Article

In Vitro and *in Vivo* Anti-Inflammatory Effects, Protection of Gut Barrier Integrity and Stimulation of Phagocytosis of ABB C1®, a Synergistic Combination of β-Glucans and Selenium- and Zinc-Enriched *Saccharomyces cerevisiae*

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Abstract: This study evaluated the anti-inflammatory effects, protection of gut barrier integrity, and stimulation of phagocytosis in peripheral cells of a nutritional supplement based on a synergistic combination of yeast-based ingredients with a unique 1,3/1,6-glucan complex and a consortium of postbiotic *Saccharomyces cerevisiae* rich in selenium and zinc. The anti-inflammatory effect in Caco-2 cells in the presence and absence of a pro-inflammatory challenge (tumour necrosis factor alpha [TNF-α]/interferon gamma [IFN-ɣ]) showed statistically significant reductions of IFN-ɣ induced protein-10 (IP-10), and monocyte chemoattractant protein-1 (MCP-1) levels vs. controls ($p < 0.001$). Disruption of the gut integrity in the presence or absence of *Escherichia coli* (ETEC H10407) showed transepithelial electrical resistance (TEER) values higher in the ABB C1® group after 6 hours of testing. Spontaneous build-up of the gut epithelium monolayer over 22 days was also greater in the ABB C1® condition vs. a negative control. ABB C1® showed a significantly higher capacity to stimulate phagocytosis as compared with controls of algae β-1,3-glucan and yeast β-1,3/1,6 glucan ($p < 0.001$). This study supports the mechanism of action by which ABB C1® may improve the immune response and be useful to prevent infection and allergy in clinical practice.

Keywords: *Saccharomyces cerevisiae*; beta-glucans; selenium; zinc; inflammatory processes; gut barrier modulation; COVID-19; allergy; nutritional supplementation

1. Introduction

Pro-inflammatory cytokines, the microbiota and the gut barrier function, and phagocytosis are some important mediators involved in the interplay of innate-adaptive immune responses [1-4] for the elimination and clearance of infectious agents, including viruses and the newly appeared SARS-CoV-2 [5-7]. Chemokines, such as interferon (IFN)ɣ-inducible protein 10 (IP-10) are involved in acute exacerbations of asthma [8] and an increase of inflammation and keratinocyte apoptosis in atopic dermatitis [9]. The monocyte chemoattractant peptide-1 (MCP-1) participates in allergen sensitization [10] and it has been shown that MCP-1 produced by keratinocytes plays a role in the process of mononuclear cell infiltration in occupational allergic contact dermatitis [11]. On the other hand, dysregulation of the intestinal barrier has been associated with chronic immune diseases (e.g. food allergy, inflammatory bowel disease, celiac disease), but

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bacterial pathogens and components of innate and adaptive immunity have been identified in the underlying regulation pathways of the gut barrier function [12].

In the current pandemic era of the coronavirus disease 2019 (COVID-19), a focus of interest has been the use of nutritional supplements with anti-inflammatory and immunomodulatory activity relevant to maintain a strong healthy immune system [13,14]. In this line, results of a randomized controlled trial in volunteers vaccinated against influenza or COVID-19 validated the capacity to stimulate trained immunity of a nutritional supplement composed of a synergistic combination of yeast-based ingredients with a unique β-1,3/1,6-glucan complex and a consortium of postbitoic *Saccharomyces cerevisiae* rich in selenium and zinc (ABB C1®) [15]. Selenium is a potent antioxidant, enhances the function of cytotoxic effector cells, and is important for maintaining T cell maturation, functions, and T-cell dependent antibody production [16]. A recent systematic review of selenium deficiency and COVID-19 based on 11 studies has shown that lower serum selenium levels are associated with worse outcomes [17]. In a similar way, zinc is a critical trace mineral for antiviral immunity and results of five studies with 1506 participants included in a meta-analysis showed that zinc supplementation led to a significant lower risk of mortality in COVID-919 patients when it was compared with non-supplemented controls [18]. Nutrition intervention securing an adequate supply of zinc and selenium, as well as vitamin D, has been recommended for rising antiviral resistance against progressive COVID-19 [19]. On the other hand, β-glucan is a polysaccharide that is abundantly found in the cell wall of *S. cerevisiae* and primes the immune system to respond better to any viral infection [20]. Also, the use of oral β-glucan has been hypothesized to boost immune responses and abrogate symptoms in COVID-19 [21], as well in other viral attacks [22].

