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Direct comparison of immunological effects of various nutritional supplements

Vaclav Vetvicka

(Department of Pathology, University of Louisville, Louisville, KY, USA)

Abstract **Objective:** Immunonutrition represents a diet based on the knowledge of basic principles of the immune system and its functions. Despite numerous claims, the direct comparison of the immunostimulating effects of natural modulators is limited, making any conclusions difficult. Our study focused on most common vitamins and immunonutrients and directly compared their effects on various branches of the immune system. **Methods:** In this study we used a mouse model to evaluate various aspects of immune reactions, namely phagocytic activity, IL-2 secretion, NK cell activity, antibody formation and growth of breast cancer in order to find out the possible effects of six different immunonutrients and their combinations. **Results:** We found that glucan was in all tests the most active immunomodulator. Synergistic effects were observed only in glucan-selenium and glucan-vitamin C combinations. The rest of immunonutrients had only small activity or no activity at all. **Conclusion:** Based on our results, we concluded that most of vitamins and minerals have only limited, if any, effects on immune activities including cancer. The current study managed to confirm synergistic effects of the beta glucan-vitamin C and beta-glucan-selenium combinations. More studies on possible positive or negative effects of such combination and on mechanisms of action are important.

Keywords vitamin C; vitamin D; vitamin B6; glucan; selenium; zinc

The role of immunonutrition has been recognized way before this term itself appeared. Looking into history, Galen of Pergamum, the physician of Marcus Aurelius, recommended the treatment of various health problems by the specific diet[1]. Proper nutritional combination offers simple, effective, and cheap way to decrease possible problems associated with numerous diseases. In general, immunonutrition is best described as the effects of nutrients on the functions of the immune system and represents an important part of nutrition and overall health status. It represents a diet based on the knowledge of principles of the immune system and its individual functions and mechanisms. Supplementation of our nutrition means an addition of essential nutrients to the food that might contains them naturally, but in suboptimal amounts. To supplement the food means to add the micronutrients such as vitamins or immunostimulators. Fortification of food with soluble fibers can serve as one of the most common examples.

Some of the most common nutraceuticals (minerals, vitamins, supplements) used in general nutrition are various antioxidants used for protection of fetal brain and cardiovascular diseases[2-3]. However, despite extensive research, most if not all clinical trials evaluating exogenous antioxidants failed to find positive results. Our study focused on most common vitamins and immunonutrients and directly compared their effects on various branches of the immune system.

Vitamin D is probably the most important vitamin out of the whole spectrum of vitamins potentially involved in regulation of immune reactions. Dynamics of serum levels of vitamin D is in humans influenced by season,

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Corresponding author: Vaclav Vetvicka. Email: vaclav.vetvicka@gmail.com

location, quality as well as quantity of sunshine, age, health, and social status of an individual[4]. Nutritional effects on vitamin D levels in various countries varies from 5% to 35% of normal values. In European countries were the normal levels found in 10–80% of population. Vitamin D insufficiency is not only involved in induction of numerous autoimmune diseases, chronic disease, inflammatory problems of the gastrointestinal tract, asthma bronchiale, diabetes and multiple sclerosis, but also plays a role in increased mortality[5]. This relation of low levels and development of various diseases results in a push for direct supplementation not only in children or general population, but most of all in senior population, with described strong improvement of general health and measurable reduction of the risk of infectious diseases. Supplementation with vitamin D is therefore recommended in population with higher risk of COVID-19 infection[6].

Vitamin C is one of essential micronutrients for humans with numerous and even pleiotropic functions connected with is ability to donate electrons. Major role of vitamin C in immunity is its antioxidant activity and subsequently defense of respiratory apparatus against oxidative stress caused by pathogens. Vitamin C can stimulate phagocytosis, T cell proliferation, interferon induction and lower viral replication[7]. Ability of vitamin C to improve immune response against COVID-19 infection was repeatedly observed[7-8]. Low levels of vitamin C result in lower resistance to infections, supplementation on the other hand results in stimulation of phagocytosis, activation of lymphocytes and changes in neutrophil chemotaxis. In addition, vitamin C is necessary for collagen biosynthesis and for sustaining integrity of epithelial tissue, which helps to reduce some diseases. Supplementation in a blanket manner in children from highly polluted areas of the Czech Republic resulted in strong reduction of respiratory tract diseases.

