

mg CPE group and 35.3% of 400-mg CPE group) at 7 days after vaccination. Similar trends were observed for the analyses of participants achieving 4-fold or greater increases in antibody levels and for the proportions achieving an antibody titre of 40 or more reciprocal dilution (data not shown). There were no differences or trends among participants over 55 years of age.

### Interpretation

The dietary supplement CPE extracted from the microalga *C. pyrenoidosa* was well tolerated by the healthy adults in this study. Adverse events were similar among recipients of CPE and those who received placebo. In the primary outcome analysis, there was no difference in antibody response between CPE recipients and the control group. Failure to demonstrate an effect of the supplement could be the result of product inactivity, inadequate dose or schedule, recipient unresponsiveness or inadequate sample size. The lack of any trend toward a greater response with increased dose indicates that dosing might not explain the lack of effect; it is not possible to determine the role of schedule because only one schedule was studied. The smaller-than-expected number of subjects means that the study did not have the planned power to ensure that any lack of demonstrated difference was not the result of a type II error. However, there were no suggestions of trends toward an effect of the product, which suggests that the lack of benefit was not the result of underenrolment.

The biological activity of CPE was demonstrated when the study subjects were stratified by age. Because of decreased immunogenicity of the influenza vaccine with subject age,<sup>20,21</sup> we hypothesized that the effect of CPE would be more apparent in older than in younger participants; in fact, we observed the opposite. The as-yet-unknown mechanism leading to diminished antibody response to influenza vaccine in elderly people might also result in lack of responsiveness to CPE, perhaps because the supplement exerts its effect through the same deficient mechanism or pathway. Alternatively, the lack of effect of CPE might be the result of prior experience with similar influenza viruses in the older group leading to an enhanced anamnestic response, which would in turn offset any immunoenhancing effect of the supplement. This latter possibility is less likely, given that prevaccination titres were higher in the older cohort only for influenza A/Panama (Fig. 2).

We have demonstrated an immunoenhancing effect of CPE in a subset of human subjects after influenza vaccination, which corroborates the results of in vivo and in vitro preclinical studies. The doses and dosing regimen chosen for clinical testing in this study were based on preclinical studies performed in laboratory animals and were tested in a phase 1 safety study (unpublished data). Whether these doses and schedules are sufficient to produce a maximum effect of the antibody response is unknown; other schedules might provide a greater (or lesser) effect. These results in-

dicating that, as in the animal models, CPE does have a biological effect in humans and encourage further investigation to determine whether there is any clinically significant effect. Influenza vaccination was selected for study, not because we anticipated the eventual use of the product as an "oral adjuvant," but rather because it serves as a model of antigenic stimulation by a respiratory pathogen; however, the supplement could potentially be used as an oral adjuvant if further studies support its efficacy. The results obtained from this double-blind, placebo-controlled trial indicate that CPE should be studied further in other models of infection (such as the rhinovirus challenge model<sup>11,12</sup>) or under natural conditions of exposure (such as household contacts of cases of influenza<sup>22</sup>) to identify potential clinical applications. It is clear from this study and others<sup>2-11</sup> that some dietary supplements may have therapeutic utility and should be subjected to further rigorous experimental evaluation to clarify their clinical benefits.

This article has been peer reviewed.

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**Competing interests:** Coleen Nolan is a Clinical and Regulatory Affairs Associate with Ocean Nutrition Canada, Ltd., and has been with the company since January 2000. Janet Shay is a Senior Manager of Clinical and Regulatory Affairs with Ocean Nutrition Canada, Ltd., and has been with the company since January 1999. Jaroslav Kralovec is Associate Director of Research-Chemistry and Principal Scientist with Ocean Nutrition Canada, Ltd., and has been with the company since February 1997; he invented and developed the product tested in this study.

**Contributors:** Scott Halperin was the principal investigator and was responsible for the study concept, design of the protocol, acquisition of clinical data, data analysis and interpretation, and writing and revision of the manuscript. Bruce Smith was the study statistician and was responsible for design of the protocol, planning of the statistical analysis, the statistical analysis itself, and review and revision of the manuscript. Coleen Nolan, Janet Shay and Jaroslav Kralovec were responsible for the study concept, design of the protocol, and review and revision of the manuscript. Jaroslav Kralovec was also the developer of the product.

