Effect of Beta-Glucan on the Improvement of Immunity in Healthy Individuals during the Flu Season: A Pilot, Prospective and Open-label Clinical Trial

Bianca Souza Bagatela[1], Bryan Y. Liu[2,3], Dingfu Zhong[4], Jiayi Ni[5], Li Zhang[6], Hao Li[3], Sven Rohmann[7], Andrey Pereira Lopes[1]

[1] Department of Hematology and Oncology, FMABC, Santo Andre, Sao Paulo, Brazil,
[2] College of Biotechnology, East China University of Science and Technology, Shanghai, P. R. China,
[3] inBiome Sciences LLC, Apex, NC, United States of America,
[4] Jinhua People's Hospital, Jinhua, Zhejiang Province, P. R. China,
[5] Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada,
[6] SPRIM China, Shanghai, P. R. China,
[7] ImmuDyne Nutritional LLC, Jacksonville, FL, United States of America.

Abstract

Beta-Glucans are heterogeneous polysaccharides of glucose polymer, and its activity depends on the molecular structure, size, branching frequency, structural modification, conformation, and solubility. Recent findings indicate that β-glucans enhance the immune system underlying the activation of lymphocytes, monocytes, macrophages, granulocytes, and natural killer (NK) cells. This study aims to evaluate the effect of yeast (1,3)-(1,6)-beta-glucan with 90-day consumption on the improvement of immunity in healthy individuals during the flu season. 12 healthy participants received oral yeast (1,3)-(1,6)-beta- D-glucan dose (30 mg) per day over a course of 90 days. The subjects were examined by the investigator at the study visits of enrollment (baseline), 30-day, 60-day and 90-day. Serum biomarkers and Peripheral Blood Mononuclear Cells (PBMC)s were measured at baseline, 30-day, 60-day and 90-day visits. In the study population, supplementation with yeast (1,3)-(1,6)-beta-glucan reduced the number of symptomatic upper respiratory tract infections (URTIs) by 44% as compared to the same winter time period (December through March) from the previous year ($p = 0.027$). The duration of sickness of URTIs reduced by 50% as compared to the previous year ($p < 0.0001$). The serum levels of IFN-γ, IL-2 and IFN-γ/IL-4 ratio statistically increased as compared to the baseline. The cell numbers from in vitro PBMC Cell proliferation assay for IFN-γ and IFN-γ/IL-4 ratio statistically increased. The findings from the present study suggest that yeast beta-glucan dietary supplement preparation reduced the upper respiratory tract infection by improving the body's immunity to defend pathogens.

Key words: *dietary supplement, efficacy, immune system, beta-glucan, PureMune*

Introduction

Beta-glucans are mainly found in the extracts of some species of mushrooms and in microbes, such as black yeast, and possess some unique immunological activities (1, 2). Beta-Glucans are heterogeneous polysaccharides of glucose polymer, consisting of a backbone of beta-(1-3)-linked beta -D-glucopyranosyl units with beta-(1-6)-linked side chains of varying distribution and length. The activity of beta-glucan depends on the molecular structure, size, branching frequency, structural modification, conformation, and solubility. It appears that the most active forms of β- glucans contain beta-(1-3)(1-6) linkages (3). Beta-glucans have been shown to exert cytotoxic activity against cancer cells (4) accompanied by activating the production of interleukin-2 (IL-2), IL-4, IL-6, IL-12, CD44, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) (5,6). These findings indicate that β-glucans enhance the immune system underlying the activation of lymphocytes, monocytes, macrophages, granulocytes, and natural killer (NK) cells (7). Beta- Glucan has been shown to protect against infection by bacteria, viruses, and pathogenic microorganisms (8).

Beta-Glucan also prevents cancer promotion and progression and has synergistic anti-tumor effects with monoclonal antibodies and cancer chemotherapeutics (9). Beta-Glucan promotes antibody- dependent cellular cytotoxicity through a biological pathway involved in carcinogenesis (10). In general, the anticancer actions of β-glucans are not attributable to their direct actions on cancer cells, as is the case with chemical anti-cancer drugs, but depends on the immunological enhancement of the host, e.g., by acting as a biological response modifier (BRM) (4).

