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SPIRULINA PLATENSIS EXPOSURE ENHANCES MACROPHAGE PHAGOCYTIC FUNCTION IN CATS

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ABSTRACT

Bronchoalveolar lavage macrophages isolated from cats were cultured on glass coverslips. Macrophages were exposed to a water-soluble extract of *Spirulina platensis* in concentration range of 0 to 60 μg per mL for two hours. *Spirulina*-extract exposure did not cause significant macrophage cytotoxicity over untreated control cultures. Macrophage monolayers from treated and control cultures were incubated with sheep red blood cells (SRBC) as well as viable *Escherichia coli*. The percentages of phagocytic macrophages for both of these particulate antigens were higher (a two-fold increase in SRBC phagocytosis and over 10% increase in *Escherichia coli* uptake) in cultures treated with various concentrations of *Spirulina*-extract. However, the numbers of either types of particles internalized by phagocytic macrophage were not different between the control and treated cultures. These data which showed that *Spirulina platensis* extract enhances macrophage phagocytic function imply that dietary *Spirulina* supplementation may improve the disease resistance potential in cats.

INTRODUCTION

The cyanobacteria *Spirulina platensis* (blue-green algae) is commercially produced for human consumption and as an agricultural feed ingredient (1,2). *Spirulina* is typically used as a health food source for humans (3). *Spirulina platensis* is considered as a suitable nutrient supplement due to its high protein content (60.5%) and its abundance of vitamins and minerals (4). We have previously shown that water soluble extract of *Spirulina* enhances macrophage phagocytic and tumoricidal functions after *in vitro* exposure (5). In addition, chicks on dietary *Spirulina* were shown to mount a higher anti-sheep red blood cells antibody response, exhibited enhanced natural killer cell and macrophage phagocytic

function, as well as higher response to PHA-P mitogen (6) as compared to the control chicks fed a basal diet. Furthermore, clearance of *Escherichia coli* and *Staphylococcus aureus* from circulation was improved in chicks on *Spirulina* diet as compared with the controls (7).

The present study was conducted to determine the effects of *Spirulina platensis* exposure on cat macrophages.

MATERIAL AND METHODS

Source of Macrophages

Bronchoalveolar lavage (BAL) was used as a source of alveolar macrophages from cats. The BAL was collected from 28 cats as previously described (8). The BAL cells were collected in RPMI-1640 medium supplemented with 10% fetal bovine serum. The cell suspension was adjusted to 1 x 10^6 cells in one mL volume. This cell suspension from individual cats was then added to a Petri dish containing four sterile glass coverslips and incubated overnight in a humidified incubator at 37 C and 5% CO_2. After this incubation period, the coverslips were washed with RPMI to remove non-adherent cells. The glass-adherent cells constituted approximately 80 to 90 % macrophages.

Spirulina platensis Extract Preparation

Spirulina platensis in dried powdered form was obtained from Earthrise Trading Company, Tollhouse, CA. A water-soluble extract of *Spirulina platensis* was prepared as previously described (5). Briefly, one gram of *Spirulina* powder was weighed and suspended in 10 mL of RPMI-1640 growth medium. This mixture was shaken vigorously on a vortex mixer and sonicated for 10 minutes at 35 output using a Fisher Model 300 sonicator. A one mL aliquot of the sonicated suspension was then transferred to fresh nine mL of RPMI medium. The mixture was then filtered twice through Whatman #1 filter. Approximately five mL of filtrate was recovered which was refiltered through 0.45 micron syringe filter (Gelman). The final recovery was approximately 2 mLs. The filtrate was greenish in color with no solid residue or sediment. The sterility of the filtrate was checked by culturing 10 μL volume on nutrient agar plate overnight which exhibited no bacterial colonies. The filtrate was stored in the refrigerator and used as stock for *in vitro* cell exposure experiments. The approximate concentration of *Spirulina* in the final extract was 10 μg per μL.

Preparation of *Escherichia coli*

Escherichia coli were prepared as previously described (9). Briefly, stock *Escherichia coli* culture was grown in several continuous passages in nutrient broth. For phagocytosis assay, bacteria were grown upto log phase (~6 hours), pelleted by centrifugation at 450 x g, washed three times with sterile phosphate buffered saline (PBS) and resuspended in RPMI-1640 growth medium without antibiotic and serum supplementation. The bacterial concentration was adjusted to approximate 2.5 x 10^7 per mL using a bacterial counter.

Preparation of Sheep Red Blood Cells

Freshly collected sheep red blood cells were washed three times with PBS. A 1% suspension of sheep red blood cells (SRBC) was made in RPMI-1640 growth medium for phagocytosis assay.

Macrophage Exposure to *Spirulina platensis* Extract

Glass-coverslip macrophage monolayers were exposed to 0, 10, 20, 40, and 60 μg/mL of *Spirulina* extract. After a two hour incubation, the monolayers were washed and then replenished with fresh RPMI-1640 growth medium.

Quantification of Cytotoxic Effects

Spirulina extract-treated and control macrophage coverslips were inverted onto a drop (~10 μL) of 0.4 % trypan blue prepared in 0.85 % saline placed on a glass microscopic slide. The slides were examined immediately under 40 x microscope objective. The cells stained blue were recorded as dead and their percentage calculated out of total adherent macrophages in three randomly selected microscopic fields per coverslip as previously described (9).