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tions.<sup>18,19</sup> An oral supplement with immunoenhancing activity might also be useful for people with known hyporesponsiveness to vaccines, as is the case with influenza vaccine administered to elderly people and hepatitis B vaccine to people who smoke.<sup>20-23</sup>

We conducted a single-centre, randomized, placebo-controlled, double-blind clinical trial to determine the immunoenhancing effect of CPE by determining the proportion of participants achieving a 4-fold or greater increase in antibody levels and measuring the geometric mean antibody titre after influenza vaccination. We also explored whether immune responsiveness to CPE as a dietary supplement was related to age.

## Methods

Healthy adults 50 years of age or older were recruited from the Halifax community in autumn 2000. Posters in local hospitals and physicians' offices, at the local university and in homes for senior citizens informed the community of the study. We excluded anyone with a known allergy to eggs or influenza vaccine, those with known immunodeficiency or malignant disease, those who were using immunosuppressive medications, those with a history of an unstable chronic medical condition and pregnant women.

As described above, CPE is a dietary supplement derived from *C. pyrenoidosa*. Under current good manufacturing process guidelines, the extract was removed from dried cells with hot water (80°C); it was then separated from the solid residue by centrifugation, and the supernatant was microfiltered and spray-dried. The resulting powder was yellow with a greenish tinge. Gelatin capsules were filled with either 200 or 400 mg CPE along with microcrystalline cellulose of sufficient volume to fill the capsule. Placebo capsules contained only the microcrystalline cellulose. Commercially available trivalent influenza vaccine recommended for the 2000/01 season and containing inactivated influenza A/New Caledonia, A/Panama and B/Yamanashi was purchased from the manufacturer (FluViral, Biochem Pharma, Laval, Que.).

The primary objective of the study was to measure participants' antibody response to influenza vaccination. The primary hypothesis was that the recipients of CPE would have a greater antibody response as indicated by the proportion of participants with a 4-fold or greater increase in antibody levels after vaccination, by the geometric mean antibody titre obtained and by the rapidity of the antibody response (response at day 7 compared with day 21 after vaccination). The secondary objectives were to determine the safety and tolerability of CPE (as indicated by the proportion of participants reporting adverse events) and to identify any age-related immunoenhancement. Antibodies against each of the 3 viruses in the vaccine were measured by hemagglutination inhibition according to standard methods;<sup>24</sup> the testing was done on coded specimens at the National Institute for Biological Standards and Control (Potters Bar, UK). Clinical safety was monitored by means of a daily subject diary and included both solicited (fever, rash, headache, body aches, sore joints, fatigue, abdominal pain, nausea, anorexia, vomiting and diarrhea) and unsolicited events. Physiological safety was monitored through serial liver enzyme determinations; complete blood counts; quantitative measurement of immunoglobulins, complement, antinuclear antibodies, anti-DNA antibodies and rheumatoid factor; and Coombs tests.

The study product (200- and 400-mg doses of CPE) and the placebo were packaged separately, in a 1:1:1 ratio, labelled with a

participant number, and dispensed by the study pharmacist before study commencement according to a computer-generated list with a block size of 6 provided by the study statistician (B.S.). After providing written informed consent, each participant was assigned the next sequential participant allocation number and was given the corresponding prefilled pill bottle. Although the study was designed to be completely blinded, it was discovered before the first enrolment that the capsules containing the study product had a subtle greenish hue whereas the placebo capsules did not; the difference was discernible only when both types of capsule were viewed simultaneously. Therefore, to preserve blinding, staff were designated as blinded and potentially unblinded. Potentially unblinded staff were responsible for dispensing the medication according to the next available allocation number, observing the first dose being taken and performing capsule counts to assess compliance. The potentially unblinded staff who dispensed the medication were able to view the capsules only after randomization had occurred and the pill bottle had been opened; therefore, even if they could discern the difference in capsule hue, this would have occurred only after randomization and allocation. All data collection and data analysis, as well as all clinical aspects of the study, were restricted to fully blinded personnel. Enrolment was limited to one participant per household to ensure that participants could not compare capsules. All study documents referred to the study as completely blinded so that participants would not be aware of the possibility of unblinding by capsule comparison.