Therefore, it was considered of interest to assess the anti-inflammatory effect of ABB C1® on intestinal cells, preservation of the gut barrier integrity, and activity in stimulating phagocytosis of peripheral cells. Confirmation of favourable effects of ABB C1® in these experimental studies may explain some of the mechanisms of action by which ABB C1® exerts its clinical benefits and would further support its use as a dietary supplement for improving the immune response to COVID-19 and other infectious diseases, as well as to prevent allergic processes.

2. Materials and Methods

2.1. Investigational product

The investigation product (ABB C1®, AB Biotek Human Nutrition & Health, Peterborough, UK) was composed of a synergistic combination of yeast-based ingredients with a unique β-1,3/1,6-glucan complex and a consortium of heat-treated postbiotic *S. cerevisiae* rich in selenium and zinc.

2.2. Anti-Inflammatory Effect of ABB C1® on Intestinal Cells/Epithelial Signalling Assay

The anti-inflammatory effect of ABB C1® on intestinal cells was studied by chemokine production by Caco-2 cells in the presence and absence of a pro-inflammatory stimulus. Caco-2 were cultured to confluence in 96-well plates in culture medium (modified Eagle's medium MEM]), supplemented with 20% (v/v) fetal bovine serum (FBS), 1% non-essential amino acids (NEAA), 1% Glutamax™, 1% sodium pyruvate, with or without 1% penicillin-streptomycin and gentamicin (50 µg/mL) (all obtained from Invitrogen, Breda, The Netherlands). At the start of the experiment, cells were washed once with antibiotic-free culture medium. The monolayers were incubated with test components in triplicate for 1 hour at 37°C in antibiotic-free medium. Then, cells were stimulated with and without a mixture of recombinant tumor necrosis factor alpha (TNF-α) (10 ng/mL) and recombinant

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interferon (IFN-ɣ) (5 ng/mL) in the presence of the test components and 50 µg/mL gentamicin. Supernatants were collected 24 hours after stimulation and stored at -20°C. A Bio-Plex Multiplex Immunoassay System (Bio-Rad, Hercules, CA, USA) was used to measure IFN-ɣ induced protein-10 (IP-10), and monocyte chemoattractant protein-1 (MCP-1) levels according to the manufacturer's instructions. IP-10 and MCP-1 levels were expressed as pg/mL. All experiments were performed in triplicate.

2.3. Gut Barrier Integrity Assay

Protection of epithelium disruption after a challenge: The effect of ABS C1® on gut barrier function upon a challenge was studied by transepithelial electric resistance (TEER) over a gut cell layer. Caco-2 cells were cultured in MEM medium in the same conditions than in the previous experiment. Then, the cells were seeded (2 x 104 cells/cm2) on Transwell polycarbonate cell culture inserts with a mean pore size of 0.4 µm and a diameter of 0.33 cm2 until full differentiation (± 1000 ohms [Ω]) (Greiner Bio-one, Alphen aan de Rijn, The Netherlands). As indicative measure for barrier integrity, TEER was measured with an EVOM2 Epithelial Volt/Ohm Meter (World Precision Instruments). On the day of the experiment, the cells were washed and incubated for 1 hour at 37°C with antibiotic- and serum-free medium containing the test components. Subsequently, the wells were exposed to *Escherichia coli* (ETEC H10407) infected at a multiplicity of infection (MOI) of 200 in the presence of the test components. TEER was measured before the start of the experiment (t = -1), 1 hour after exposure to the test components before addition of the pathogens (t = 0), and after 1 (t = 1), 2 (t = 2), 4 (t = 4), and 6 (t = 6) hours after exposure to the pathogens, as well as over 22 days. The TEER values of the individual conditions after exposure to the pathogens were compared to their own TEER value at t = 0 and expressed as ΔTEER (Ω/cm2). A negative control (ETEC H10407 only) was included. All experiments were performed in triplicate.

Spontaneous build-up of the epithelium: On the day of the experiment, the cells were washed and incubated for 1 hour at 37°C with antibiotic- and serum-free medium containing the test components. TEER was measured before the start of the experiment (t = -1) every 2 days during 22 days. The TEER values of the individual conditions were compared to their own TEER value at t = 0 and expressed as ΔTEER (Ω/cm2). A negative control without the study product was included. All experiments were performed in triplicate.