Vitamin B6 deficit is involved in insufficient maturation of lymphocytes and in depression of their numbers. In addition, it affects thymus and spleen atrophy[9]. Vitamin B6 also influences the numbers and function of lymphocytes. In addition, low levels of this vitamin can result in lower activity of NK cells and neutrophils.

Trace elements are essential cofactors important for several important proteins[7,10]. It is necessary to maintain their optimal concentration, as both low and to high levels might be dangerous or even toxic. At the same time, interaction between host and microorganism is dependent on the level of minerals, as trace elements module this interaction. In case of disbalance, physiological functions of the organism might be disrupted[4,11]. In addition to physiological functions, trace elements are also involved in regulation of immune reactions. They play role not only in the quality of various barriers, often playing the role of first defense, but also in the whole spectrum of immune reactions, both nonspecific and specific[7-8,12].

Zinc is an important mineral often labelled as “essential trace element” and its deficiency is known since 1960s. It functions as a molecule with catalytic, structural, and regulatory effects involved in both proteins and enzymes synthesis and function. Zinc supplementation needs to be carefully done, so it reduces potential insufficiency and at the same time rule off overdose, which might result in dysbiosis and predisposition to various infections due to the compromised salivary immune response[13]. Zinc supplementation was found to improve antibody response and induction of production of cytotoxic T cells, cytokines and Tregs. In addition, zinc is involved in signal transduction by T and B lymphocytes[14]. Additional roles are reduction of oxidative stress and regeneration of intracellular killing with induction of macrophage-derived cytokines[15]. Zinc homeostasis-related effects on the activation of key signaling molecules together with epigenetic modification clearly summarizes the important role of zinc. It is not surprising that it is considered to be a gatekeeper of immune functions[16].

Selenium is another potent micronutrient playing role in various facets of health including immune response. Low levels of selenium result in reduction of NK cell activities and higher risk of microbial infections. In addition, mutation of viral particles with increased virulence has been described[6]. Long-term deficit results in increase oxidative stress and suppressed numbers of monocytes. In addition, selenium was found to have significant suppressive effects on development of breast cancer, probably acting via epigenetic mechanisms. Selenium was found to increase phagocytic activity[17] and stimulate function of NK cells[18]. Recent studies found that addition of selenium improved immunodulation caused by beta glucan. Some studies suggested increased risk of COVID-19 infection. Numerous papers suggested significant role of selenium in cancer reduction[19].

Beta glucans are natural polysaccharides isolated from various sources including mushroom, yeast, grain and seaweed. Optimal nutritional composition might offer some intake of beta glucan, but current nutritional trend of using industrial type of nutrition constantly lowers the amount of beta glucan in our food. Extensive research of beta glucans and their actions undertaken in last several decades helped to elucidate numerous mechanisms of action and their involvement in improvement of our health, including microbiome, probiotic, and prebiotic effects and most of all, immune system[20-22]. Our studies suggested the use of orally-given beta glucan both in prevention and in therapeutical interventions in various types of diseases. In these studies, the individual fractions have activity similar to low molecular heparin. In COVID-19 patients is a high risk od development of diseminal intravascular coagulation. Thrombocythemia and elevated levels of D-dimer induce higher activation of platelets and higher coagulation. Beta glucan effects might be elevated by addition of adequate dose of vitamin C and D. These studies suggest that beta glucan is one of the most prominent immunomodulators.

As there are numerous studies suggesting that beta glucan act in synergy with some additional nutrients such as vitamin C, resveratrol[23], vitamin D[24] or selenium[25], we decided to test the samples both individually and together with beta glucan.

We present the following article in accordance with the ARRIVE reporting checklist (available at https://dx.doi.org/10.3978/j.issn.2095-6959.2021.12.001).

1 Materials and Methods

1.1 Animals

Female, 8-week-old BALB/c mice (average weight app. 27 g) were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal work was done according to the University of Louisville IACUC (Institutional Animal Care and Use Committee) protocol. Mice were kept in cages with 24/7 access to food and water under constant supervision of animal facility crew and veterinarian. Animals were sacrificed by cervical dislocation after CO_2 asphyxiation. For subsequent experiments, mice were randomly divided into groups. All experiments were repeated 3 times with a total of 15 mice/group.

1.2 Ethical statement

Experiments were performed under a project license #16477 granted by institutional board of IACUC (Institutional Animal Care and Use Committee), in compliance with national and institutional guidelines for the care and use of animals. A protocol was prepared before the study without registration.

