, diabetes, Alzheimer's, Parkinson's and a host of other life-threatening maladies.

Finally, this study led to a patent for Drs. Chew and Park on the use of Astaxanthin to prevent DNA damage from oxidation. The patent states that administering as little as 2mg per day of Natural Astaxanthin over a four week period is sufficient to reduce DNA damage by approximately 40% (Chew and Park, 2006). Similar to systemic inflammation, oxidative damage of our cells' DNA can also manifest as serious health issues including the development of cancerous cell lines.

At this point, after examining the results of this study which indicate five positive effects on immune markers, the reduction of CRP and the excellent effects on DNA damage, two conclusions emerge:

1) This certainly is a landmark study and…
2) Persons wishing to improve their immunity or employ preventative health measures should strongly consider supplementing with Natural Astaxanthin each day.

Other Human Clinical Research

In addition to the landmark human clinical trial by Drs. Chew and Park, another clinical trial was done last year which further substantiates improvement of the immune response in humans. This study was done in Europe on young athletic men. Forty men were randomly separated into two groups, one which took 4mg of Natural Astaxanthin each day for 90 days, and the other which took placebos.

One goal of this study was to examine the effect of Astaxanthin on people subjected to physiological stress induced by athletic activity. A high level of physical activity can have many precarious effects on our bodies: It can alter our systems and challenge our immune response; create very high levels of oxidation; and can also increase muscle injury and inflammation levels in the body. Additionally, extensive physical exertion can change biochemical parameters.

The researchers were fair and accurate in their measurements. They ran saliva and blood samples at the commencement of the study and again at the endpoint after 90 days. Importantly, they drew their samples before the young athletes began to exercise to prevent any immediate spikes from exertion from inaccurately skewing the results. The following benefits were found in the group supplementing with Natural Astaxanthin:

- Immunoglobulin levels increased (immunoglobulins are proteins present in immune cells that function as antibodies, chemically combining with bacteria, viruses and foreign substances invading the body)
- The Pro-Oxidant / Antioxidant balance decreased (pro-oxidants create more harmful free radicals in the body while antioxidants combat these destructive substances)
- CRP levels increased in the placebo group while no increase was found in the Astaxanthin group
- Plasma muscle enzyme levels were reduced in the Astaxanthin group

The summary stated, "This study indicates that Astaxanthin supplementation improves immunoglobulin response and attenuates muscle damage, thus preventing inflammation induced by rigorous physical training. Our findings also point that Astaxanthin could show significant physiologic modulation in individuals with mucosal immunity impairment or under conditions of increased oxidative stress and inflammation" (Baralic, et al, 2015).

In addition to this excellent study, another human clinical trial is of interest to our discussion here. This study was done in Japan at the Tsurumi University School of Dental Medicine in patients suffering from an autoimmune disorder called Sjogren's syndrome. Sjogren's syndrome is a systemic chronic inflammatory condition. What happens to people with Sjogren's is that lymphocytes infiltrate glands such as salivary and sweat glands. The symptoms manifest as conditions such as dry mouth and eyes.

This study was done in three parts: In-vitro, a mouse model, and lastly as a human clinical trial in both patients suffering from Sjogren's syndrome as well as in healthy subjects that did not suffer from Sjogren's.

1. In the in-vitro study, Astaxanthin partially suppressed hydrogen peroxide-induced oxidation in human salivary gland epithelial cells.
2. In the mouse model, Astaxanthin helped keep the animals' mouths salivating after they were exposed to irradiation.
3. In both the healthy human group and the group with Sjogren's syndrome, Astaxanthin appeared to increase salivary output and decreased the level of an oxidative stress marker.

The study concluded, "These results suggest that Astaxanthin might act as a reactive oxygen species scavenger, providing benefits to Sjogren's syndrome patients with impaired salivary secretion" (Yamada, et al, 2010). While not a "normal" immunity study, the benefits for these patients with this autoimmune disorder add additional weight to our discussion here.

Additional Pre-Clinical Trials on the Immune Response

As mentioned in the Introduction, there are approximately 50 published pre-clinical trials showing potential for Astaxanthin for cancer prevention and tumor reduction. We will not review them here due to space constraints; however, persons interested in learning more about this research should contact us at support@bggworld.com

There have also been dozens of pre-clinical trials specifically on improvements to the immune response. Again, due to space constraints, we will not elaborate on each of these here; we will, however, give summary highlights of some of these studies to give our Readers a more thorough background on the mass of research in this area:

- Astaxanthin improves the function of human neutrophils (a type of white blood cells) (Macedo, et al, 2010).
- Astaxanthin stimulates immune response in-vitro and in mice (Lin, et al, 2015).
- Astaxanthin reduces immune liver injury in rat model of autoimmune hepatitis (Li, et al, 2015).
- Astaxanthin suppresses lymphocyte activation more effectively than ginkgolide B in-vitro in cells from patients with allergic rhinitis and pollen-related asthma (Mahmoud, et al, 2012).
- Astaxanthin improves the tumor immune response in mice (Kurihara, et al, 2002).
- Astaxanthin modulates the immune system of fish (Jagruthi, et al, 2014).
- Astaxanthin changes the immune response to *H. pylori* bacteria (Akyon, 2002).
- Astaxanthin superior to other carotenoids in enhancing immunity in-vitro (Okai and Higashi-Okai, 1996).