2.4. Stimulation of Phagocytosis of Peripheral Blood Monocytes and Leukocytes and Peritoneal Macrophages in Mice

In this experiment of phagocytosis of peripheral blood monocytes and leukocytes and peritoneal macrophages in mice, 10 BALB/c nude mice of both sexes and 8-weeks-old were included in each study group. The products (control yeast β-1,3/1,6-glucan, control algae β-1,3-glucan, and ABB C1®) were given for 10 days by forced feeding. Eight samples of peripheral blood per mice were extracted (0.1 mL) from the mice fed with various doses of the products or PBS (negative control). Samples were incubated in vitro with 0.05 mL of 2-hydroxyethyl methacrylate particles (HEMA) (5 x 108 mL). The tubes were incubated at 37°C for 60 min with intermittent shaking. Smears were stained with Wright's stain (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). The cells with three of more HEMA particles were considered positive. At least 300 cells were examined in each experiment. Results were standardized to reflect the β-glucan dosage received by the mice: 100% for the positive controls β-1-3/1,6 glucan from yeast and β-1,6-glucan from algae, and 68.89% for ABB C1®.

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Handling of mice and all experimental procedures were conducted under regular conditions in accordance with the European Convention for the Care and Use of Laboratory Animals as approved by the Czech Animal Care and Use Committee. Last approval June 2021.

2.5. Statistical Analysis

Quantitative data are expressed as mean and standard deviation (± SD). The Student's *t* test (two-sided) or the one-way analysis of variance (ANOVA) with Dunnett's procedure was used for the comparison of data according to conditions of application. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Anti-inflammatory effect on intestinal cells

The anti-inflammatory effect evaluated in the presence and absence of a pro-inflammatory challenge (tumour necrosis factor alpha [TNF-α]/interferon gamma [IFN-ɣ] showed statistically significant reductions of IP-10 and MCP-1 levels (Figure 1 and Table 1).

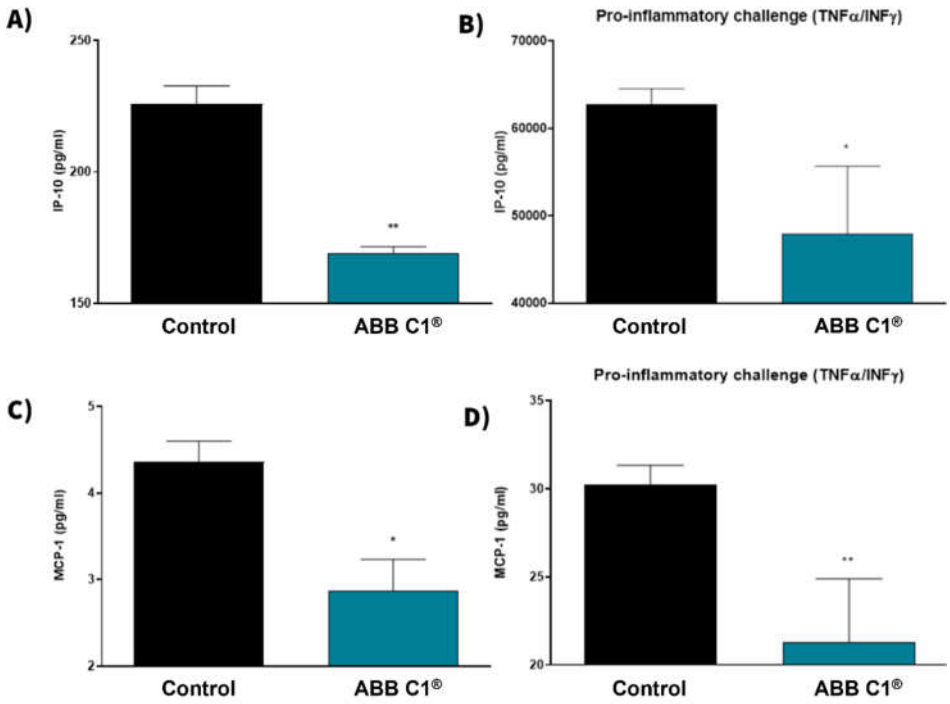

Figure 1. Mean differences in IP-10 levels between negative control and ABB C1® 56.79 (95% confidence interval [CI] 20.74 to92.85), $^{**}p < 0.001$ (panel A) and between negative control and ABB C1® after TNF-α/IFN-ɣ challenge 14880 (95% CI 1599 to 28170), $^{*}p = 0.0004$ (panel B), and in MCP-1 levels between negative control and ABB C1® 1.49 (95% CI 0.17 to 2.81), $^{*}p = 0.014$ (panel C) and between negative control and ABB C1® after TNF-α/IFN-ɣ challenge 3.72 (95% CI 2.17 to 15.73, $^{**}p < 0.0001$) (panel D).