Macrophages and dendritic cells have typical cell surface receptors called pattern recognition receptors (PRRs) that detect innately non-self-molecules including pathogen-associated molecular patterns (PAMPs) (11). β-Glucans might act as PAMPs and are recognized by PRRs, because β-glucans cannot directly penetrate cell membrane due to their large molecular size (12). The major PRRs for β-glucans might be dectin-1 and the roll-like receptor (TLR). After binding with β- glucan, dectin-1 and TLR may induce signaling cascade and activate immune cells. Other receptors, such as complement receptor 3 (CR3), scavenge receptors (SR), and lactosylceramide (LacCer), may be involved as well (11, 13).

The biological effects beta-1,3/1,6-glucans depends on the interaction between beta-1,3/ 1,6-glucan and specific receptors on epithelial surfaces (14). However, beta-1,3/1,6-glucans may stimulate the gut immune system, such as suppressive effects on asthma and allergy symptoms. Beta-1,3/1,6-glucan may interact indirectly with the gut microbiota by affecting intestinal barrier function and LPS toxicity, and by enhancing the production and secretion of components such as lysozyme, antimicrobial peptides and IgA.

Several double-blind, randomized and controlled clinical studies have demonstrated that beta- glucans could reduce the incidence of common cold, upper respiratory infection and improve the mood state in young children, adults and stressed women (15, 16, 17, 18).

This study is designed to examine the effect of PureMune with beta-glucan on the improvement of immunity in healthy individuals during the flu season. The immunity can be measured by analyzing the immune biomarkers of IFN γ, IL-2, TNF-alpha/beta, IL-4, IL-5, IL-10, IL-13, IL- 12, IL 17, IL-6 and CD44 at baseline, 30-day, 60-day and 90-day visits. In addition, in vitro cell proliferation assay of PBMCs can be measured to assess immune cells DNA synthesis at baseline, 30-day, 60-day and 90-day visits.

Methods

Study population and investigational product

Twelve subjects with at least four common colds within the last twelve months were enrolled into the study. They must meet the following inclusion criteria: written consent to participate, age ≥ 18–80 years, at least four common cold infections within the last 12 months and agree not to take any nutritional medications or supplements during the study. The main exclusion criteria were as follows: diseases of cardiovascular, cerebrovascular, liver, kidney, hematopoietic system and other serious diseases, congenital or acquired immunodeficiency diseases, pregnant or lactating, has an allergy to beta-glucan, takes any drugs or food supplement related to the study product in recent days, participated another clinical trial in the past three months, has any diseases or takes any drugs or nutrition products that can affect the evaluation of the study product.

The beta-glucan is an insoluble (1,3)-(1-6)-beta glucan which was made from Baker's yeast (S. cerevisiae), with a purity of at least 80% on dry matter (branching factor approximately: 1,3[backbone]: 1,6 [side chain]: 1,3/1,6 [branching] = 10:1:0.6). In addition, it contains <2% α-D-Mannose; < 6% fat; < 3% protein; <6% moisture; and < 3% ash on dry matter. The dry matter is more than 80%.

Subjects received a total of 30 mg of insoluble yeast beta-glucan (PureMune provided by ImmuDyne Nutritional LLC, USA) in one sachet bag per day for 90 days.

The clinical study was approved by the Ethics Committee of Shanghai Nutrition Society. The written informed consent was obtained from all participants prior to entering the study. The study was registered in the Chinese Clinical Trial Registry at https://www.chictr.org.cn/index.aspx ChiCTR2000040893.

Study design

The study was a single center, open label, prospective pilot trial which was carried out in accordance with the Helsinki declaration and ICH GCP E6 from December 2020 to April 2021 in the Department of Gastroenterology of Jinhua People's Hospital, Jinhua, Zhejiang Province, China.

The eligible twelve subjects were enrolled at one study site. There were four study visits of baseline, 30-day, 60-day and 90-day during the study period of 12 weeks. A common cold episode was defined by the occurrence of at least two of the following cold symptoms: fever, cough, runny nose and throat pain. During the common cold, the subjects were instructed to record and assess their cold symptoms at home for a period of 14 days for each occurring episode. Their cold symptoms include fever, cough, runny nose, throat pain, headache, muscle pain, week feeling, hard breathing, retrosternal pain and lack of appetite. In addition, the subjects the duration of sickness by counting the start date of the beginning of any of the symptoms and the stop date of the last symptom. The duration of one URTI is defined as the number of days from the date of onset of any of the abovementioned symptoms to the date that all the symptoms are relieved.

