

## Effects of a *Spirulina*-Based Dietary Supplement on Cytokine Production from Allergic Rhinitis Patients

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**ABSTRACT** *Spirulina* represents a blue-green alga that is widely produced and commercialized as a dietary supplement for modulating immune functions, as well as ameliorating a variety of diseases. We have previously shown that the *in vitro* culture of *Spirulina* with human peripheral blood mononuclear cells (PBMCs) modulated the production of cytokines. In the present study, we evaluated the impact of a *Spirulina*-based dietary supplement (Earthrise Nutritionals, Inc., Irvine, CA) on patients with allergic rhinitis by assessing the production of cytokines [interleukin (IL)-4, interferon (IFN)- $\gamma$ , and IL-2] critical in regulating immunoglobulin E-mediated allergy. In a randomized double-blinded crossover study versus placebo, allergic individuals were fed daily with either placebo or *Spirulina*, at 1,000 mg or 2,000 mg, for 12 weeks. PBMCs isolated before and after the *Spirulina* feeding were stimulated with phytohemagglutinin (PHA) prior to determining the levels of cytokine from cell culture supernatants. Although *Spirulina* seemed to be ineffective at modulating the secretion of Th1 cytokines (IFN- $\gamma$  and IL-2), we discovered that *Spirulina*, administered at 2,000 mg/day, significantly reduced IL-4 levels by 32% from PHA-stimulated cells. These results indicate that *Spirulina* can modulate the Th profile in patients with allergic rhinitis by suppressing the differentiation of Th2 cells mediated, in part, by inhibiting the production of IL-4. To our knowledge, this is the first human feeding study that demonstrates the protective effects of *Spirulina* towards allergic rhinitis.

**KEY WORDS:** • antioxidants • blue-green algae • interleukin-4 • *Spirulina*

### INTRODUCTION

**S**PIRULINA IS A TYPE OF CYANOBACTERIA with a history of human consumption dating as far back as the 16<sup>th</sup> Century, where it has been reported that Aztecs harvested algal biomass resembling *Spirulina* to be consumed as part of their diet.<sup>1</sup> *Spirulina* is a filamentous blue-green alga that apparently flourishes in alkaline lakes with an extremely high pH.<sup>2</sup> This cyanobacterium can potentially represent an important staple in the human diet due to its overall nutritional qualities, which includes a high content of protein (60–71% according to strain), phenolic acids, tocopherols,  $\beta$ -carotenes, and  $\alpha$ -linolenic acid.<sup>1</sup> In addition to its nutritional values, *Spirulina* lacks cellulose cell walls and therefore does not require chemical or physical processing in order to become digestible.<sup>1</sup> Moreover, *Spirulina* is relatively easy to cultivate, thereby sparking the early interest in it as a commercial food supplement with potential therapeutic health benefits.

Not only can the consumption of *Spirulina* be healthy by supplying proteins and other nutrients, but a number of re-

ports have suggested *Spirulina* to be an effective immune modulator. It has been well documented that *Spirulina* exhibited anti-inflammatory properties, in particular, by inhibiting the release of histamine from mast cell-mediated allergic reactions.<sup>3–5</sup> The active ingredient found in *Spirulina* responsible for its anti-inflammatory activities is C-phyco-cyanin, a pigment commonly found in blue-green algae.<sup>6–9</sup> It is known that C-phyco-cyanin can selectively inhibit the activity of cyclooxygenase-2, a critical enzyme in the biosynthesis of prostaglandins.<sup>9</sup> Because of its chemical structure, C-phyco-cyanin also displays antioxidative and free radical scavenging properties, which may contribute, at least in part, to its anti-inflammatory activities.<sup>6,10–13</sup> Additional studies have also indicated that *Spirulina* exposure enhances the phagocytic functions of macrophages in cats,<sup>14</sup> chickens,<sup>15</sup> and channel catfish.<sup>16</sup> Furthermore, dietary *Spirulina* was shown to have protective effects toward orally induced food allergy in mice by preventing the increase of immunoglobulin (Ig) E, while increasing the IgA antibody level to protect against allergic reactions.<sup>17</sup> More recently, we have shown that *in vitro* culture of *Spirulina* extract was effective in modulating the secretion of cytokines [interleukin (IL)-1, IL-4, and interferon (IFN)- $\gamma$ ] from human peripheral blood mononuclear cells (PBMCs).<sup>18</sup>

In continuation of such promising studies, with particular emphasis on inflammatory-mediated diseases such as al-

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ergy, the present study evaluates the impact of dietary *Spirulina* on cytokine production from patients with allergic rhinitis. In a randomized double-blinded crossover study versus placebo, subjects diagnosed with clinical allergic rhinitis were fed daily with either *Spirulina* (at 1,000 or 2,000 mg) or placebo for 12 weeks. PBMCs, collected from the subjects before and after *Spirulina* feeding, were cultured in the presence and absence of phytohemagglutinin (PHA) for 48 hours, after which cytokine (IL-4, IFN- $\gamma$ , and IL-2) levels were determined from cell culture supernatants.