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Table 1. Anti-inflammatory effect of ABB C1® on intestinal cells/epithelial signalling assay.

Experimental conditions	Mean ± SD
IP-10 levels, pg/mL	
ABB C1®	169.08 ± 4.33
Control	225.87 ± 15.39
ABB C1® with TNF-α/IFN-ɣ challenge	42369.44 ± 13446.14
Control with TNF-α/IFN-ɣ challenge	62363.65 ± 4611.93
MCP-1 levels, pg/mL	
ABB C1®	2.87 (0.63)
Control	4.36 (0.53)
ABB C1® with TNF-α/IFN-ɣ challenge	19.50 (7.62)
Control with TNF-α/IFN-ɣ challenge	30.62 (2.80)

SD: standard deviation, IP-10: IFN-ɣ-induced protein 10, MCP-1: monocyte chemoattractant protein 1.

3.2. Gut Barrier Integrity Assay

The capacity of ABB C1® to protect the gut epithelium disruption caused an infectious agent *Escherichia coli* (ETEC H10407) was evaluated. Transepithelial electrical resistance (TEER) values were higher for ABB C1® after 1 and 6 hours of testing, with $p < 0.05$ and $p < 0.1$ respectively (Figure 2, top panel). The total area under the curve (AUC) showed a statistical trend towards a significant increase in the ABB C1® condition ($p < 0.1$). In addition, spontaneous build-up of the gut epithelium monolayer over 22 days was also greater in the ABB C1® condition as compared with a negative control (Figure 2, bottom panel). Numerical values are shown in Tables 2 and 3.

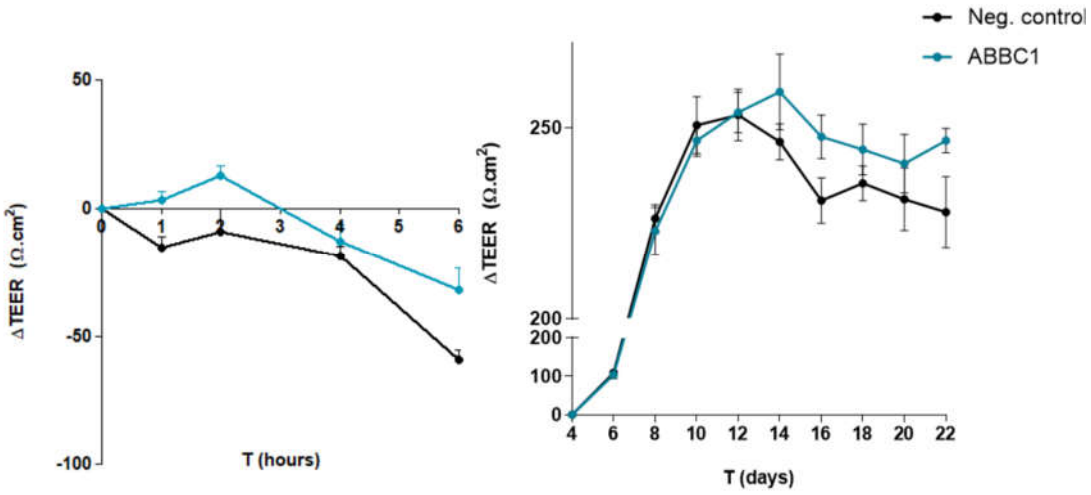

Figure 2. Gut barrier integrity assay in the presence of an infectious agent causing disruption of the gut epithelium;. 1 hour difference in the reduction of ΔTEER was statistically significant in favour of ABB C1® ($p < 0.05$). A trend towards statistical significance in favour of ABB C1 was found at 6 hours difference in the reduction of ΔTEER and in the total negative AUC ($p < 0.1$) (left panel). The comparison of ΔTEER values over the course of 22 days indicates a higher spontaneous build-up of the epithelium monolayer for the ABB C1® condition versus a negative control, even if it did not reach statistical significance (right panel).

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Table 2. Results of gut barrier integrity over the course of 6 hours.

