

model in mice was employed. Consistent with its in vitro effect on mast cell IL-5 production, treatment of mice with fraction 2 inhibited OVA-induced eosinophil infiltration in the airways. Moreover, the recruitment of neutrophils to the allergic airways was also inhibited by fraction 2 treatment, suggesting an anti-allergic effect in mice.

In an attempt to examine the mechanisms involved in fraction 2-induced inhibition of airway eosinophilia, the IL-5 level in the bronchoalveolar lavage fluid was examined. Although there was a decreasing trend of OVA-induced IL-5 production in fraction 2-treated mice, the difference was not statistically significant when compared to that in those OVA-challenged, non-treated mice. Thus, additional mechanisms likely contribute to fraction 2-induced inhibition of inflammatory cell infiltration in the allergic airways. Although the mechanisms of the allergy remain incompletely defined, it is well-known that the production of antigen specific IgE and dysregulation of type 1 and type 2 cytokine production are essential in the development of allergic inflammation. Interestingly, *Chlorella* extracts reduce IgE production in mice [20]. It remains to be determined whether fraction 2 affects OVA-specific IgE production. The development of allergy is primarily driven by type 2 cytokines such as IL-5, and counter regulated by type 1 cytokines such as IFN- γ and IL-12. *Chlorella* extracts have been shown to increase the production of type 1 cytokines IFN- γ and IL-12 [36]. It is therefore likely that fraction 2 inhibits the development of allergic airway inflammation through enhancing the type 1 cytokine production and down-regulating type 2 cytokines. Further studies are necessary to elucidate the mechanisms involved in fraction 2-mediated inhibition of allergic inflammation.

The molecular components in 2 responsible for the inhibitory effects on allergic inflammation are unclear. The immunostimulating effects of 2 on B cell proliferation are almost exclusively induced by the large molecular mass fraction 2a. Similarly, 2a demonstrated more potent inhibitory effects on IgE-dependent cytokine production by mouse and human mast cells than the smaller molecular mass fragments. Thus, it is likely that the large molecular mass components of 2 play a major role in the anti-allergic airway inflammation in vivo.

Recently, an immunoenhancing effect of *Chlorella* extract in human subjects has been demonstrated [18]. We attempted to examine the potential anti-allergic effect of *Chlorella* extract fractions in a human system. Given that human CBMC have a phenotype similar to that of human lung mast cells [37], CBMC were used to determine the effects of *Chlorella* extract fractions in IgE-dependent mast cell activation. Interestingly, IgE-dependent production of cytokine GM-CSF by CBMC was inhibited by all *Chlorella* extract fractions tested.

In summary, we demonstrated an anti-eosinophilia effect of *Chlorella* extract fraction 2 in mouse allergic airway in vivo and an inhibitory effect on IgE-dependent cytokine production by mast cells in vitro. These results suggest a potential beneficial role for *Chlorella* extract or its fractions in allergic inflammation. Further studies are needed to identify the role of *Chlorella* extract on the regulation of Th1 and Th2 immune systems and its impact on human allergy disorders.

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BSA stimulation (Fig. 3). Mast cell viability as tested by Trypan blue assay was not affected by treatment of 2 or its components after an 18h treatment (IgE-treated: 96%; IgE+TNP: 97%, IgE+TNP+ 2: 96%; cells treated with fragments of 2 with or without TNP showed $\geq 95\%$ viability). Interestingly, antigen-induced mast cell degranulation and IL-4 production was not affected by 2 (Table 1), suggesting a selective effect of 2 and its fragments on mast cells.

3.4. Effects on ovalbumin-induced airway inflammation in mice in vivo

To evaluate the potential anti-allergic effect of 2 in vivo, an allergic airway inflammation model in mice was employed. Mice were sensitized with OVA i.p. and challenged with OVA intranasally. During the sensitization and challenging processes, mice in the experimental groups received 2 orally (4 mg/mouse, once every 2 days from day 1 to day 29). Mice in the control groups received saline. Inflammatory cell infiltration is one of the characteristics of allergic airway inflammation. OVA sensitization and challenge induces a significant eosinophil and neutrophil recruitment in the lung as determined by the increase of EPO and MPO activities. Treatment of mice with 2 inhibited OVA-induced EPO and MPO activities in the lung (Figs. 4 and 5).