## MATERIALS AND METHODS

### Randomized double-blinded crossover versus placebo study

A total of 36 patients ranging between 18 to 55 years of age with a clinical history of allergic rhinitis, but no other health problems, were enrolled in this study. The patients were equally divided into three groups, each consuming either placebo (Group D) or a proprietary, *Spirulina*-based dietary supplement of Earthrise Nutritionals, Inc. (Irvine, CA) at doses of 1,000 mg/day (Group C) or 2,000 mg/day (Group U) for 12 weeks. All subjects were instructed to ingest four capsules per day. Therefore, the group who received the *Spirulina* supplement at 1,000 mg/day (Group C) was also taking inert placebo as part of their dosage. The placebo was a color-matched capsule containing inert material. It should be noted that two subjects from group U dropped out of the study, leaving a group size of 10. This study was approved by the Institutional Review Board at the University of California at Davis (Protocol number 200210882-1).

### PBMC isolation

Peripheral blood was collected at week 0 pre-feeding and week 12 post-feeding into sodium citrate-containing tubes. The blood was diluted 1:1 with Hanks' Balanced Salt Solution (HBSS; Invitrogen, Carlsbad, CA) without calcium chloride, magnesium chloride, or magnesium sulfate. The diluted blood was then layered over a Histopaque<sup>®</sup>-1077 gradient (Sigma, St. Louis, MO) and centrifuged at 500 g for 30 minutes at room temperature. PBMCs were harvested from the interface layer, washed twice with HBSS, and then counted. The cells were resuspended in RPMI-1640 (Invitrogen) containing 10% fetal bovine serum and supplemented with 0.1% of a 50 mg/mL gentamicin solution (Invitrogen). PBMC concentration was adjusted to  $2 \times 10^6$  viable cells/mL after estimation of viability by trypan blue exclusion assay. Viability was consistently greater than 96%.

### Culture of PBMCs

In 48-well plates, a cell suspension containing  $1.0 \times 10^6$  PBMCs was cultured in the presence or absence of PHA (25  $\mu$ g/mL) at 37°C with 5% CO<sub>2</sub>. Following a 48-hour incubation, the supernatant fractions were harvested for enzyme-linked immunosorbent assay (ELISA) analysis. All treatments were performed in duplicate.

### ELISA analysis of cytokines

Levels of IL-4, IFN- $\gamma$ , and IL-2 were measured in supernatants from PHA-stimulated and unstimulated PBMCs. For quantitation of secretory IL-4, a High Sensitivity Quantikine Human IL-4 ELISA kit (R&D Systems, Minneapolis, MN) was used with a detection limit of 0.25 pg/mL. Standard ELISA kits used to measure levels of IFN- $\gamma$  and IL-2 (R&D Systems, Minneapolis, MN) had a detection limit of 15.6 pg/mL.

### Statistics

Results obtained following the *Spirulina* feeding (week 12) were compared with baseline (week 0) by Student's paired *t* test with a two-tailed *P* value. Significance was taken as *P* < .05.

