Reactivity of the immunological system of rats stimulated with Biolex-Beta HP after cyclophosphamide immunosuppression

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Abstract

The objective of this study was to determine the stimulating effect of the Biolex-Beta HP ($\beta$-1,3/1,6-D-glucan) dietary supplement on selected parameters of specific and non-specific humoral and cellular immunity in rats immunosuppressed with cyclophosphamide. The experimental material comprised 40 Wistar rats, divided into two equal groups: control and experimental. In the course of 3 successive days, the rats from the experimental group were administered cyclophosphamide intramuscularly at a rate of 50 mg/kg BW per day. On the 8th day of the experiment, 10 control and 10 experimental rats were sacrificed, and total protein and $\gamma$-globulin levels, lysozyme and ceruloplasmin activity were determined in the blood serum. The proliferative response of blood lymphocytes after stimulation with lipopolysaccharide or concanavalin A, respiratory burst activity and the potential killing activity of phagocytes were determined in whole heparinised blood. Starting on the 8th day of the experiment, the feed of the remaining rats from the experimental and control groups was supplemented for 14 consecutive days with Biolex-Beta HP at a rate of 50 mg/kg BW per day. On day 22, arterial blood samples were collected and immune parameters were determined. The results indicate that $\beta$-1,3/1,6-D-glucan has a positive effect on the analysed parameters of non-specific cellular and humoral immunity after cyclophosphamide-induced suppression. Nevertheless, the observed effect only marked a return to the norm, as most of the analysed parameters were merely restored to their initial levels, with the exception of lysozyme activity, which considerably exceeded the level noted before immunosuppression.

Key words: Biolex-Beta HP, immunosuppression, total protein levels, humoral and cellular immunity parameters.

Introduction

Immunosuppression, a dysfunction of the immune system, is triggered by a variety of biological (viruses, bacteria, fungi, parasites), chemical (water and feed contamination, antibiotics, heavy metals, disinfectants) and physical factors (stress, suboptimal temperature, excessive concentrations of dust and harmful gases). Prolonged exposure to those adverse factors can lead to the deterioration of health, lower body weight gains, lower effectiveness of preventive vaccination, higher susceptibility to cancer, infectious and parasitic diseases, induction of latent infections [1-5] and increased mortality that contributes to economic losses. To effectively counteract those adverse effects, scientists are searching for substances that augment the body’s immune response. Those substances are known as immunostimulants or immunostimulators. Immunostimulants can accelerate, enhance or prolong the immune response subject to the immune status of the host, the route of administration and the applied dose [6-10]. Recent years have witnessed the growing popularity of immunostimulants, in particular substances of natural origin [11-15]. This group of natural stimulants includes $\beta$-1,3/1,6-D-glucan (Biolex Beta-HP), which is analysed in the presented experiment. The branched $\beta$-1,3/1,6-glucan polysaccharide found in the Biolex-Beta HP product has the structure of a $\beta$-1,3 main chain connected to a $\beta$-1,6 side chain. This polysaccharide can be isolated from mannanproteins without the use of aggressive alkalis or acids in the hydrolysis process, and it occurs in an unmodified, natural form that guarantees the highest level of biological activity [16]. Due to their varied and complex structure, $\beta$-glucans can bind to various receptors ($\beta$-glucan receptors) located on the surface of effector cells (macrophages, monocytes, neutrophils, NK cells, dendritic cells, T cells...
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Material and methods

Animals. Animal experiments were carried out in conformance with the Animal Protection Law (Journal of Laws of 24 February 2005, no. 33, item 289) and the recommendations of the Animal Ethics Committee of the University of Warmia and Mazury in Olsztyn. During the experiment, animals were kept in Faculty premises and adequate experimental conditions were observed.