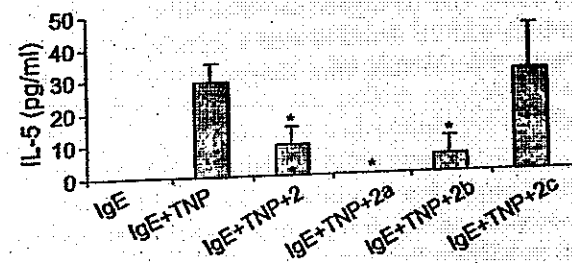


Fig. 3. Effect of *Chlorella* extract fraction 2 and its sub-fractions on IgE-dependent IL-5 production by mouse mast cells. Anti-TNP IgE-sensitized mouse bone marrow-derived mast cells were treated for 18 h with 100 $\mu\text{g/ml}$ of the *C. pyrenoidosa* extract (fraction 2) or its subfractions 2a, 2b or 2c. These cells were then stimulated with antigen TNP-BSA (10 ng/ml) for an additional 18 h. IL-5 production in the cell free supernatants were determined by ELISA. Results are mean \pm S.E., $n=3$. * $p<0.05$ compared with mast cells stimulated with TNP-BSA alone.

Table 1
No effects of *Chlorella* extracts on IgE-dependent mast cell degranulation and IL-4 production

	Degranulation (%)	IL-4 production (pg/ml)
Without treatment	6.4 \pm 1.6	2.8 \pm 2.8
Treated with IgE+TNP	23.4 \pm 1.2	75.8 \pm 14.9
IgE+TNP+ <i>Chlorella</i> extract fraction 2	26.8 \pm 9.0	68.0 \pm 8.2
IgE+TNP+ <i>Chlorella</i> extract fraction 2a	19.2 \pm 1.4	68.3 \pm 10.6
IgE+TNP+ <i>Chlorella</i> extract fraction 2b	29.4 \pm 3.3	75.8 \pm 12.8
IgE+TNP+ <i>Chlorella</i> extract fraction 2c	31.4 \pm 8.2	79.6 \pm 10.9

Mouse bone marrow-derived mast cells (BMMC) were sensitized with anti-TNP IgE overnight. For mast cell degranulation assay, BMMC were treated with 100 $\mu\text{g/ml}$ of the dialyzed *Chlorella pyrenoidosa* extract (fraction 2) or its subfractions 2a, 2b or 2c for 1 h. Then BMMC were stimulated with TNP-BSA (10 ng/ml) for 30 min. Mast cell degranulation was determined by measuring β -hexosaminidase activities in cell free supernatants and cell pellets. For testing IL-4 production, anti-TNP-IgE-sensitized BMMC were treated for 18 h with 100 $\mu\text{g/ml}$ of 2 or its subfractions 2a, 2b or 2c. These cells were then stimulated with antigen TNP-BSA (10 ng/ml) for an additional 18 h. IL-5 production in the cell free supernatants were determined by ELISA.

Given the importance of IL-5 in eosinophil survival and recruitment, effects of 2 on IL-5 production in the airway were determined. As shown