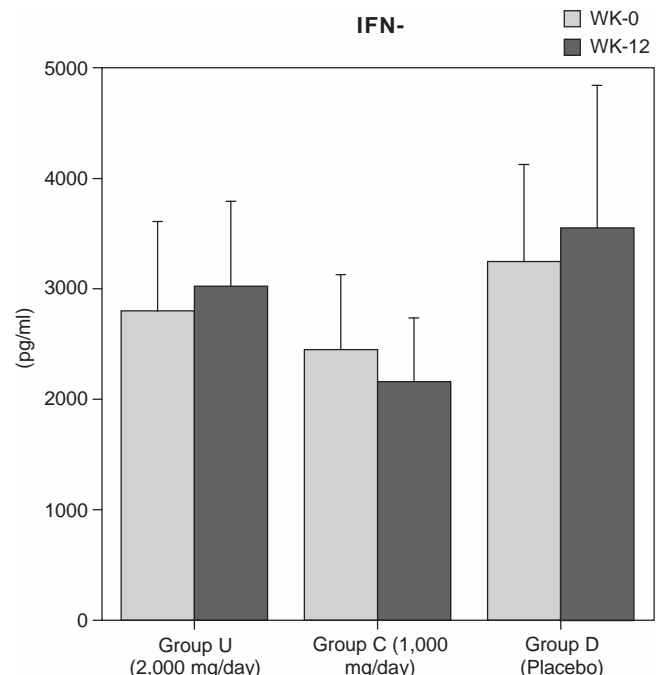
## RESULTS

### Cytokine production from unstimulated PBMCs

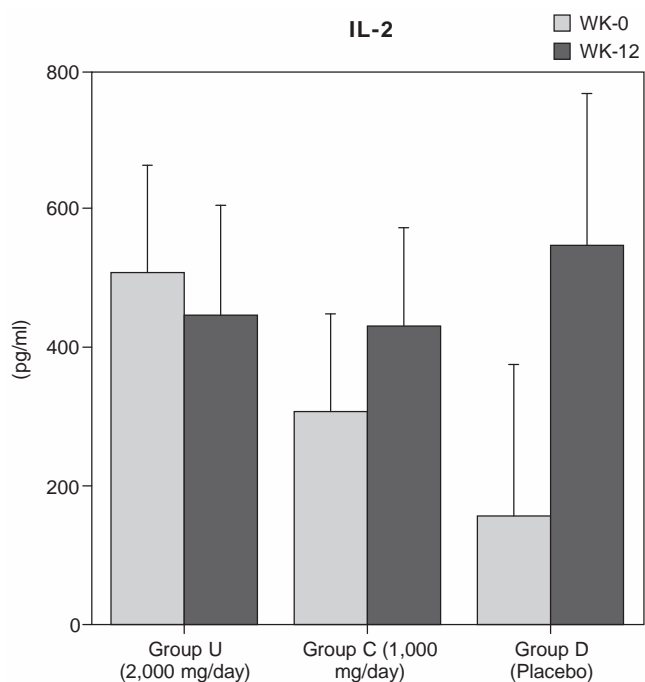
PBMCs cultured for 48 hours under non-stimulatory conditions (*i.e.*, media only) did not produce any detectable levels of IL-4, IFN- $\gamma$ , and IL-2 in any groups.

### Cytokine production from PHA-stimulated PBMCs

When cells were cultured in the presence of a T-cell mitogen, PHA was able to induce measurable levels of cy-



**FIG. 1.** Effect of dietary *Spirulina* on secretion of IFN- $\gamma$  from PHA-stimulated PBMCs. Data are expressed as IFN- $\gamma$  concentration from supernatants harvested at the 48-hour timepoint. Because of high interindividual variability, no statistical significant differences were detected.



**FIG. 2.** Effect of dietary *Spirulina* on secretion of IL-2 from PHA-stimulated PBMCs. Data are expressed as IL-2 concentration from supernatants harvested at the 48-hour timepoint. Although Group D displayed a significant increase following week 12, the standard error of the mean is too high for statistical significance.

tokines in all groups. Statistical analysis was determined by comparing the baseline level (week 0) of cytokine production with week 12 post-feeding levels of the corresponding group.

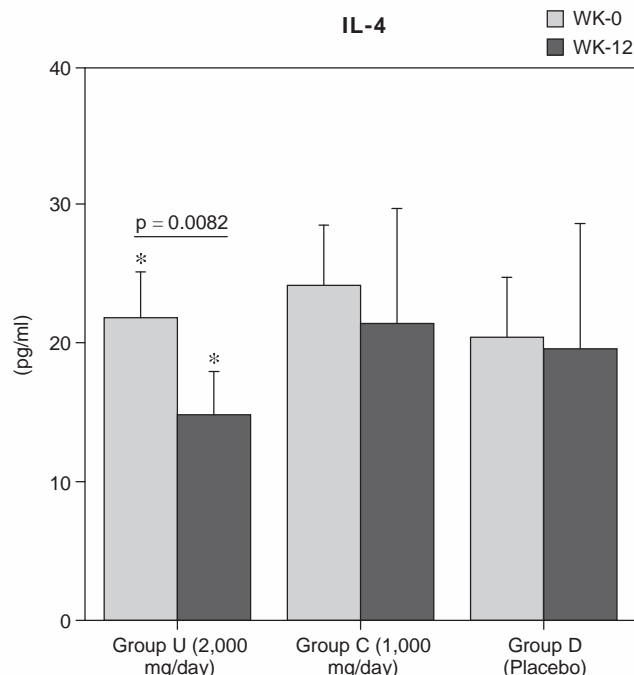
Dietary *Spirulina* was unable to significantly alter the levels of IFN- or IL-2 at either dosage (Figs. 1 and 2, respectively). For IFN-, *Spirulina*-fed groups showed levels of 3,024 780 pg/mL (Group U; *n* = 10) and 2,173 572 pg/mL (Group C; *n* = 12), which did not differ significantly from corresponding baseline levels (2,810 799 and 2,469 667 pg/mL, respectively). Similarly, for IL-2, exposure to *Spirulina* produced levels of 448 155 pg/mL (Group U) and 431 142 pg/mL (Group C), which measured comparably to baseline levels of 508 168 and 308 127 pg/mL, respectively. However, it appeared as though *Spirulina* significantly reduced the secretion of IL-4 by 32% (21.9 3.2 pg/mL pre-feed vs. 14.9 3.0 pg/mL post-feed; *P* = 0.0082) only when allergic patients were administered the higher dose (Group U) (Fig. 3), while the group consuming the lower dosage (Group C) of *Spirulina* did not significantly lower the production of IL-4 (24.2 4.4 2 pg/mL pre-feed vs. 21.5 8.3 pg/mL post-feed).