Differences Between *Natural* Astaxanthin and *Synthetic*

It is extremely important to understand the vast differences between Natural and Synthetic Astaxanthin since this distant synthetically-produced cousin of Natural Astaxanthin is now being falsely promoted in the supplement industry as "Nature Identical." While a full review of the vast differences between these two molecules would be too comprehensive for this paper, it is important that our Readers understand that these are two completely distinct molecules. In fact, other than sharing the same chemical formula, they are almost exact opposites in all other respects.

A critical finding of one of the head-to-head antioxidant experiments we mentioned earlier is the clear superiority of Natural Astaxanthin to Synthetic Astaxanthin in antioxidant strength. In both university research at Creighton University under the auspices of acclaimed antioxidant researcher Debasis Bagchi, PhD as well as in independent laboratory testing at Brunswick Laboratories, Natural Astaxanthin extracted from microalgae was found to be a minimum of 20X stronger in antioxidant strength than Synthetic Astaxanthin produced from petrochemicals (Capelli, et al, 2013).

To summarize, the primary differences between the Natural and Synthetic Astaxanthin are:

- **Shape**: The Natural Astaxanthin molecule's stereochemistry is unique (it is shaped differently than the Synthetic Astaxanthin molecule).
- **Esterification**: Natural Astaxanthin is 95% esterified (it has a fatty acid molecule attached to either one or both ends of the molecule). Synthetic Astaxanthin is exclusively "free" Astaxanthin and does not have fatty acid molecules attached to it.
- **Synergy**: Natural Astaxanthin from *Haematococcus pluvialis* microalgae comes complexed in nature with supporting carotenoids. There are consistently small amounts of other antioxidant carotenoids such as lutein, beta-carotene and canthaxanthin ranging from 3% - 15% of the total carotenoid fraction which may provide a synergistic effect when ingested. Synthetic Astaxanthin does not contain supporting carotenoids.
- **Source**: Synthetic Astaxanthin is synthesized from petrochemicals in an elaborate process. Natural Astaxanthin is extracted from natural *Haematococcus pluvialis* microalgae.
- **Safety**: Natural Astaxanthin has an extensive portfolio of human safety studies and a history of over 15 years of safe use as a commercially-sold nutritional supplement. Synthetic Astaxanthin has never been directly tested in humans for safety. (This is an overriding concern due to serious safety issues with related synthetic carotenoids beta-carotene and canthaxanthin.)
- **Efficacy**: Amazingly and perhaps most importantly, Synthetic Astaxanthin has <u>never</u> been shown to have any health benefit in human clinical research. It is completely untested and may turn out to not have any health benefit at all (which leads to the logical question as to why the company that released it to the human nutritional supplement market made this groundless decision). Meanwhile, Natural Astaxanthin has been shown to have diverse health benefits in approximately 100 different positive human clinical trials.

- **Antioxidant Strength**: To expand on what we mentioned above, Natural Astaxanthin is 20X stronger than Synthetic Astaxanthin as an antioxidant in scavenging free radicals. In another antioxidant head-to-head comparison, it was shown that Natural Astaxanthin is over 50X stronger than Synthetic Astaxanthin in singlet oxygen quenching.
- **Dosage**: In the event that Synthetic Astaxanthin is ultimately proven safe for long-range human consumption, dosages would logically be a minimum of 20 times greater than corresponding dosages of Natural Astaxanthin due to its vastly inferior antioxidant profile. This high dosage requirement would most likely put Synthetic Astaxanthin out of reach economically for most consumers (Capelli, et al, 2013).

Conclusion

We started this review with a chronological tour of the published literature of Dr. Jyonouchi, the pioneer of Astaxanthin immunity research. While her work was limited to in-vitro experiments and rodent studies, it clearly established the potential for Astaxanthin as an immune modulating supplement.

The next set of studies reviewed were under the auspices of the highly regarded carotenoid research Boon Chew, PhD and his colleague Jean Soon Park, PhD. These professors at Washington State University found Astaxanthin to be a much more capable molecule than other carotenoids, leading them to embark on a series of experiments in a variety of mammals. These pre-clinical trials further validated Astaxanthin's capabilities in this area and led Dr. Chew to his landmark human clinical trial. The landmark trial established five different improvements in blood markers for immunity in humans by Natural Astaxanthin. Additional results found a reduction in the key marker for systemic inflammation (CRP) as well as establishing Astaxanthin's ability to reduce oxidative damage of DNA. Drs. Chew and Park were awarded a patent for Astaxanthin as a method to reduce DNA damage in humans, declaring from their research that a 2mg per day dose could reduce DNA damage by approximately 40%.

Finally, we reviewed additional human, animal and in-vitro research that confirm the results of the landmark immunity clinical trial.

Based on the research cited above, the authors recommend that persons wishing to improve their immune response should strongly consider supplementing with 4mg of Natural Astaxanthin each day.

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