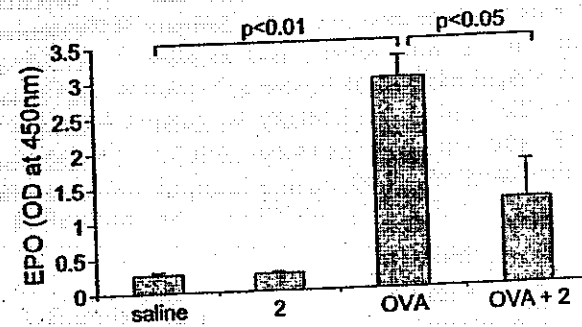


Fig. 4. Effect of *Chlorella* extract fraction 2 on allergen-induced airway eosinophilia in mice. Mice were sensitized by i.p. injection of ovalbumin (OVA) at days 1 and 5 and stimulated by OVA intranasally on days 21, 25 and 29. For the treatment with *Chlorella* extract fraction 2, mice received 4 mg/mouse of the tested substance orally once every 2 days from day 1 to day 29. Twenty-four hours after the last OVA stimulation, mice were sacrificed and lung tissues were collected to determine the eosinophil infiltration by determining EPO activities in the lung. Results are mean \pm S.E. from 8 mice (OVA treated or OVA+fraction 2 treated) or 5 mice (saline treated or fraction 2 treated).

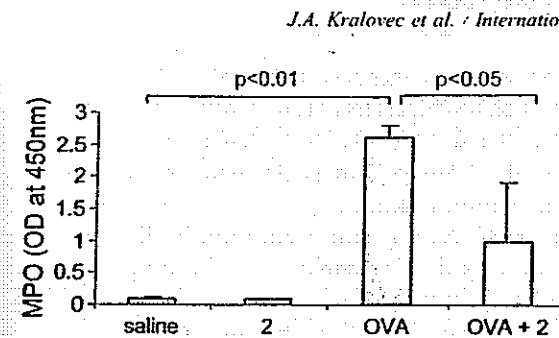


Fig. 5. Effect of *Chlorella* extract fraction 2 on allergen-induced neutrophil recruitment in the airways of mice. Mice were treated as described in Fig. 4. Twenty-four hours after the last OVA stimulation, mice were sacrificed and bronchoalveolar lavage fluids were collected to determine the neutrophil infiltration by determining MPO activities. Results are mean \pm S.E. from 8 mice (OVA treated or OVA+fraction 2 treated) or 5 mice (saline treated or fraction 2 treated).

in Fig. 6, there was a decreasing trend of OVA-induced IL-5 production in mice treated with 2. However the difference did not reach statistical significance, suggesting that the preparation may have additional effects to induce inhibition of eosinophil infiltration in OVA-induced lung inflammation.

3.5. Effect on GM-CSF production by human mast cells

CBMC are able to produce GM-CSF [32], which is abundant in allergic airways and is believed to be important for the development of allergic airway inflammation [33]. To examine the effects of *Chlorella* extract fragments on GM-CSF production by human mast cells, CBMC were treated with fragments

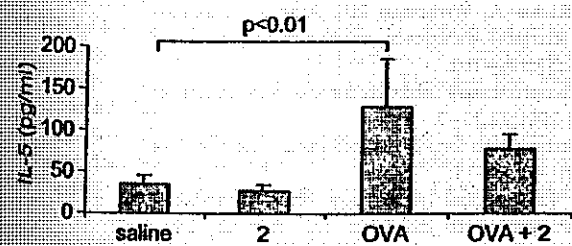


Fig. 6. Effects of *Chlorella* extract fraction 2 on allergen-induced IL-5 production in the airways in mice. Mice were treated as described for Fig. 4. Twenty-four hours after the last OVA stimulation, bronchoalveolar lavage fluids were collected to determine IL-5 production by ELISA. Results are mean \pm S.E. from 8 mice (OVA treated or OVA+fraction 2 treated) or 5 mice (saline treated or fraction 2 treated).

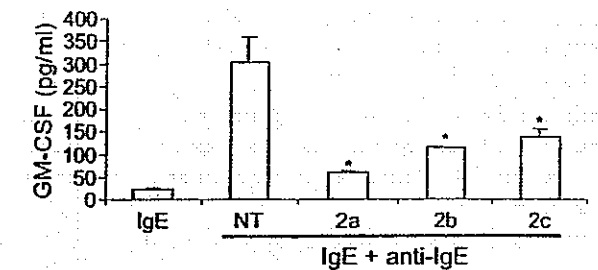


Fig. 7. Effect of *Chlorella* extract fractions on IgE-dependent GM-CSF production by human mast cells. IgE-sensitized human cord blood-derived mast cells were treated for 18 h with 100 $\mu\text{g/ml}$ of *Chlorella* extract fractions including 2a, 2b or 2c. These cells were then stimulated with anti-IgE for an additional 18 h. GM-CSF production in the cell free supernatants were determined by ELISA. Results are mean \pm S.E., $n=3$. * $p<0.05$ compared with mast cells stimulated with anti-IgE without treatment with the tested substances (NT, not treated).