ΔTEER Ω/cm2	**Experimental condition**		
	ABB C1®	Control	T-test
	Mean (SD)	Mean (SD)	*p*-value
t = 0	0	0	-
t = 1	3.33 (5.77)	-15.33 (7.51)	**0.030**
t = 2	12.67 (6.66)	-9.33 (16.01)	0.126
t = 4	-13.0 (11.14)	-19.00 (7.21)	0.484
t = 6	-32.33 (15.57)	-59.33 (6.81)	**0.078**
AUC			
Area of negative peaks	198.53 (57.38)	97.62 (53.56)	**0.090**

AUC: Area Under the Curve; SD: Standard deviation; TEER: transepithelial electrical resistance, t: time (hours).

Table 3. Spontaneous build-up of gut epithelium monolayer during 22 days.

ΔTEER (Ω/cm2)	**Experimental condition**		
	ABB C1®	Negative control	T-test
	Mean (SD)	Mean (SD)	*p*-value
t = 4	0	0	-
t = 6	104.33 (17,62)	109.0 (17.69)	0.940
t = 8	223.0 (10,82)	226.33 (8.82)	0.497
t = 10	246.67 (7,10)	250.67 (18.11)	0.464
t = 12	254.0 (9,17)	253.33 (16.51)	0.780
t = 14	259.33 (17,10)	246.33 (11.43)	0.455
t = 16	247.67 (9,82)	231.0 (14.45)	0.169
t = 18	244.33 (11,50)	235.50 (11.11)	0.315
t = 20	240.67 (13,20)	231.33 (19.90)	0.530
t = 22	246.67 (5,51)	228.0 (22.70)	0.141
AUC			
Area of positive peaks	1913.00 (62.48)	1943.67 (48.00)	0.451

AUC: Area Under the Curve; SD: standard deviation, TEER: transepithelial electrical resistance. t: time (days).

3.3. Stimulation of Phagocytosis of Peripheral Cells

In relation to stimulation of phagocytosis of peripheral blood monocytes and leukocytes and peritoneal macrophages cells in vivo, all samples showed activity, although the highest activity was observed in the ABB C1® condition as compared with controls of algae β-1,3-glucan and yeast β-1,3/1,6 glucan (Figure 3 and Table 4).

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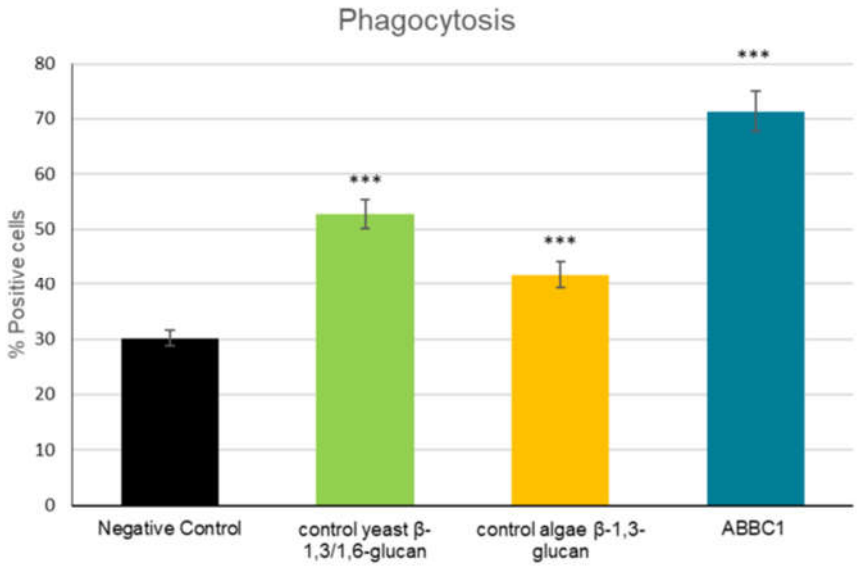

Figure 3. Percentage of positive (phagocytosing) cells in the four experimental conditions. ABB C1® was significantly different from the negative control and from the eyast and algae β-glucan controls (*** $p < 0.001$).

Table 4. Stimulation of phagocytosis of peripheral cells in mice.