1.3 Material

RPMI 1640 medium, sodium citrate, Wright stain, Limulus lysate test E-TOXATE, Concanavalin A, HEPES, penicillin and streptomycin were obtained from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) was from Hyclone Laboratories (Logan, UT). For our study, we used yeast-derived insoluble beta glucan (Biorigin, Brazil), vitamin B6 (Life extension, Ft. Lauterdale, USA), zinc (Puritan Pride, USA), vitamin C (Nature Made, USA), sodium selenite (Spectrum, Gardena, USA), and vitamin D (BioTech, USA). Daily doses were 100 mg of beta glucan, 10 mg of selenium, 25 IU of vitamin D, 0.4 mg of vitamin B6, 5 mg of zinc, and 5 mg of vitamin C.

1.4 Cell lines

The BALB/c mouse-derived mammary tumor cell line Ptas64 was generously provided by Dr. Wei-Zen Wei of the Michigan Cancer Foundation, Wayne State University, Detroit, MI. YAC cells were obtained from the ATCC (Manassas, VA). The cells were cultivated in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) medium containing HEPES (Sigma) and 10% heat-inactivated FCS (Hyclone Lab., Logan, UT), in disposable tissue culture flasks at 37 ℃ in a 5% CO_2/95% air incubator.

1.5 NK cell cytotoxicity assay

Spleen cells were isolated from spleen of mice from individual groups. The NK cell assay using YAC-1 cells as a target was described in Ref. [23]. Briefly, splenocytes (10^6/mL; 0.1 mL/well) in V-shaped 96-well plastic microplates were incubated with 50 μL of target cell line YAC-1 and further incubated for 4 hours at 37 ℃. The cytotoxic activity of cells was determined by CytoTox 96 Non-Radioactive Cytotoxicity Assay from Promega (Promega, Madison, WI, USA) according to the manufacturer's instructions.

The optical density was evaluated by STL ELISA reader (Tecan U.S., Research Triangle Park, NC) at 492 nm. Specific cell-mediated cytotoxicity was calculated using the formula: Percent-specific killing (% cytotoxicity) = 100 × [(OD_{492} experimental – OD_{492} spontaneous) divided (OD_{492} maximum – OD_{492} spontaneous)].

1.6 Tumor inhibition in vivo

One million of Ptas64 cells suspended in PBS were injected into mammary fat pads. Fourteen days after injection, mice were checked for development of palpable tumors and randomly divided into experimental groups. For these experiments, the individual samples were diluted in PBS and injected (i.p.) daily for 2 weeks. At the end of treatment, the mice were sacrificed, tumors removed and weighed[26].

1.7 Phagocytosis

Test of phagocytic activity using synthetic HEMA-based polymeric microspheres was described earlier[27]. Briefly: 0.1 mL of peripheral blood from mice previously injected with tested samples or PBS was incubated *in vitro* with 0.05 mL of microspheres diluted to a concentration of 5×10^8/mL. After incubation at 37 ℃ for 60 min with intermittent shaking, smears were stained with Wright stain (Sigma) and evaluated. The cells with three or more HEMA particles were considered positive. All experiments were repeated three times with 5 mice/group.

1.8 IL-2 secretion

Purified spleen cells (2×10^6/mL in RPMI 1640 medium with 5% FCS) obtained from mice previously injected with 100 mg of tested samples or PBS were added into individual wells of a 24-well plastic disposable tissue culture plate. Cells were incubated for 48 hours in a humidified incubator (37 ℃, 5% CO_2/95% air). As a positive control, we used 1 μg of Concanavalin A (Sigma). At the end of incubation, supernatants were collected, filtered through 0.45 μm filters and subsequently the level of IL-2 was evaluated a Quantikine mouse IL-2 kit (R&D Systems, Minneapolis, MN) using STL ELISA reader.

1.9 Antibody formation

The technique was described earlier[28]. Briefly: formation of antibodies was evaluated using ovalbumin (Sigma) as an antigen. Mice were injected twice with 100 μg of albumin and the serum was collected 7 days after last injection. Experimental groups were getting daily ip. injection of tested materials. Level of specific antibodies against ovalbumin was detected by ELISA using STL ELISA reader. As positive control we used a combination of ovalbumin and Freund's adjuvant (Sigma).

1.10 Statistical analysis

Statistical significance was evaluated by a pair *t*-test using a GraphPad Prism 502 software (GraphPad Software, USA).