2a, 2b and 2c for 18 h. Cell free supernatants were used for GM-CSF production. Treatment of CBMC with *Chlorella* extract fragments significantly reduced anti-IgE-induced GM-CSF production (Fig. 7). CBMC with or without *Chlorella* extract treatment showed $\geq 95\%$ viability as tested by Trypan blue assay.

4. Discussion

Algae have been widely used as foods, food additives, or nutraceuticals. The beneficial effects include improvements of immune response to various pathogens [13], tumor antigens [12,16] or allergens [34]. The potential use of algae as anti-allergic agents was proposed based on the finding that algae products demonstrate inhibitory effects on antigen-induced mediator secretion from mast cells [34,35]. Mast cells play an essential role in the development of allergy through secretion of various potent mediators. We demonstrated that a *Chlorella* extract fraction 2 significantly inhibited antigen-induced IL-5 production by mouse BMMC. This effect may be important because IL-5 is one of the key cytokines involved in the development of allergic inflammation through regulation of eosinophil survival and migration in vivo. To determine the in vivo effects of fraction 2 on allergen-induced allergic inflammation, an OVA-induced airway inflammation

10 µg OVA adsorbed to 1 mg alum. A booster injection of the same dose of alum-adsorbed OVA was given on day 5. Mice in groups 1 and 2 received 2 (4 mg/mouse) every 2 days, while mice in groups 3 and 4 received saline injection. Mice in groups 2 and 3 were intranasally challenged with OVA (20 µg in saline) on days 21, 25 and 29 after primary immunization, while mice in groups 1 and 4 received saline intranasally. Twenty-four hours after the last OVA challenge, all mice (groups 1 to 4) were sacrificed. Lung tissues were examined for eosinophil and neutrophil infiltration and cytokine production, measures of airway inflammation.

2.8. Preparation of bronchoalveolar lavage fluid (BALF) and lung tissue and assays for eosinophil peroxidase (EPO) and myeloperoxidase (MPO)

Lung tissues were homogenized in 50 mM HEPES buffer (4 µl/mg lung) containing soybean trypsin inhibitor (100 µg/ml). The homogenate was centrifuged at 4 °C for 30 min at 14,000 rpm. The pellet was re-suspended and homogenized in 0.5% cetyltrimethylammonium chloride (CTAC) (4 µl/mg tissue) and centrifuged as above. The clear extract was used for EPO and MPO assays.

To obtain the bronchoalveolar lavage fluid (BALF), mice were sacrificed by cardiac puncture and phosphate buffer solution (PBS) was infused into the heart to remove blood from the lungs. The BALF was obtained by lavaging the lung with 3×1 ml PBS containing soybean trypsin inhibitor (100 µg/ml). BALF was centrifuged at 1500 rpm for 5 min at 4 °C. The pellets were resuspended in 1 ml NH₄Cl and spun as before to lyse red blood cells. The supernatants were discarded, the pellets resuspended in 0.5% CTAC (250 µl/mice) and centrifuged and the clear extracts were used for MPO assay.

EPO was specifically measured with *o*-phenylene diamine as the chromogen at pH 8.0 in the presence of 3 mM KBr and MPO with tetramethylbenzidine as the chromogen at pH 5.0 in the absence of KBr but with the EPO inhibitor, resorcinol, as previously described [31]. For the EPO assay, samples (75 µl) were added to an equal volume of substrate mixtures containing KBr (6 mM), *o*-phenylene diamine (3 mM) and H₂O₂ (8.8 mM). The reaction was allowed to develop for 30 s and stopped by adding 150 µl of 2 M H₂SO₄

containing 2 mM resorcinol. The optical density was measured at 490 nm.