Experimental condition	Percentage of positive cells, mean ± SD	T-test *p*-value vs. control
ABB C1®	71.37 (3.68)	$p < 0.001$
Control yeast β-1,3/1,6 glucan	52.75 (2.61)	$p < 0.001$
Control algae β-1,3-glucan	41.63 (2.44)	$p < 0.001$
Negative control	30.13 (1.40)	-

SD: standard deviation.

4. Discussion

In the two *in vitro* studies and in the experimental study in mice, the product based on a synergistic combination of β-glucans and selenium- and zinc-enriched *S. cerevisiae* (ABB C1®) showed significantly more favourable effects as compared with the control conditions regarding an anti-inflammatory effect, a protection of the gut barrier disruption, and as a stimulation of phagocytosis in peripheral blood monocytes and leukocytes and peritoneal macrophages.

The anti-inflammatory effect was shown by significantly lower levels of IP-10 and MCP-1 as compared to controls in both testing conditions, with and without TNF-α/INF-ɣ challenge mimicking inflammatory conditions. Elevated IP-10 and MCP-1 levels in COVID-19 patients and their possible usefulness as biomarkers of disease severity have been reported in different studies [23-29]. In a study in the third wave of the pandemic of immunological and laboratory signatures in 139 consecutive SARS-CoV-2 positive patients hospitalized in Northern Italy, serum concentration of IP-10 at baseline (> 4271 og/mL) predicted a worsening in clinical condition and could be useful in driving clinical decisions tailored to expected clinical course of COVID-19 disease [24]. High levels of MCP-1 have been reported to be useful to identify poor outcomes in COVID-19 patients on hospital admission [25]. Thus, the ability of ABB C1® to counteract these pro-inflammatory cytokines seems to be clinically relevant in the recovery and prognosis of COVID-19 and other infections.

Gut microbiome dysbiosis and gut barrier dysfunction in COVID-19 patients represent a source of bacteremia, which may contribute to worsening COVID-19

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outcomes [30]. The COVID-19 patients presenting poor outcomes are also those in which the immune system's hyperresponsiveness and a severe inflammatory condition (cytokine storm) are particularly evident, and have been associated with impaired microbiota phenotype [331.] Alteration of gut microbiota during COVID-19 increases the risk for microbial translocation and reflects disease severity and dysfunctional immune response [32]. In a study of plasma samples of 60 individuals tested positive for SARS-CoV-2, severe COVID-19 was associated with high levels of markers of tight junction permeability and translocation of bacterial and fungal products into the blood, with microbial translocation linked to markers of systemic inflammation [33]. Results of our *in vitro* study of the protective effect of ABB C1® against disruption of the gut barrier and enhancement of spontaneous build-up of the gut epithelium monolayer support a plausible beneficial effect of dietary supplementation with ABB C1® in COVID-19 and other infectious diseases. Based on the combined anti-inflammatory effect and preservation of gut barrier function, nutritional supplementation with ABB C1® may play a role contributing to improve symptoms and reduce severity of infections.

In the experimental study of stimulation of phagocytosis of peripheral cells in mice, two positive controls previously tested in the laboratory were included in the experiment: a β-1,3/1,6 glucan from yeast and a β-1,3-glucan from algae. The algae glucan had the lower effect, which is consistent with its chemical structure, comprising a linear carbohydrate chain with β-1,3 bonds [34]. Phagocytosis is known to play a crucial role in initiating the innate immune response against infection. Although phagocytic functions are performed by binding of pathogen-associated molecular patterns (PAMP) with their cell surface receptors on the phagocytes, leading to signal transduction and the release of inflammatory mediators, β-glucan-induced phagocytosis is mediated by various phagocytic receptors, mainly dectin-1 and complement receptors (CR3), and contributes to initiation of immune responses [35,36].

5. Conclusions

Taken together, the findings of these three experimental studies show that ABB C1®, which is a synergistic combination of yeast-based ingredients with a unique 1,3/1,6-glucan complex and a consortium of heat-treated probiotic *S. cerevisiae* rich in selenium and zinc, exhibited anti-inflammatory properties, protection of the gut barrier, and stimulated phagocytosis. Given the safety and tolerability of the product shown in a previous randomized controlled trial in healthy volunteers after getting vaccinated against the COVID-19 [9] and considering the present findings, the use of dietary supplementation with ABB C1® in patients with COVID-19 and other infections may be useful to ameliorate symptoms and improve prognosis in clinical practice.

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Conflicts of Interest: M.T., J.C. and C.d.L. are full time employees of AB Biotek Human Nutrition & Health.

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