2 Results

First, we evaluated the effects of tested materials on phagocytosis. Phagocytosis is usually the first reaction to test in case of natural immunomodulators. We used a well-established test employing synthetic microbeads based on 2-hydroxyethylmethacrylate polymers, which have very low spontaneous adhesion to cell membrane, eliminating false positivity. Our data are summarized in Table 1 and showed that only beta glucan, vitamin D and selenium stimulated phagocytic activity. When combined, vitamin C and selenium increased the already significant effects of glucan.

Next, we focused on the role of the tested supplements on NK cell activity. For these experiments, we used human YAC-1 cells and incubated them with spleen cells isolated from mice injected with tested samples. Results shown were obtained using the 50:1 effector-target ratio, similar results were obtained when using additional experimental ratio, i.e., 10:1 and 100:1 ratio, respectively (data not shown). Data summarized in Figure 1 showed that only vitamin C and beta glucan alone significantly improved NK cell activity (Figure 1). Combinations with glucan showed synergistic effects for glucan and vitamin C and selenium.

In next part of the experiments, we decided to evaluate

Table 1 Effects on phagocytic activity

Sample	Macrophages	Monocytes	Neutrophils
PBS	18.8±1.1	28.4±1.8	31.9±2.6
Glucan	39.9±2.2	41.2±2.8	44.3±3.6
Vitamin B6	17.3±1.6	29.5±2.1	31.0±3.4
Glucan + vitamin B6	41.4±2.9	43.1±4.4	46.2±2.4
Vitamin C	18.0±1.0	29.9±2.3	32.0±3.1
Glucan + vitamin C	45.1±2.5*	44.1±4.1	50.1±3.4*
Vitamin D	30.9±3.1	27.2±1.9	30.9±2.9
Glucan + vitamin D	43.9±2.4	42.4±4.1	43.9±4.1
Se	30.3±1.2	27.5±2.2	30.4±2.7
Glucan + Se	48.2±2.8*	49.9±3.8*	48.2±1.1*
Zn	18.7±1.7	30.8±3.7	29.5±2.7
Glucan + Zn	28.26±2.1	42.3±3.5	44.1±4.0

Stimulation of phagocytic activity between glucan alone and glucan plus vitamins/minerals was significant at $P<0.01$ level.

the possible changes in humoral immunity. First, we tested the production of IL-2 after 3 days *in vitro* incubation of splenocytes isolated from animals supplemented with tested samples. Since the secretion of control (i.e., mice supplemented with PBS only) was zero, all tested materials (with exception of selenium) show some activity. However, with exception of beta glucan, this activity was low, and the stimulation was in the 10–20 ng/mL range. In case of combinations, only glucan-vitamin C and glucan-selenium combinations showed significantly higher activity than glucan alone (Figure 2).

The evaluation of humoral immunity was followed

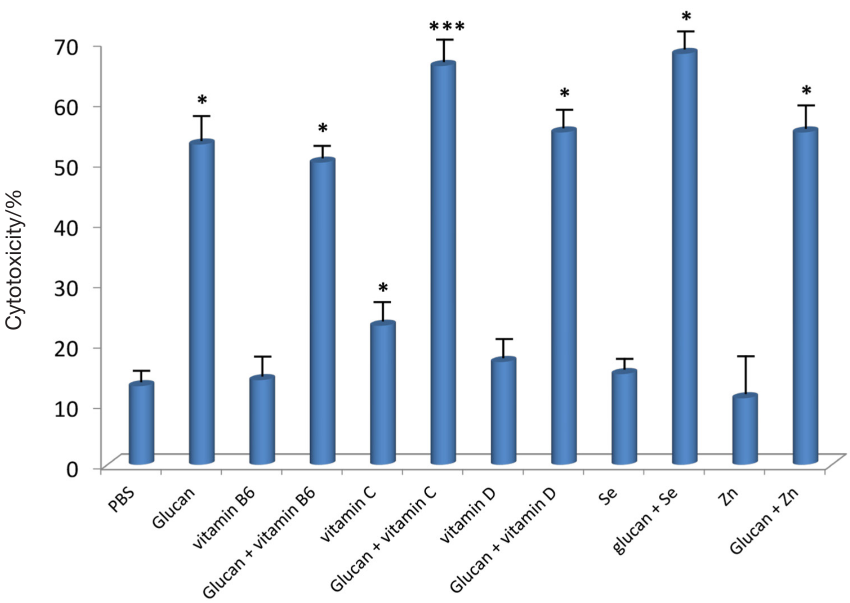

Figure 1 Effects of tested materials on natural killer cell activity. For each experiment, 4 wells per sample were evaluated, each experiment was repeated at least three times. Control mice were injected with PBS only. *, significant differences between the control (ovalbumin alone) and samples at $P \leq 0.05$. **, significant differences between glucan alone and glucan combined with individual samples at $P \leq 0.05$ level.