The study product compliance was determined by counting the returned unconsumed sachets. The subjects were instructed to record each sachet that they took. Sufficient compliance was defined if 75 and 110 % of the sachets were consumed.

Outcome measures

The primary objective of the study is to evaluate the effects of 90-day beta-glucan consumption on the improvement of immunity in healthy individuals with having more than four times of common colds within the last 12 months. The incidence of URTIs was defined as the number of URTIs during the study period. The severity and duration of URTIs episodes, the incidence of medically confirmed adverse events and concomitant medications were assessed.

Processing of the blood and serum samples: blood samples of 12 normal individuals (5 males and 7 females) were obtained at Jinhua People's Hospital (Jinhua, Zhejiang Province, China). The serum samples were prepared using the standard method and the sera were stored at -80°C until analysis.

Serum Immune Biomarkers Measurement and Analysis: IFN γ, IL-2, and TNF-alpha/beta, IL-4, IL-5, IL-10 and IL-13, ((Th1: Th2 ratio (percentage of IFN γ and IL-4)), IL-12, IL 17, IL-6, IgE and CD44 at enrollment (baseline), 30-day, 60-day and 90-day visits.

In vitro cell proliferation assay (innate immunity): Peripheral Blood Mononuclear Cells (PBMCs) are cultured with or without con-canavalin A (Con A) for 72 h at 37C and label with [3H] thymidine, then assess DNA synthesis by measuring thymidine uptake at enrollment (baseline), 30-day, 60-day and 90-day visits.

Statistical analysis

This is a prospective, open label study in which all 12 subjects taking the same product. Data were summarized as mean ± standard deviation or median (the 25th percentile, the 75th percentile) for continuous variables, and frequency (percentage) for categorical variables. The summary URTIs symptoms were presented by age groups.

The comparison of the number and the duration of URTIs at the end of the study versus baseline were performed using paired t-test. The comparison of post-intervention blood biomarkers versus baseline were performed using paired t-test for each time point (day 30, 60 and 90). All statistical tests were performed at a significance level of 0.05.

Statistical analysis in this study was performed using SAS 9.3 statistical software (SAS Institute Inc., USA). All tests employed a 0.05 significance level.

Results

A total of 12 participants (18 to 80 years old) who sustained common cold (collectively URTIs) at least four times in the previous years were enrolled. All 12 subjects completed the trial. The gender ratio of completed participants was 5 males to 7 females (41.7%:58.3%), as shown in Table 1. The related symptoms of URTIs for all subjects are presented in Table 2.

Table 1. Baseline characteristics

Baseline characteristics	Summary statistics
Number of subjects	12
Males, %	5 (41.7%)
Females, %	7 (58.3%)
Age (years), mean (SD), median (q1, q3)	49.4 ± 17.2, 51.0 (38.0, 59.8)
18-30 years, %	2 (16.7%)
30-40 years, %	3 (25.0%)
50-60 years, %	4 (33.3%)
70-80 years, %	3 (25.0%)
Number of medical confirmed upper respiratory tract infections from the previous year during 3-month winter from December through March	1.33 ± 0.49, 1 (1, 2)
Average duration of upper respiratory tract from the previous year during 3-month winter from December through March	5.42 ± 1.16, 5 (4.75, 6.25)

Data are presented as mean±SD and median (Q1, Q3), or frequency (%).

SD: standard deviation; Q1: the 25th percentile; Q3: the 75th percentile.

Table 2. Symptoms of upper respiratory tract infections

Symptom	Overall (n=12)			18-30 years (n=2)			30-40 years (n=3)			50-60 years (n=4)			70-80 years (n=3)		
	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90
a. Fever	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)
b. Cough	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
c. Runny nose	6 (50.0)	2 (16.7)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	2 (50.0)	1 (25.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)
d. Throat pain	4 (33.3)	2 (16.7)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	1 (25.0)	0 (0.0)	1 (33.3)	1 (33.3)	0 (0.0)
e. Headache	5 (41.7)	1 (8.3)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)
f. Muscle pain	1 (8.3)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
g. Week feeling	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
h. Hard breathing	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
i. Retrosternal pain	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)
j. Lack of appetite	3 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)