For the MPO assay, samples (75 µl) were mixed with equal volumes of the substrate (3,3',5,5'-tetramethylbenzidine dihydrochloride, 3 mM; resorcinol, 120 µM; and H₂O₂, 2.2 mM) for 2 min. The reaction was stopped by adding 150 µl of 2 M H₂SO₄. The optical density was measured at 450 nm.

2.9. Cytokine production and mast cell degranulation

The concentrations of IL-5, GM-CSF or IL-4 in the lung tissues or the BALF were determined by ELISA as previously described using antibody pairs from R&D Systems (Minneapolis, MN) [30].

Mast cell degranulation was determined by β -hexosaminidase release into the cell free supernatant. BMDC were treated with 100 µg/ml of CPE for 1 or 18 h and followed by stimulation with TNP-BSA (10 ng/ml). Cell free supernatants and cell pellets were used to determine β -hexosaminidase activity. Briefly, 50 µl of each sample was incubated with 50 µl of 1 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide (Sigma) dissolved in 0.1 M citrate buffer, pH 5, in a 96-well microtiter plate at 37 °C for 1 h. The reaction was stopped with 200 µl/well of 0.1 M carbonate buffer, pH 10.5. The plate was read at 405 nm in an ELISA reader.

2.10. Statistical analysis

Data are presented as mean±S.E.M. of the indicated number of experiments or animals. Statistical significance was determined by assessing means with ANOVA or by using an unpaired *t*-test. Differences were considered significant at *p*<0.05.

3. Results

3.1. Extraction of CP cells

Lyophilized extract 1 was compared with commercial samples of *Chlorella* extract (Respondin™) to ensure that as a source of 2 it is in full compliance with the specifications embodied in the Certificate of Analysis of this immunomodulator. Material 1 was produced with an average yield of 10.2%

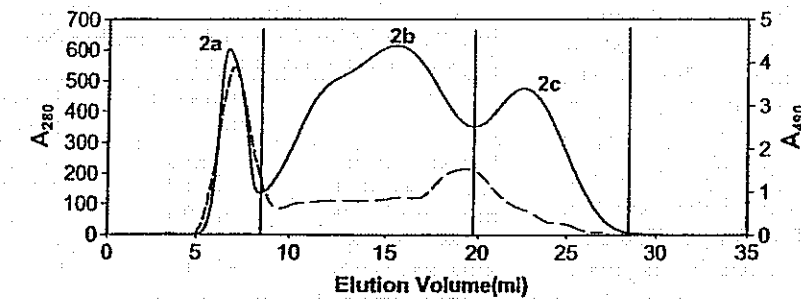


Fig. 1. Chromatography of 2 using Sephadex G100 column. Two grams of sample were dissolved in 100 mM acetate buffer, pH 4.5 (20 mg/ml), filtered, applied on a column of dimensions 5.0×100 cm and eluted with the same buffer at a flow rate of 0.36 ml/min. The fractions were monitored at 280 nm (—), and at 490 nm (---) after mixing with phenol-sulfuric acid reagent.

Dialysis against a 12 kDa membrane followed by lyophilization provided 2 with a 6.8% yield (mass was reduced by 30%), and specific immunoactivity measured as stimulation of undifferentiated spleen cells increased by about 25%. The polysaccharides and other carbohydrate based biopolymers of 1 and 2 contain predominantly arabinose, glucose, rhamnose, ribose and galactose with 1 having higher amounts of glucose than 2 (48% versus 19% of total polysaccharide).