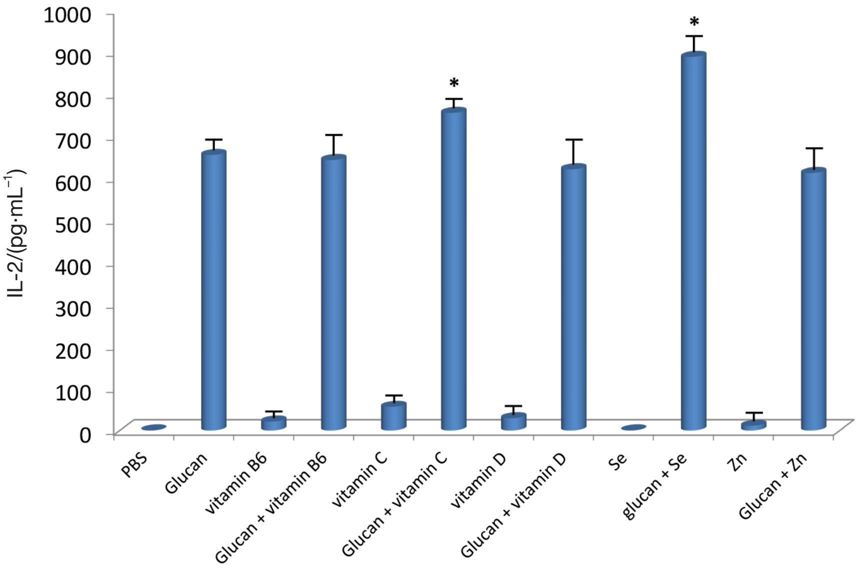

Figure 2 Effects of tested material on secretion of IL-2 by spleen cells. As the control (PBS) production of IL-2 is zero, all columns represent significant differences between control (PBS) and samples at $P \leq 0.05$ level. All experiments were performed in triplicates.

by testing the potential effects on antibody formation. Immunization with ovalbumin was used as an experimental model. Mice were injected twice with 0.1 mg of ovalbumin and serum was collected seven days after the second dose. Our results showed that only beta glucan stimulated antibody production. Again, only glucan-vitamin C and glucan-selenium combination showed some synergy (Figure 3).

In addition, we focused on the possible role of tested supplements in inhibition of cancer. Using a model of breast cancer, we showed that beta glucan and zinc suppressed the cancer growth, but the effects of beta glucan were much stronger. When we tested the combinations, no synergistic effects were found (Figure 4).

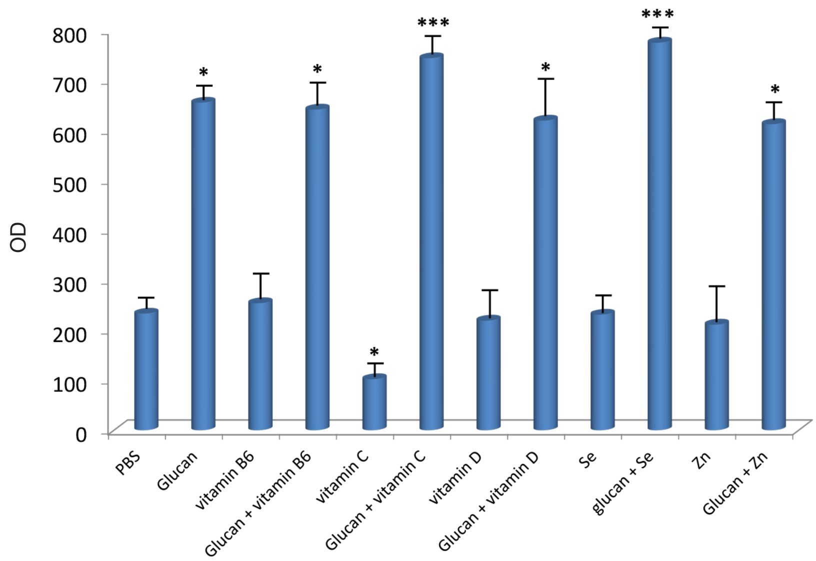

Figure 3 Effects of two *ip*. injections of tested samples on formation of antibodies against ovalbumin. Mice were injected twice (two weeks apart) and the serum was collected 7 days after the last injection. The level of specific antibodies against ovalbumin was detected by ELISA. As positive control, Freund's adjuvant was used. *significant differences between the control (ovalbumin alone) and samples at $P \leq 0.05$. **significant differences between glucan alone and glucan combined with individual samples at $P \leq 0.05$ level. Individual substances were used at 100 μg/dose. All experiments were performed in triplicate.