Table 3. Number and duration of upper respiratory tract infections and treatment

Outcomes	In the past year of baseline	During the study	Post-intervention vs. Baseline Difference (95% CI)	p-value
Number of medical confirmed upper respiratory tract infections	Per 3 months (December to March): 1.33±0,49, 1 (1, 2)	Per 3 months (December to March): 0.75±0.62, 1 (0, 1)	Per 3 months (December to March): -0.58 (-1.09, -0.08)	Per 3 months (December to March): 0.027
Average duration of sickness per URTI	Per 3 months (December to March): 5.42±1.16, 5 (4.75, 6.25)	Per 3 months (December to March): 2.71±2.22, 3 (0, 4.5)	Per 3 months (December to March): -2.71 (-3.61, -1.81)	Per 3 months (December to March): <0.0001
Total duration of sickness	Per 3 months (December to March): 7.42±3.78, 5.5 (5, 9)	Per 3 months (December to March): 2.92±2.31, 3.5 (0, 5)	Per 3 months (December to March): -4.50 (-6.39, -2.61)	Per 3 months (December to March): 0.0003

Data are presented as mean±SD and median (Q1, Q3).

SD: standard deviation; Q1: the 25th percentile; Q3: the 75th percentile.

The symptoms of URI for all subjects are recorded and summarized in Table 2. The durations of the time from start to stop of the common cold are recorded through the study but not for each single symptom.

The number and duration of upper respiratory tract infections and treatment for all subjects were recorded and summarized in Table 3.

The results presented in Table 3 showed that the incidence of the sickness for all subjects during the study are significantly less as compared with the same 3-month winter period from December through March from the previous year (p=0.027). The duration of the sickness per URTIs during the study are significantly less than from the previous year (p<0.0001). The total duration of sickness including URTIs are significantly less than the previous year (P=0.0003).

The results presented in Table 4 showed that statistically significant higher serum IFN-γ levels at day 30, day 60 and day 90 were observed as compared with the baseline (p<0.0001). Table 4 shows that statistically significant higher serum IL-2 levels at day 30 and day 60 (p<0.05) and day 90 (p>0.05 NOT statistical significant) were observed as compared with the baseline. Table 4 also

Table 4. Serum biomarkers

Biomarkers	Baseline	Day 30	Day 60	Day 90	Change compared to baseline (p-value)		
					Day 30 vs. Baseline	Day 60 vs. Baseline	Day 90 vs. Baseline
IgE (IU/ml)	19.54±3.32 18.95 (17.73, 21.35)	20.40±5.87 18.85 (17.20, 21.03)	20.85±3.08 19.80 (18.45, 22.35)	19.14±3.41 18.65 (16.10, 20.93)	0.563	0.074	0.676
IFN-γ (pg/ml)	1.16±0.06 1.17 (1.13, 1.21)	1.31±0.13 1.33 (1.23, 1.41)	1.29±0.11 1.32 (1.22, 1.37)	1.27±0.10 1.28 (1.21, 1.37)	<0.0001	0.0001	<0.0001
IL-4 (pg/ml)	1.46±0.16 1.45 (1.35, 1.55)	1.42±0.13 1.39 (1.31, 1.54)	1.45±0.15 1.42 (1.36, 1.53)	1.50±0.16 1.48 (1.39, 1.61)	0.357	0.904	0.188
IFN-γ: IL-4 ratio	0.80±0.09 0.81 (0.72, 0.85)	0.93±0.12 0.89 (0.84, 0.98)	0.89±0.11 0.91 (0.82, 0.97)	0.86±0.12 0.87 (0.78, 0.92)	0.005	0.010	0.043
IL-2 (pg/ml)	1.29±0.07 1.28 (1.25, 1.34)	1.71±0.65 1.39 (1.24, 1.97)	1.63±0.56 1.50 (1.23, 1.68)	1.40±0.21 1.35 (1.21, 1.58)	0.034	0.039	0.058
TNF-α (pg/ml)	1.92±0.72 1.72 (1.40, 2.19)	2.00±0.73 1.72 (1.49, 2.63)	1.93±0.73 1.75 (1.33, 2.31)	1.90±0.64 1.73 (1.45, 2.21)	0.204	0.789	0.489
IL-5 (pg/ml)	1.43±0.10 1.44 (1.35, 1.48)	1.51±0.26 1.43 (1.34, 1.60)	1.41±0.18 1.35 (1.30, 1.53)	1.41±0.17 1.35 (1.29, 1.49)	0.212	0.697	0.505
IL-6 (pg/ml)	4.41±0.48 4.62 (4.20, 4.70)	4.49±0.63 4.45 (4.15, 5.01)	4.42±0.52 4.63 (4.18, 4.71)	4.39±0.53 4.55 (4.25, 4.76)	0.379	0.903	0.397
IL-10 (pg/ml)	2.72±0.45 2.66 (2.36, 3.03)	2.91±0.66 2.71 (2.37, 3.36)	2.83±0.64 2.67 (2.30, 3.12)	2.70±0.46 2.67 (2.29, 2.97)	0.079	0.202	0.302
IL-12P70 (pg/ml)	2.10±0.32 2.17 (1.87, 2.34)	2.25±0.53 2.30 (1.82, 2.56)	2.12±0.44 2.26 (1.79, 2.38)	2.06±0.44 2.18 (1.72, 2.32)	0.123	0.588	0.449
IL-17A (pg/ml)	1.28±0.07 1.29 (1.24, 1.33)	1.20±0.15 1.24 (1.09, 1.33)	1.29±0.12 1.29 (1.20, 1.34)	1.32±0.10 1.31 (1.24, 1.39)	0.117	0.745	0.074
CD44 (pg/ml)	481.58±114.94 470.5 (403.5, 538)	486.42±117.67 459 (359.75, 559)	475.42±86.40 484.5 (395, 527.25)	477.00±101.80 474 (400.25, 536.25)	0.822	0.734	0.822
IL-13 (pg/ml)	56.55±17.29 62.25 (41.70, 70.30)	56.06±13.24 55.44 (51.43, 60.25)	58.21±13.31 (58.35 (49.95, 65.34)	57.59±11.12 56.43 (52.58, 61.88)	0.910	0.471	0.850
TNF-β (pg/ml)	1.53±1.01 1.35 (0.93, 1.80)	1.48±0.86 1.10 (0.90, 2.00)	1.58±0.88 1.55 (0.85, 2.33)	1.55±1.10 1.25 (0.80, 2.05)	0.744	0.696	0.859