3.2. Chromatography of 2

Size exclusion chromatography of 2 using a Sephadex G100 column resolved the mixture into two distinct portions, a large molecular mass component 2a eluting in the void volume and smaller molecular mass components 2b and 2c, that appeared in most cases in the form of two partially resolved peaks (Fig. 1). The immunostimulatory activity measured by stimulation of [³H]-thymidine incorpo-

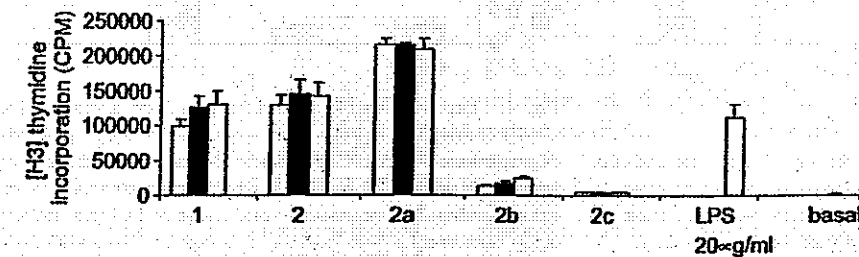


Fig. 2. Stimulation of proliferation of undifferentiated spleen cells by *Chlorella* extract (1) and its components. The stimulation effect was measured by [³H]-thymidine incorporation into mouse splenocytes. Three different concentrations of sample are shown: 31.25 µg/ml (gray bar), 125 µg/ml (filled bar) and 250 µg/ml (open bar). The controls were untreated cells (negative control) and cells challenged with 20 µg/ml LPS (positive control). All fractions were dialyzed against distilled water, lyophilized and then dissolved in tissue culture medium, prior to adding to cells. Results are mean±S.E., *n*=3.

ration into undifferentiated spleen cells or its B cell sub-population was associated almost exclusively with fraction 2a (Fig. 2). This fraction contained primarily arabinose, galactose and rhamnose in the polysaccharide and carbohydrate-based portion, without ribose, suggesting total removal of RNA fragments. Based on amino acid analysis, fraction 2a contained 25% protein by weight.

3.3. Cytokine production by mouse mast cells

Since mast cells play an essential role in the development of allergy through secretion of mast cell-derived mediators such as IL-5, effects of 2 on IgE-dependent IL-5 production by mast cells were determined. Mouse BMDC were sensitized with anti-TNP IgE and treated with 2 (100 µg/ml) for 18 h, followed by TNP-BSA stimulation for 18 h. BMDC treated with 2 and its higher end molecular mass components 2a and 2b demonstrated a significant reduction in IL-5 production after antigen TNP-

both fresh and marine water and is widely used as a food supplement [4]. It is a valuable source of nutrients, relatively low in cellulose, and exhibits a remarkable diversity of physiological and biochemical properties [5]. The beneficial effects of *Chlorella* preparations include improvement of immune function and a better control of ulcerative colitis and hypertension [6,7]. *Chlorella* preparations associated with the prevention and treatment of cancer, bacterial, fungal and viral infections appear to be mediated via the immune system rather than its direct toxicity against the tumor or pathogen [8–17]. An aqueous extract of *Chlorella pyrenoidosa* (CP) 1 (Respondin™; Ocean Nutrition Canada, Halifax, NS, Canada) has recently been shown to be safe and to enhance the antibody response in subjects receiving influenza vaccination [18]. In vitro, the dialyzed CP extract, fraction 2, and its large molecular mass fractions stimulate B cell proliferation and macrophage activity [19], indicative of an immunomodulating effect. Interestingly, oral administration of a *Chlorella* preparation in mice suppressed the production of immunoglobulin E (IgE), suggesting a potential effect for *Chlorella* in the regulation of allergic response [20].

Allergy is a multicellular-involved immune disorder. Mast cells play a central role in the development of both early and late-phase allergic inflammation [21]. This is because mast cells express high levels of receptors for IgE that recognize specific antigens. Upon activation by antigens, mast cells release various potent mediators [22]. Many of the mast cell's effects are mediated by secretion of mast cell-derived mediators such as interleukin-5 (IL-5) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are important for the survival and function of eosinophils. Eosinophils and neutrophils are major inflammatory cells in the late phase of allergic disorders, such as asthma [23].