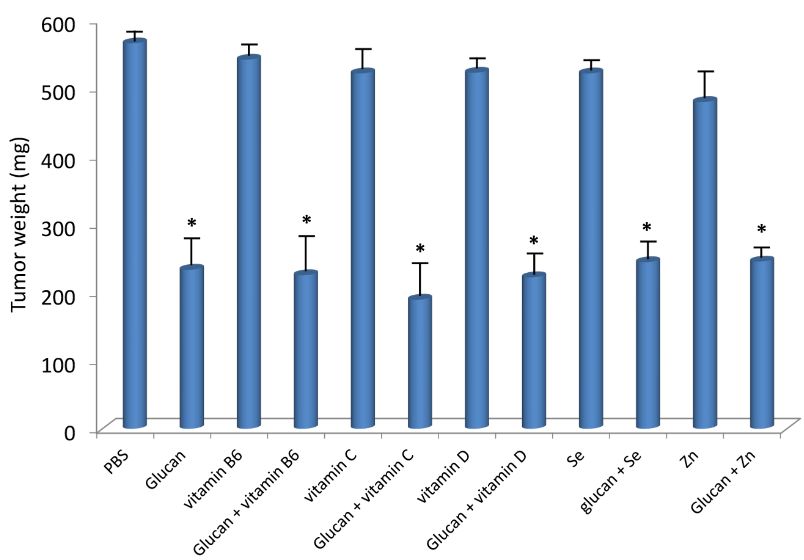

Figure 4 Effects of glucan supplementation on the weight of primary breast cancer tumors. Results represent mean from three experiments ± SD. *significant differences between the control (ovalbumin alone) and samples at $P \leq 0.05$. Individual substances were used at 100 μg/dose. All experiments were performed in triplicate.

3 Discussion

Effective and safe natural immunomodulators remains to be the holy grail of complementary or integrative medicine. Numerous nutraceuticals have been intensively studied for several decades. Some with consistent results (e.g., beta glucan), some with rather conflicting data probably based on differences in biological material and isolation techniques (e.g., *Echinacea*), as no consensus on preparation of extracts exist[29]. Several natural immunomodulators already reached clinical studies. However, the search for nutrients or their combinations with higher biological activities continue.

Current study focused on six common immunomodulators, vitamins and minerals, all with claims of supporting immune system. With limited number of studies directly comparing immunological effects of various immunomodulators or botanicals[30-31], we decided to evaluate their effects on both cellular and humoral immunity.

Most immunomodulators act via stimulation of cellular branch of immunity, therefore we started with phagocytosis. The data confirmed the previously published synergy with vitamin C. In case of selenium, the combination of beta glucan with organic selenium derivatives was already been found to be beneficial in several murine tumor models[25]. Similarly to older studies, only beta glucan alone and in combination with vitamin C or selenium significantly stimulated phagocytic activity of several blood types.

When we focused on NK cell activity, we expected higher activities, as numerous modulators such as curcumin, resveratrol or ginseng were found to stimulate NK cells[32], but only beta glucan and vitamin C showed significant effects. When combined, beta glucan-vitamin C and beta glucan-selenium combinations showed higher activity that individual components.

Selenium has been associated with several disease such as Kashin-beck disease, cardiovascular problems or cancer[19]. The effects on cancer research are ascribed to their direct and/or indirect antioxidant properties[33]. Despite numerous clinical trials and various variations of selenium being tested, none of selenium compounds have been clinically recognized, making search for a better composition with improved biological effects even more important. Selenium had significant synergy with beta glucan. Previous studies showed significant suppressive effects on breast cancer development, probably manifested via epigenetic mechanisms[34]. Our previous study found not only the direct effects on two different types of experimental cancer, but also a synergy with beta glucan[35]. The results were, however, dependent on type of selenium with sodium selenite being superior.