**DISCUSSION**

For thousands of years, humans have been incorporating *Spirulina* as part of their diet on the assumption that this prehistoric plant can combat various ailments. Yet, it has

only been within the last decade that the scientific community has corroborated some of the alleged health benefits of *Spirulina*. In particular, it is well documented that this cyanobacterium exhibits anti-inflammatory properties, and therefore can potentially alleviate certain diseases mediated by inflammation, such as colitis<sup>8</sup> and allergy.<sup>3-5</sup> In the present study, we examined the therapeutic application of dietary *Spirulina* towards allergic rhinitis by measuring production of cytokines important in regulating IgE-mediated allergy.

Allergic rhinitis is thought to be initiated by an allergen breaching the mucosal epithelium, where antigen-presenting cells, particularly dendritic cells, can take up, process, and present allergen peptides to type-2 T helper (Th2) cells.<sup>19</sup> This cellular interaction stimulates Th2 cells to release cytokines that promote the IgE production, as well as the growth and activation of eosinophils, mast cells, and basophils.<sup>19-21</sup> In particular, IL-4 is a critical cytokine involved in all aspects of inducing type I hypersensitivity reactions. It is well known that IL-4 can effectively promote IgE production from B cells, which can sensitize an individual to a particular allergen by binding to high-affinity Fc RI present on mast cells. Therefore, subsequent allergen binding to IgE on the surface of mast cells can culminate in the release of inflammatory mediators, initiating symptoms characteristic of allergic rhinitis. Hence, aberrant production of IL-4 has been implicated in allergy for it favors Th2 differentiation in correlation with humoral-mediated immunity.<sup>22</sup> In con-



**FIG. 3.** Effect of dietary *Spirulina* on secretion of IL-4 from PHA-stimulated PBMCs. Data are expressed as IL-4 concentration from supernatants harvested at the 48-hour timepoint. Values from week 12 were compared with week 0 using Student’s paired *t* test with a two-tailed *P* value. \*Significance was taken as *P* < .05.

trast, Th1 cells can secrete IL-2 and IFN- $\gamma$  to antagonize the cytokine milieu that promotes allergic inflammation. IFN- $\gamma$  is a macrophage-activating cytokine that promotes Th1-biased responses associated with cell-mediated immunity.<sup>23</sup> Consequently, the balance of Th1/Th2 type cytokines is critical in determining whether an immune response is to be dominated by macrophage activation or by antibody production, particularly IgE, which mediates symptoms of allergic rhinitis.

In the present double-blinded crossover study, we have shown that allergic patients consuming 2,000 mg of *Spirulina* daily can reduce the production of IL-4 from PHA-stimulated PBMCs by 32%. According to several reports claiming that *Spirulina*-fed animals can inhibit mast cell function,<sup>3-5</sup> it is now conceivable such activity may, in part, have resulted from reduced levels of IL-4. Although we cannot confirm that such inhibition observed is dose-dependent, it may be possible that increasing the daily regimen above 2,000 mg may also enhance the suppressing capability of *Spirulina*.

In our previous investigation, we reported that *in vitro* culture of resting and PHA-stimulated PBMCs with *Spirulina* extract significantly increased the levels of IL-4.<sup>18</sup> However, it is important to consider that the subjects participating in our prior study were healthy individuals that did not suffer from allergic rhinitis. Furthermore, the baseline value for IL-4 from PHA-stimulated PBMC cultures of all allergic patients (22.1  $\pm$  2.3 pg/mL,  $n = 34$ ) in our current study (prior to the *Spirulina* feed) is 69.5% higher than similar cultures observed with healthy individuals (13.1  $\pm$  6.9 pg/mL,  $n = 12$ ; values taken from Mao *et al.*<sup>18</sup>). Hence, it is conceivable that the *Spirulina* feed modulated the Th profile in allergic individuals either by preventing the differentiation of Th2 cells or by enhancing the population of Th1 cells to overcome the effects of Th2 cells. Since we did not observe any significant difference in IFN- $\gamma$  and IL-2 (both Th1-type cytokines) production following 12 weeks, it is most likely that *Spirulina* exerted its effects by suppressing the pathway leading to Th2-committed cells mediated, in part, by inhibiting the secretion of IL-4.

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