Phagocytosis Assay

Phagocytosis assay was performed as previously described (9,10). Briefly, one mL of bacterial or SRBC suspension was added to *Spirulina*-exposed or control macrophage coverslip cultures in Petri dishes. After one hour incubation, the coverslip cultures were washed with sterile saline, fixed in methanol, stained with Leukostat stain (Fisher Scientific, PA), and mounted on microscopic slides. The numbers of phagocytic macrophages and internalized particles per phagocytic macrophage were scored microscopically at 100 x magnification. Approximately 200 cells were scored from each coverslip.

Statistical Considerations

All data were analyzed using ANOVA (11), and treatment means were separated by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The data on macrophage viability after exposure to *Spirulina* extract are given in Table 1. The levels of cytotoxicity observed in macrophage cultures exposed to various doses of *Spirulina* extract were comparable to the unexposed (medium only) cultures.

The phagocytic potential of macrophages after exposure to *Spirulina* extract was determined using two different antigens: SRBCs and *Escherichia coli*. The data are given in Table 2 and 3.

As shown in Table 2, all doses of *Spirulina* extract doubled the percentage of BAL macrophages capable of phagocytizing SRBC as compared with the untreated controls. However, the numbers of SRBC internalized per phagocytic macrophage did not differ between the treatment and control groups. The percentage of *Spirulina*-treated macrophages capable of internalizing *Escherichia coli* also increased as compared with the controls (Table 3). However, this difference was observed at *Spirulina* doses of 20 to 60 μg/mL with an overall increase of approximately 10% over the untreated controls. As with the SRBC, the number of *Escherichia coli* internalized per phagocytic macrophage was not significantly different.

The findings of this study suggest that *Spirulina* exposure significantly enhance the phagocytic potential of cat brochoalveolar macrophages. These findings are in agreement with our previous studies (5) showing a significant elevation of chicken macrophage phagocytic function after *Spirulina* exposure *in vitro*. Furthermore, *Spirulina*-extract exposure induced tumor necrosis-like factor in chicken macrophages after *in vitro* exposure (5) which was not quantitated in the present study with cat macrophages. Our *in vivo* studies which employed dietary inclusion of various levels of *Spirulina* have shown that *Spirulina* fed chickens challenged with SRBC show higher antibody response as well as enhanced macrophage phagocytic functions (6), without compromising the lymphoid organ integrity or performance end points (body weights, feed conversion). In addition, chickens fed a diet containing 1,000 ppm *Spirulina* showed increased *Escherichia coli* and *Staphylococcus aureus* clearance from circulation and spleen after an intravenous challenge (7). The *Spirulina*-fed chickens when injected with Phytohemagglutinin-P showed enhanced cutaneous basophilic hypersensitivity (CBH) response suggesting an improvement in cell-mediated arm of the immune system (6,7).

TABLE 1

Macrophage Viability After Exposure to *Spirulina platensis* Extract *in vitro*.[1]

Spirulina Extract (μg/mL)	Cytotoxicity (Percent)
0	3.5 ± 1.3
10	4.5 ± 2.1
20	3.9 ± 2.3
40	5.6 ± 2.0
60	ND[2]

1. Macrophage monolayers were exposed to *Spirulina* extract for a period of one hour. The cell viability was determined by trypan blue exclusion. The data are the means and SEM from three coverslips per treatment from two cats.

2. ND = Not Done.

TABLE 2

Phagocytosis of Sheep Red Blood Cells by Macrophages After Exposure to *Spirulina platensis in vitro*.[1]

Spirulina Extract (μg / mL)	Phagocytic Macrophages (Percent)	SRBC/Macrophage (Number)
0	15.6 ± 2.0[b]	2.2 ± 0.3
10	30.4 ± 2.0[a]	2.6 ± 0.3
20	33.4 ± 2.0[a]	2.6 ± 0.3
40	35.6 ± 2.0[a]	2.6 ± 0.3
60	33.8 ± 2.9[a]	2.4 ± 0.4

1. Macrophages from each of the six cats were cultured on triplicate coverslips and incubated with SRBC for one hour.

[a-b]Means ± SEM in a column with different superscripts are significantly different ($P \leq .0001$).

TABLE 3

Phagocytosis of *Escherichia coli* by Macrophages After Exposure to *Spirulina platensis in vitro.*[1]

Spirulina Extract (μg / mL)	Phagocytic Macrophages (Percent)	*E. coli* / Macrophage (Number)
0	63.1 ± 3.1[b]	3.98 ± 0.8
10	68.9 ± 3.1[ab]	3.94 ± 0.6
20	72.8 ± 3.1[a]	4.07 ± 0.7
40	73.3 ± 3.1[a]	3.90 ± 0.7
60	75.4 ± 3.6[a]	3.79 ± 0.6

1. Macrophages from each of the 22 cats were cultured on triplicate coverslips and incubated with *Escherichia coli* for one hour.

[a-b]Means ± SEM in a column with different superscripts are significantly different ($P \leq .06$).

Spirulina is rich in nutrients that modulate the immune system (beta carotene, vitamin E, iron, zinc). However, additional factors present in *Spirulina* such as lipopolysaccharide (12) and C-phycocyanin (13) have been shown to possess macrophage activation and stem cell differentiation potential. Taken together, our studies suggest that *Spirulina platensis* can be considered as a potent immunomodulator which has the potential to upregulate both humoral and cell-mediated arms of the immune system both in the chickens and cats. A possible inclusion of *Spirulina* in cat food may therefore be considered as a dietary mean of enhancing disease resistance potential of cats. It would be interesting to follow up these *in vitro* studies with *in vivo* dietary supplementation in cats. However, based on our previous studies in the chicken system it may be appropriate to include 10,000 ppm *Spirulina platensis* in cat diets without compromising the performance characteristics.

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