Vitamin D deficiency has been implicated in pathophysiology of various diseases including rheumatoid arthritis and Crohn disease. In addition, vitamin D supplementation is beneficial in prevention of diabetes mellitus[36]. Other effects of vitamin D are improved intake of glucose in cells and regulation of insulin resistance[37]. Despite findings of beneficial synergy between beta glucan and vitamin D in patients with diabetic retinopathy[24], our current study found no stimulation of immunological reaction by this vitamin. The effects on diabetic retinopathy are most probably cause by some mechanisms not related to immune system.

Zinc deficiency was originally supposed to be rare, but lately it was found to be quite common with approximately 2 billion people being affected worldwide[16]. Among diseases partially connected to zinc deficiency are diabetes, arthritism. macular degeneration, problems with immunity and chronic inflammation[38]. In our study, however, no effects of zinc alone or any synergistic effects when combined with beta glucan have been found.

To most extend, the results of this current study confirmed older findings comparing effects of various bioactive combinations and showing that only beta glucan-based combinations have significant immunological activities[39]. Several conclusions can be drawn—most of vitamins and minerals have only limited, if any, effects on immune activities including cancer. It might be possible that higher doses of minerals or vitamins could show increased activities, but no study comparing the biological dose-dependency of these compounds exists. In view of the already established synergy between beta glucan and vitamin C[40] and glucan and resveratrol[39], the use of combined extracts might be beneficial. The current study managed to confirm synergistic effects of the beta glucan-vitamin C and beta-glucan-selenium combinations. More studies on possible positive or negative effects of such combination are important.

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Footnote

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References

1. Ackerknecht EH. Therapeutics from the primitives to the 20th century (with an appendix: history of dietetics)[M]. London: MacMillan Publishers, 1973.
2. Murcia M, Espada M, Julvez J, et al. Iodine intake from supplements and diet during pregnancy and child cognitive and motor development: the INMA Mother and Child Cohort Study[J]. J Epidemiol Community Health, 2018, 72(3): 216-222.
3. Speeckaert MM, Speeckaert R, Wierckx K, et al. Value and pitfalls in iodine fortification and supplementation in the 21st Century[J]. Br J Nutr, 2011, 106(7): 964-973.
4. Alexander J, Tinkov A, Strand TA, et al. Early nutritional interventions with zinc, selenium and vitamin D for raising anti-viral resistance against progressive COVID-19[J]. Nutrients, 2020, 12(8): 2358.
5. Williams J, Williams C. Responsibility for vitamin D supplementation of elderly care home residents in England: falling through the gap between medicine and food[J]. BMJ Nutr Prev Health, 2020, 3(2): 256-262.
6. Calder PC. Nutrition, immunity and COVID-19[J]. BMJ Nutr Prev Health, 2020, 3(1): 74-92.
7. Garvin MR, Alvarez C, Miller JI, et al. A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm[J]. Elife, 2020, 9: 59177.
8. Gombart AF, Pierre A, Maggini S, et al. A Review of Micronutrients and the Immune System-Working in Harmony to Reduce the Risk of Infection[J]. Nutrients, 2020, 12(1): 236.
9. Crespi B, Alcock J. Conflicts over calcium and the treatment of COVID-19[J]. Evol Med Public Health, 2021, 9(1): 149-156.
10. Horowitz RI, Freeman PR. Three novel prevention, diagnostic, and treatment options for COVID-19 urgently necessitating controlled randomized trials[J]. Med Hypotheses, 2020, 143: 109851.
11. Zittermann A, Pilz S, Hoffmann H, et al. Vitamin D and airway infections: a European perspective[J]. Eur J Med Res, 2016, 21: 14.
12. Lopez CA, Skaar EP. The impact of dietary transition metals on host-bacterial interactions[J]. Cell Host Microbe, 2018, 23(6): 737-748.
13. Jawhara S. How to boost the immune defence prior to respiratory virus infections with the special focus on coronavirus infections[J]. Gut Pathog, 2020, 12: 47.
14. Duchateau J, Delepesse G, Vrijens R, et al. Beneficial effects of oral zinc supplementation on the immune response of old people[J]. Am J Med, 1981, 70(5): 1001-1004.
15. Chowdhury MA, Hossain N, Kashem MA, et al. Immune response in COVID-19: A review[J]. J Infect Public Health, 2020, 13(11): 1619-1629.
16. Wessels I, Maywald M, Rink L, et al. Zinc as a gatekeeper of immune function[J]. Nutrients, 2017, 9(12): 1286.
17. Milad K, Racz O, Sipulova A, et al. Effect of vitamin E and selenium on blood glutathione peroxidase activity and some immunological parameters in sheep[J]. Vet Med, 2001, 46: 1-5.
18. Ravaglia G, Forti P, Maioli F, et al. Effect of micronutrient status on natural killer cell immune function in healthy free-living subjects aged ≥90 y[J]. Am J Clin Nutr, 2000, 71(2): 590-598.
19. Tan HW, Mo HY, Lau ATY, et al. Selenium species: current status and potentials in cancer prevention and therapy[J]. Int J Mol Sci, 2018, 20(1): 75.
20. Melvin JA, Bomberger JM. Compromised defenses: exploitation of epithelial responses during viral-bacterial co-infection of the respiratory tract[J]. PLoS Pathog, 2016, 12(9):