In this study, we examined the effect of 2 on mast cell cytokine production in vitro and allergic lung inflammation in mice in vivo. Fraction 2 inhibited IgE-dependent IL-5 production by mast cells. Furthermore, we report that oral administration of 2 blocked ovalbumin (OVA)-induced eosinophil and neutrophil infiltration in the airways of mice, suggesting an anti-allergic effect of this dietary supplement.

2. Materials and methods

2.1. Materials

Chemicals (analytical-grade) and dialysis membranes with molecular weight cutoff 12 kDa were purchased from Sigma-Aldrich Chemical (St. Louis, MO), unless specified otherwise. Lyophilized *C. pyrenoidosa* cells were obtained from Taiwan *Chlorella* Manufacturers (Taipei, Taiwan). The moisture content of the cells was established with a moisture balance and was below 3%. All dialyses were performed against several changes of distilled water until the conductivity of the dialysate in a salinity mode was less than 1 ppm. Chromatography was performed using columns and accessories purchased from Amersham Bioscience (Baie D'Urfe, PQ). RPMI 1640 medium was obtained from Invitrogen (Burlington, ON). The BCA kit for protein determination was purchased from Pierce (Rockford, IL). Human IgE was obtained from Chemicon International (Temecula, CA) and anti-human IgE Ab was purchased from DAKO Diagnostic Canada (Mississauga, ON). Appropriate antibody pairs for determination of cytokines, including IL-5 and GM-CSF, by enzyme linked immunosorbent assay (ELISA) were obtained from R&D Systems (Minneapolis, MN).

2.2. Extraction of *Chlorella* cells

CP cells (300 g) were extracted with 1500 ml of water at 80 °C for 1 h. After cooling to 22 °C the mixture was centrifuged at 20,700 ×g for 20 min and the resulting pellet was washed twice in 750 ml of distilled water and re-pelleted. The supernatants of the extraction and wash steps were combined and filtered through a 0.45 µm membrane, evaporated under vacuum to 500 ml and the concentrate was split into two equal portions. The first portion was microfiltered and directly lyophilized to obtain 1. The second portion was dialysed against water using a 12 kDa cutoff membrane (Sigma) and lyophilized to obtain 2.

To determine the carbohydrate based polymers, selected preparations were stirred in 1 M trifluoroacetic acid (3.0 mg/ml) in a capped vial under N₂ at 100 °C for 3 h, dried and reduced at room temperature using 0.5 M NaBH₄ in 0.6 ml of 1 M NH₄OH under stirring overnight. After quenching with 20% acetic

acid in methanol and drying, 3.0 ml of acetic anhydride was added. The sample was then heated at 80 °C for 2 h, evaporated to dryness and extracted with acetyl acetate. The retention times and mass spectra of the tested samples were compared with those of derivatized pure standards of individual sugars and/or commercially available mixtures of alditol acetates. Standards of methyl 3-*O*-methyl- α -L-rhamnopyranoside and methyl 2-*O*-methyl- α -L-rhamnopyranoside were prepared according to literature with minor modifications [24,25]. Determination of uronic acid content was performed as described by Blumenkrantz and Asboe-Hansen [26] and fresh *m*-hydroxydiphenyl reagent was used for each analysis. Quantitative determination of *N*-acetyl amino sugar content was performed using a standard protocol developed by Reisig [27].

2.3. Chromatographic fractionation of 2

The solution of 2 filtered through 0.45 µm filter was applied to a Sephadex G100 XK50/100 column equilibrated in 0.1 M sodium acetate in 0.02% sodium azide, pH 4.5. The elution proceeded at a linear flow rate of 0.36 ml/min. Fractions were monitored at 280 nm and after mixing with phenol-sulfuric acid reagent they were re-tested at 490 nm [28]. The appropriate fractions were pooled, dialyzed, lyophilized and tested for their ability to stimulate proliferation of spleen cells.

2.4. Animals

Female BALB/c and C57BL/6 mice age 6–8 weeks were purchased from Charles River Canada (Saint-Constant, PQ) and maintained in the animal care facility of the IWK Health Center, Dalhousie University (Halifax, NS) under a 12 h light/dark cycle. The studies were carried out in full compliance with regulations of the Canadian Council on Animal Care.