Data are presented as mean±SD and median (Q1, Q3).

SD: standard deviation; Q1: the 25th percentile; Q3: the 75th percentile.

shows that statistically significant higher serum IFN-γ/IL-4 ratio at day 30, day 60 and day 90 were observed as compared with the baseline ($p<0.05$). However, no statistically significant changes were observed for other biomarkers at day 30, day 60 and day 90 as compared with the baseline.

The results presented in Table 5 showed that statistically significant higher IFN-γ levels at day 30, day 60 and day 90 were observed as compared with the baseline ($p<0.05$) from the in vitro PBMC cell proliferation assay. Table 5 also shows that statistically significant higher IFN- γ/IL-4 ratio at day 30 and day 60 ($p<0.05$) and day 90 ($p>0.05$ NOT statistical significant) were observed as compared with the baseline. However, no statistically significant changes were observed for other biomarkers at day 30, day 60 and day 90 as compared with the baseline (data from other biomarkers were not show).

Percentages of INF- γ and IL-4-producing CD4+ T cells (Th1:Th2 ratio) were determined by single-cell measurement of intracellular cytokines using flow cytometry as described (Openshaw P et.al. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. (J Exp Med. 1995; 182: 1357–67).

Regarding adverse events, five events were observed for all subjects enrolled in the study: allergic rhinitis, frequent defecation, fracture, torticollis and cardiac murmurs. All adverse events were not related to the study products and none of these subjects were withdrawn from the study because of adverse events.

Conclusion & discussion

This is a Pilot, Open-label Study to evaluate on the effect of PureMune with Beta-Glucan on the improvement of immunity in healthy individuals during the flu season. A total of 100 people were screened and 24 of them were scheduled and visited the study site. A total of 12 subjects met the inclusion and exclusion criteria and enrolled into this study, and all 12 subjects finished this study with zero dropout.

The incidence of the sickness for all subjects during the study are significantly less as compared with the same 3-month winter period from December through March from the previous year ($p=0.027$). The duration of the sickness per URTI during the study are significantly less than from the previous year ($p<0.0001$). The total duration of sickness including URTI are significantly less than the previous year ($P=0.0003$).