Each subject took one capsule each morning for 28 days and recorded any adverse events in a daily diary. Symptom data were collected by telephone on day 10 and during visits on days 21, 28 and 42; compliance was determined by capsule counts. On study day 21, baseline antibody levels were measured and the subject received a single intramuscular injection of the influenza vaccine. Antibody levels were retested on study days 28 and 42 (i.e., 7 and 21 days after vaccination). Baseline physiological blood tests were done on study entry and on the last day of administration of study product or placebo (day 28).

The ideal sample size was calculated on the basis of the primary serological outcome; we determined that 150 participants would permit the detection of a 20% difference in the proportion of participants undergoing seroconversion with a power of 0.8. This number of participants would provide greater than 90% power to detect differences of 15% or more in any uncommon clinical adverse events (occurring in less than 5% of participants) and 80% power to detect such differences for more common events (occurring in more than 10% of participants). The study analysis was an intent-to-treat analysis of all subjects who received the influenza vaccine. Baseline characteristics of the treatment groups were compared with *t*-tests for continuous variables and Fisher's exact test for categorical variables. The proportions of participants who underwent seroconversion (4-fold or greater and 2-fold or greater increase in antibodies), the geometric mean antibody titres and the proportions of participants with clinical or laboratory abnormalities were assessed by calculating binomial point estimates and 95% confidence intervals;<sup>25</sup>  $p < 0.05$  was taken as statistically significant. The age analysis was undertaken to determine whether there was an effect of age on immune response to the dietary supplement. Although the initial study plan was to compare subjects 65 years of age or older with those younger than 65 years, lower-than-expected enrolment in the older age group precluded this analysis. Therefore, the age used for this comparison was determined by the age distribution of the enrolled participants to achieve roughly half of the participants in each age group; the age cutoff was determined before the study was unblinded.

## Results

A total of 124 participants were enrolled in the study and received study product or placebo; we terminated enrolment before reaching the target sample size of 150 to avoid enrolment during the influenza season. Seven participants withdrew from the study, but only one withdrawal was because of side effects (nausea and abdominal discomfort) (Fig. 1). The study groups were similar in terms of age, sex (Table 1), medical history, vital signs, physical findings, concomitant medications and physiological blood test results (data not shown).

Adverse events reported during the initial 28-day period (while subjects were taking the supplement or the placebo) were similar between the study groups. The only difference related to fatigue, which was reported more frequently by participants receiving the 200-mg dose than by those re-

ceiving placebo or the 400-mg dose (Table 1). No serious adverse events were reported.

No effects of CPE were found in the overall antibody analysis. Four-fold or greater antibody increases were uncommon in all study groups (11.1% to 28.2%) and were not more frequent among recipients of CPE (Table 2). A greater proportion of participants underwent seroconversion by the less stringent definition of a 2-fold or greater increase in antibodies (40.5% to 59.0%); however, there was still no difference between the groups given CPE or placebo (Table 2). Differences were also not observed for the geometric mean antibody titres achieved at 7 or 21 days for any of the 3 viral strains in the vaccine (data not shown).

Significant differences were detected in the preplanned by-age analysis. Participants up to 55 years of age who received CPE had higher levels of antibody against influenza A/New Caledonia at 7 and 21 days after vaccination; these