e1005797.
21. Vetvicka V, Vannucci L, Sima P, et al. Beta glucan: supplement or drug? From laboratory to clinical trials[J]. Molecules, 2019, 24(7): 1251.
22. Vetvicka V, Vannucci L. Glucan and its role in immunonutrition[M]// Nutrition and immunity. Switzerland: Springer, 2019: 453-460.
23. Vetvicka V, Volny T, Saraswat-Ohri S, et al. Glucan and resveratrol complex--possible synergistic effects on immune system[J]. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 2007, 151(1): 41-46.
24. Richter J, Závorková M, Vetvicka V, et al. Effects of β-glucan and vitamin D supplementation on inflammatory parameters in patients with diabetic retinopathy[J]. J Diet Suppl, 2019, 16(4): 369-378.
25. Vetvicka V, Pinatto-Botelho MF, Dos Santos AA, et al. Evaluation of a special combination of glucan with organic selenium derivative in different murine tumor models[J]. Anticancer Res, 2014, 34(12): 6939-6944.
26. Vetvicka V, Yvin JC. Effects of marine beta-1,3 glucan on immune reactions[J]. Int Immunopharmacol, 2004, 4(6): 721-730.
27. Větvicka V, Fornůsek L, Kopecek J, et al. Phagocytosis of human blood leukocytes: a simple micromethod[J]. Immunol Lett, 1982, 5(2): 97-100.
28. Vetvicka V, Vetvickova J. β1,3-glucan: silver bullet of hot air?[J]. Open Glycoscience, 2010, 3: 1-6.
29. Barrett B, Vohmann M, Calabrese C, et al. Echinacea for upper respiratory infection[J]. J Fam Pract, 1999, 48(8): 628-635.
30. Wilasrusmee C, Siddiqui J, Bruch D, et al. In vitro immunomodulatory effects of herbal products[J]. Am Surg, 2002, 68(10): 860-864.
31. Wagner H. Search for plant derived natural products with immunomodulatory activity (recent advances)[J]. Pure Appl Chem, 1990, 62: 1217-1222.
32. Vetvicka V, Vetvickova J. Combination of glucan, resveratrol and vitamin C demonstrates strong anti-tumor potential[J]. Anticancer Res, 2012, 32(1): 81-87.
33. Rahmanto AS, Davies MJ. Selenium-containing amino acids as direct and indirect antioxidants[J]. IUBMB Life, 2012, 64(11): 863-871.
34. de Miranda JX, Andrade Fde O, Conti Ad, et al. Effects of selenium compounds on proliferation and epigenetic marks of breast cancer cells[J]. J Trace Elem Med Biol, 2014, 28(4): 486-491.
35. Vetvicka V, Vetvickova J. Addition of selenium improves immunomodulative effects of glucan[J]. N Am J Med Sci, 2016, 8(2): 88-92.
36. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes[J]. Diabetes Care, 2004, 27(3): 813-823.
37. Bajaj S, Singh RP, Dwivedi NC, et al. Vitamin D levels and microvascular complications in type 2 diabetes[J]. Indian J Endocrinol Metab, 2014, 18(4): 537-541.
38. Giacconi R, Costarelli L, Piacenza F, et al. Main biomarkers associated with age-related plasma zinc decrease and copper/zinc ratio in healthy elderly from ZincAge study[J]. Eur J Nutr, 2017, 56(8): 2457-2466.
39. Vetvicka V, Vetvickova J. Comparison of immunological properties of various bioactive combinations[J]. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 2012, 156(3): 218-222.
40. Jesenak M, Majtan J, Rennerova Z, et al. Immunomodulatory effect of pleuran (β-glucan from Pleurotus ostreatus) in children with recurrent respiratory tract infections[J]. Int Immunopharmacol, 2013, 15(2): 395-399.

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