Higher serum IFN-γ levels at day 30, day 60 and day 90 were observed as compared with the baseline ($p<0.0001$). Higher serum IL-2 levels at day 30 and day 60 ($p<0.05$) and day 90 ($p>0.05$ NOT statistical significant) were observed as compared with the baseline. Higher serum IFN-γ/IL-4 ratio at day 30, day 60 and day 90 were observed as compared with the baseline ($p<0.05$). However, there are no statistical significant changes that were observed for other serum biomarkers at day 30, day 60 and day 90 as compared with the baseline.

For the in vitro PBMC cell proliferation assay, higher IFN-γ levels at day 30, day 60 and day 90

Table 5. In Vitro PBMC Cell Proliferation Assay

Biomarkers	Baseline	Day 30	Day 60	Day 90	Change compared to baseline (p-value)		
					Day 30 vs. Baseline	Day 60 vs. Baseline	Day 90 vs. Baseline
IFN-γ (% CD4 + cells)	25.82±9.15 29.34 (20.06, 32.76)	31.20±10.69 32.51 (24.99, 38.43)	29.73±10.03 29.73 (22.89, 36.82)	28.21±10.04 30.86 (22.87, 33.44)	0.001	0.005	0.028
IL-4 (% CD4 + cells)	2.70±1.16 2.73 (1.65, 3.52)	2.50±1.20 2.34 (1.55, 3.50)	2.60±1.22 2.55 (1.44, 3.43)	2.80±1.23 2.77 (1.73, 3.91)	0.260	0.499	0.411
IFN-γ: IL-4 ratio	12.28±8.29 8.56 (6.96, 18.45)	15.62±9.91 12.39 (9.75, 19.76)	14.32±8.70 11.42 (8.90, 21.53)	12.61±7.60 10.35 (8.14, 20.82)	0.048	0.040	0.607
IL-2 (pg/ml)	1.58±0.14 1.60 (1.50, 1.66)	1.74±0.37 1.68 (1.43, 1.94)	0.66±0.23 1.76 (1.50, 1.82)	1.61±0.25 1.57 (1.46, 1.70)	0.071	0.092	0.555

Data are presented as mean±SD and median (Q1, Q3).

SD: standard deviation; Q1: the 25th percentile; Q3: the 75th percentile.

were observed as compared with the baseline ($p<0.05$). Higher IFN-γ/IL-4 ratio at day 30 and day 60 ($p<0.05$) and day 90 ($p>0.05$ NOT statistical significant) were observed as compared with the baseline. There are no statistical significant changes were observed for IL-2 and IL-4 at day 30, day 60 and day 90 as compared with the baseline.

Taken together, PureMune (30mg beta-glucan) consistently stimulate immune cells through the study (at 30, 60 and 90 days of intervention) for increasing the IFN-γ production both in vivo and in vitro. In addition, higher IFN-γ/IL-4 ratio at day 30, day 60 and day 90 for both in vivo and in vitro were observed as compared with the baseline ($p<0.05$). The higher ratio suggests that Th1:Th2 ratio was higher at day 30, 60 and 90 after receiving the beta-glucan than at the baseline because of the increasing IFN-γ (Th1 cells) and no percent change in IL-4 (Th2 cells) after receiving beta-glucan than at the baseline, which further suggest that intake of PureMune (beta-glucan) may be useful for prevention and treatment of infectious and allergic diseases induced by a weak Th1-type immune response.

These study results suggest that a daily intake of PureMune augments acquired immunity, especially Th1-related immune functions in healthy subjects. Augmentation of the Th1 response may be beneficial for individuals living in modern cities, because the better public hygiene and fewer infections in these societies may reduce the Th1 response, thereby increasing the risk of developing allergies.

Acknowledgements

Funding for this study was received from ImmuDyne Nutritional LLC, 3930 Hollywood Avenue, Pensacola, FL 32505 U.S.A. We thank SPRIM China for conducting the study. We thank inBiome Sciences LLC for participating the study design, data analysis and liaising between ImmuDyne Nutritional LLC and SPRIM China.