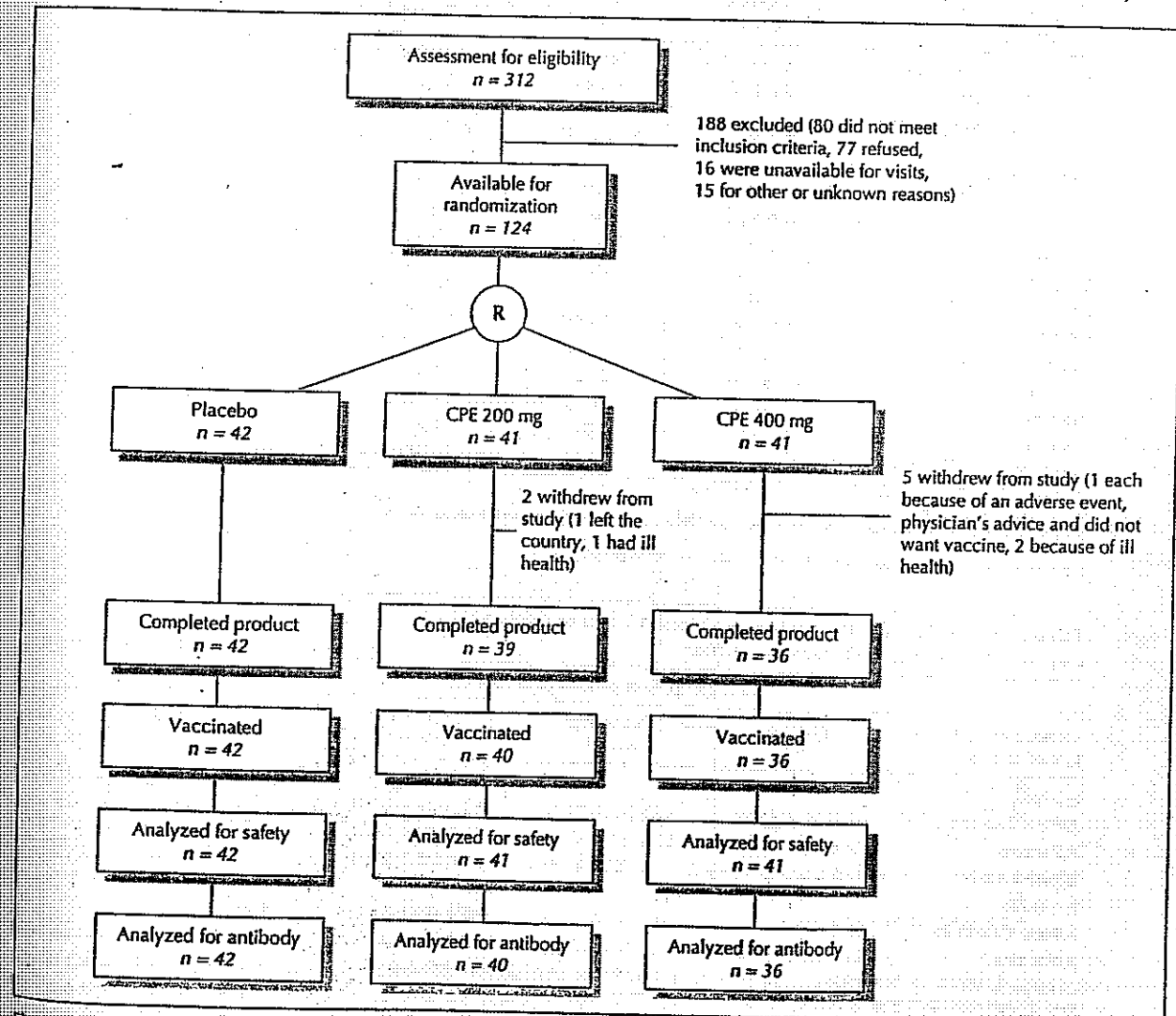


Fig 1. Clinical trial profile. CPE = *Chlorella pyrenoidosa* extract.

gia could have their level of pain (as measured by the TPI) significantly decreased by simply adding *Chlorella* to the diet suggests that further clinical testing of dietary *Chlorella* supplementation in these patients is warranted. Based on the results of this pilot study, we have determined power, sample size, and the most useful outcome variables for a more comprehensive, placebo-

controlled clinical trial. Such an investigation of dietary *Chlorella* supplementation is now underway.

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Research

Recherche

## Safety and immunoenhancing effect of a *Chlorella*-derived dietary supplement in healthy adults undergoing influenza vaccination: randomized, double-blind, placebo-controlled trial

Scott A. Halperin, Bruce Smith, Coleen Nolan, Janet Shay, Jaroslav Kralovec

#### Abstract

**Background:** Enhancement of immune function has been claimed as a benefit of some natural health products, although few have been subjected to randomized clinical trials. We evaluated the effect of an oral dietary supplement derived from the edible microalga *Chlorella pyrenoidosa* on immune response after influenza vaccination.