2.5. Stimulation of splenocyte proliferation with *Chlorella* extract fractions

BALB/c mice between 9–16 weeks of age were killed by cervical dislocation, their spleens removed under aseptic conditions, and the splenocytes isolated as previously described [29]. Splenocytes were plated

at 3×10^5 cells/well in 0.1 ml of RPMI 1640 medium in 96-well flat bottom tissue culture plates. Samples dissolved in the cell medium were added to triplicate wells to give a final volume of 0.2 ml/well and the plates were incubated at 37 °C under a humidified atmosphere of 95% O₂/5% CO₂. Lipopolysaccharide (LPS, 20 ng/ml) was added to triplicate wells as a positive control. After 48 h, the cells were pulsed with [³H]-thymidine (1 µCi/well in 10 µl RPMI) and incubated for an additional 18 h. Cells were harvested onto glass fiber filters and the associated radioactivity determined by liquid scintillation counting.

2.6. Mast cell activation, *Chlorella* extract fractions treatment and mast cell viability test

Mouse bone marrow-derived mast cells (BMMC) were cultured as previously described [30]. BMMC (1×10^6 cell/ml) were incubated with the tested substances at the concentration of 100 µg/ml for 18 h at 37 °C. For IgE-dependent mast cell activation studies, BMMC were suspended in fresh complete medium containing 25% of TIB141 (ATCC) cell supernatants that contain mouse IgE directed against trinitrophenyl moiety (TNP). Following sensitization, cells were washed and activated by the addition of 10 ng/ml trinitrophenylated bovine serum albumin (TNP-BSA, Biosearch Technologies, Novato, CA).

Human cord blood-derived mast cells (CBMC) were cultured as previously described [30]. CBMC were treated with 2 (100 µg/ml) for 18 h. For the study of IgE-dependent activation, CBMC were sensitized with human IgE (10 µg/ml) (Chemicon, Temecula, CA), and stimulated for 24 h with anti-human IgE Ab (1:200) (DAKO Diagnostic Canada, Mississauga, ON). Cell free supernatants were collected for the determination of GM-CSF production.

The viability of mast cells with or without treatment was determined by Trypan blue dye exclusion assay.

2.7. Allergic lung inflammation in mice and treatment with 2

Mice were divided into 4 groups (8 mice/group). All mice received ovalbumin (OVA, Sigma) sensitization on day 1 by an intraperitoneal (i.p.) injection of



An aqueous *Chlorella* extract inhibits IL-5 production by mast cells in vitro and reduces ovalbumin-induced eosinophil infiltration in the airway in mice in vivo

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Abstract

An aqueous extract of the edible microalga, *Chlorella pyrenoidosa* (CP) (1), has recently been tested for its immunomodulatory effects in a human clinical trial. Here, the CP extract was dialyzed and fractionated using Sephadex G 100 chromatography. The effects of a dialyzed aqueous CP extract, fraction 2, on mast cell mediator release in vitro and ovalbumin-induced allergic airway inflammation in vivo were examined. In vitro, treatment of mouse bone marrow-derived mast cells with 2 for 18 h significantly inhibited antigen (trinitrophenyl-BSA)-induced IL-5 production. In vivo, treatment of mice with 2 during ovalbumin sensitization and stimulation process significantly reduced eosinophil and neutrophil infiltration in the airways. Moreover, fractions obtained by size exclusion chromatography of 2 inhibited IgE-dependent cytokine GM-CSF production from human cord blood-derived mast cells. Taken together, these results suggest that 2 is composed of biopolymers with anti-allergic potential.

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Keywords: *Chlorella pyrenoidosa*; Allergy; Asthma; Mast cells; Inflammation; Immunomodulation

1. Introduction

A widespread increase in the prevalence of allergy and asthma has occurred during the last 20 years [1]. There is growing interest by the public in alternative medicine for the management of allergy [2], including using dietary supplements [3]. *Chlorella* is a unicellular green algae found in

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