References

1. *Ikewaki N, Fujii N, Onaka T, et al. Immunological actions of Sophy beta- glucan (beta1,3-1,6-glucan), currently available commercially as a health food supplement. Microbiol. Immunol, 2007; 51: 861-873.*

2. *Kim HS, Hong JT, Kim Y, et al. Stimulatory effect of β-glucans on immune cells. Immune. Netw, 2011; 11: 191-195.*

3. *Demleitner S, Kraus J, Franz G. Synthesis and antitumour activity of sulfoalkyl derivatives of curdlan and lichenan. Carbohydr Res, 1992; 226: 247-252.*

4. *Vetvicka V, Vetvickova J. Glucan supplementation has strong anti-melanoma effects: Role of NK cells. Anticancer Res, 2015; 35: 5287-5292.*

5. *Ostadrahimi A, Ziaei JE, Esfahani A, et al. Effect of beta glucan on white blood cell counts and serum levels of IL-4 and IL-12 in women with breast cancer undergoing chemotherapy: a randomized double-blind placebo-controlled clinical trial. Asian Pac. J. Cancer Prev. 2014; 15: 5733-5739.*

6. *Ostadrahimi A, Ziaei JE, Esfahani A, Jafarabadi MA, Movassaghpourakbari A, Farrin N. Effect of Beta Glucan on White Blood Cell Counts and Serum Levels of IL-4 and IL-12 in Women with Breast Cancer Undergoing Chemotherapy: A Randomized Double-Blind Placebo-Controlled Clinical Trial. Asian Pacific Journal of Cancer Prevention, Vol 15, 2014.*

7. *Ding J, Feng T, Ning Y, et al. β-Glucan enhances cytotoxic T lymphocyte responses by activation of human monocyte-derived dendritic cells via the PI3K/AKT pathway. Hum. Immunol, 2015; 76: 146-154.*

8. *Zhou LD, Zhang QH, Zhang Y, Liu J, Cao YM. The shiitake mushroom-derived immuno-stimulant lentinan protects against murine malaria blood-stage infection by evoking adaptive immune-responses. Int Immunopharmacol, 2009; 9: 455-462.*

9. *Harada K, Itashiki Y, Takenawa T, Ueyama Y: Effects of lentinan alone and in combination with fluoropyrimidine anticancer agent on growth of human oral squamous cell carcinoma in vitro and in vivo. Int J Oncol, 2010; 37: 623-631.*

10. *Sier CF, Gelderman KA, Prins FA, Gorter A. Beta-glucan enhanced killing of renal cell carcinoma micrometastases by monoclonal antibody G250 directed complement activation. Int J Cancer, 2004; 109: 900-908.*

11. *Brown GD, Gordon S. Immune recognition of fungal beta-glucans. Cell Microbiol, 2005; 7: 471-479.*

12. *Chen J, Seviour R. Medicinal importance of fungal beta-(1→3), (1→6)-glucans. Mycol Res, 2007; 111: 635-652.*

13. *Kim HS, Park KH, Lee HK, et al. Curdlan activates dendritic cells through dectin-1 and tolllike receptor 4 signaling. Int. Immunopharmacol, 2016; 39: 71-78.*

14. *Nishitani Y, Zhang L, Yoshida M, Azuma T, Kanazawa K, Hashimoto T, et al. Intestinal anti-inflammatory activity of lentinan: influence on IL-8 and TNFR1 expression in intestinal epithelial cells. PLoS One, 2013; 8: e62441.*

15. *Auinger A, Riede L, Bothe G, Busch R, Gruenwald J. Yeast (1,3)-(1,6)-beta-glucan helps to maintain the body's defence against pathogens: a double-blind, randomized, placebo controlled, multi-centric study in healthy subjects. Eur J Nutr, 2013; 52: 1913–1918.*

16. *Shawn M. Talbott & Julie A. Talbott. Baker's Yeast Beta-Glucan Supplement Reduces Upper Respiratory Symptoms and Improves Mood State in Stressed Women. Journal of the American College of Nutrition, 2012; Vol. 31, No. 4, 295–300.*

17. *Meng F. Baker's Yeast Beta-Glucan Decreases Episodes of Common Childhood Illness in 1 to 4 Year Old Children during Cold Season in China. J Nutr Food Sci 2016, 6: 4-*

18. *Ikewaki N, Sonoda T, Miyazawa Y, Onizuka S, Chikamori M. Serum levels of β-1,3-1,6 glucan-specific antibodies and immune biomarkers in normal individuals. J. of Kyushu Univ. of Health and Welfare, 2018; 19: 87-94.*

Corresponding Author
Bianca Souza Bagatela,
Department of Hematology and Oncology,
FMABC, Santo Andre,
Sao Paulo,
Brazil,
E-mail: biancabagatela@gmail.com