**Methods:** We conducted a randomized, double-blind, placebo-controlled community-based clinical trial in a convenience sample of 124 healthy adults at least 50 years of age randomly assigned to receive the study product (200 or 400 mg of a *Chlorella*-derived dietary supplement) or placebo. Participants took the study product or placebo once daily for 28 days. On day 21, we administered a single dose of a licensed trivalent, inactivated influenza vaccine. We obtained serum specimens to measure hemagglutination inhibition titres before and 7 and 21 days after vaccination. The primary immunological outcomes were the proportion of participants with a 4-fold or greater increase in antibodies and geometric mean antibody titres after vaccination; the proportion of participants reporting adverse events during therapy was the safety outcome.

**Results:** A total of 117 (94%) participants completed all aspects of the study. There were no differences in the proportions of recipients of 200 or 400 mg of the *Chlorella*-derived dietary supplement or placebo who achieved at least a 4-fold increase in antibodies (proportions for the 3 virus strains ranged from 17.9% to 28.2% for the 200-mg group, from 11.1% to 22.2% for the 400-mg group and from 19.0% to 21.4% for the placebo group;  $p > 0.05$  for all comparisons). Reports of adverse events were similar for recipients of the supplement and placebo, except with regard to fatigue, which was reported more frequently by recipients of 200 mg of the supplement (18/41 or 44%) than by those who received 400 mg of the supplement (8/42 or 20%;  $p = 0.032$ ) or placebo (8/42 or 19%;  $p = 0.019$ ). Recipients of 400 mg of the supplement who were 55 years of age or younger had significantly higher geometric mean antibody titres against influenza A/New Caledonia 21 days after vaccination ( $p = 0.047$ ) and against B/Yamanashi 7 days after vaccination ( $p = 0.034$ ); the trends were nonsignificant for titres against A/Panama. We also observed similar increases for the proportions of subjects with a 2-fold or greater or a 4-fold or greater increase in antibodies.

**Interpretation:** The *Chlorella*-derived dietary supplement did not have any effect in increasing the antibody response to influenza vaccine in the overall study population, although there was an increase in antibody response among participants aged 50-55 years. Adverse events were similar among those receiving the supplement and the placebo. Further studies are warranted to explore the range of clinical effects resulting from ingestion of this dietary supplement.

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Dietary supplements and alternative therapies are increasingly being used worldwide as the public strives to prevent disease and improve health.<sup>1,2</sup> The regulatory requirements for the sale of dietary supplements are less stringent than those for pharmaceuticals,<sup>3</sup> and for many of these supplements, efficacy data supporting use are the exception rather than the rule.<sup>4</sup> The health benefits attributed to dietary supplements vary widely. Enhancement of immune function has been claimed for *Echinacea*,<sup>5,6</sup> zinc<sup>7</sup> and vitamin C<sup>8</sup> (all for prevention of respiratory infections), although the results of clinical trials have been equivocal.<sup>9-13</sup>

*Chlorella* are freshwater unicellular, microscopic algae, widely used as a food supplement in Japan.<sup>14</sup> The supplement has been taken as tablets, capsules, extract liquid or a food additive; claims for health benefits have included improvement of immune function<sup>15</sup> and improvement in control of hypertension, fibromyalgia and ulcerative colitis.<sup>16</sup> An aqueous extract of the edible microalga *Chlorella pyrenoidosa* (CPE) (ONC-107, Ocean Nutrition Canada, Ltd., Halifax) was found to have both in vitro and in vivo activity. In a proliferation assay, CPE stimulated production of interleukin 6 by BALB/c mouse spleen cells and macrophages; CPE was also effective in reducing the frequency and severity of infection with *Listeria monocytogenes* and *Candida albicans* in 2 mouse infection models<sup>17</sup> (J. Kralovec and associates, manuscript in preparation). An orally administered immunoenhancer might be useful for people with impaired immune responses to infection and vaccination, such as those with HIV infection and others with immunodeficiency, or for normal individuals with depressed immune response associated with viral infec-