Experimental design. The experimental material comprised 40 adult Wistar rats aged 14 weeks, including 20 females with average body weight 200 g, and 20 males with average body weight 340 g. The animals were initially divided into two groups (control and experimental) of 10 males and 10 females each. The males and females from each group were kept in separate cages. All animals were fed Murigran pelleted feed for rodents (Akropol Motycz) and had ad libitum access to water. Over a period of 3 consecutive days (days 1-3), 20 experimental group rats were administered cyclophosphamide (N,N-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide CAS 50-18-0, SIGMA) intramuscularly at the daily rate of 50 mg/kg BW in the form of 75 g/l PBS solution (phosphate buffered saline with the addition of calcium chloride and magnesium chloride, Biomed). On the 8th day of the experiment, 10 control group rats (K) and 10 experimental group rats (C) were sacrificed by an overdose of Narcotan (Halothanum, CAS 151-67-7, ZENTIVA, lot 3081006). Samples of arterial blood were collected to determine and compare selected biochemical parameters and parameters of non-specific humoral immunity in rats (total protein levels, γ globulin levels, lysozyme activity, ceruloplasmin activity). Blood was diluted with heparin (Heparinum natricum, CAS 9005-49-6 Polfa Warsaw) to determine and compare selected parameters of non-specific cellular immunity in rats [proliferative response of blood lymphocytes (MTT) after stimulation with LPS or ConA, respiratory burst activity (RBA) and potential killing activity (PKA) of phagocytes]. Starting on the 8th day of the experiment, the feed of the remaining 10 rats from the experimental group (C+G) and 10 rats from the control group (G) was supplemented with β-1,3/1,6-D-glucan (Biolex-Beta HP, Leiber GmbH) at the daily rate of 50 mg/kg BW for 14 consecutive days (days 8-21). On day 22, all C+G and C group rats were sacrificed by an overdose of Narcotan, and samples of arterial blood were collected to determine and compare selected biochemical parameters and parameters of non-specific humoral immunity in rats. Blood was diluted with heparin to determine and compare selected parameters of non-specific cellular immunity in rats. A group treated with cyclophosphamide and tested after 22 days was not established because in our previous study [32] cyclophosphamide also exerted a suppressive effect on the analysed parameters between day 8 and day 22 of the experiment, in comparison with the control group (not treated with cyclophosphamide), and only minor variations in those parameters were noted after day 22.

Evaluation of non-specific humoral immunity parameters. Lysozyme activity in blood plasma was determined by the turbidimetric method [33] modified by Siwicki and Anderson [34], ceruloplasmin activity in blood plasma was evaluated by the method developed by Siwicki

and B cells). They include dectin-1 [17, 18], complement receptor 3 (CR3, CD11b/CD18, αMβ2-integrin, Mac-1) [19, 20], lactosylceramide ( LacCer) [21] and selected scavenger receptors (SRs) [22], including SR CD36 [23], TLR-2 (toll like receptor-2) and, probably, TLR-4 [24, 25]. The binding process activates effector cells (NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells) and increases the secretion of proinflammatory chemokines and cytokines: TNF-α (tumor necrosis factor α), interleukin-1β (IL-1β), IL-6, IL-8, IL-12, interferon (IFN) γ and IFN-β2 [26, 27]. According to Pelizon et al. [28], IL-12, which stimulates the cellular immune response, plays a vital role in this process by enhancing lymphocyte proliferation, differentiating the subpopulations of Th1 lymphocytes from Th0 lymphocytes, and activating NK cells [29] that produce IFN-γ. Interferon γ increases the phagocytic activity of macrophages (activated macrophages) and it strongly differentiates Th1 cells.

In the experiment, immunosuppression was not induced by an infection, which produces unpredictable results, but by a chemical substance that minimizes the relevant risk. The researchers opted for cyclophosphamide due to its ability to deliver reliable and repeatable results within a short period of time. Cyclophosphamide is a cytotoxic alkylating agent that cross-links DNA and prevents its separation, which results in altered protein production, decreased cell division and cell death. Rapidly proliferating cells are most susceptible to cyclophosphamide’s alkylating effects, and this observation has been used in cancer research. Cyclophosphamide also influences the cells of healthy tissues, including immune cells, bone marrow cells (in particular developing blood cells), activated lymphocytes (that proliferate and produce antibodies), foetal cells, hair follicle cells and intestinal epithelial cells [30]. Cyclophosphamide weakens both the cellular and humoral immune response. Its effect is dose-dependent, but even a single administration can temporarily impair the immune system [31]. Cyclophosphamide is widely used in the treatment of neoplastic and autoimmune diseases.

This study makes the first ever attempt to evaluate the effect of β-1,3/1,6-D-glucan isolated from Saccharomyces cerevisiae on rats immunosuppressed with cyclophosphamide. The aim of this study was to demonstrate the effect of Biolex-Beta HP on selected parameters of humoral and cellular immunity in cyclophosphamide-immunosuppressed rats.

Evaluation of non-specific humoral immunity parameters. Lysozyme activity in blood plasma was determined by the turbidimetric method [33] modified by Siwicki and Anderson [34], ceruloplasmin activity in blood plasma was evaluated by the method developed by Siwicki
Lysozyme activity. Whole blood samples were centrifuged for 5 min at 1,000 g to separate blood cells from the serum. The serum was diluted 1:1 with phosphate buffer, and 0.1 ml of the solution was placed in microplate wells. 0.5 ml of *Micrococcus lysodeikticus* bacterial suspension (25 mg bacteria/100 ml phosphate buffer) (Sigma Chemical Co.) was added. Absorbance was measured directly after the addition of bacteria (E0) and after 1, 2, 3 and 30 minutes (final E). The final absorbance was subtracted from the initial absorbance (E0) to determine lysozyme activity with the use of a standard curve. The standard curve was plotted based on the optical density values for known lysozyme concentrations.

Ceruloplasmin activity. Whole blood samples were centrifuged for 5 min at 1,000 g to separate blood cells from the serum. The following buffers were prepared: 1) acetate buffer (pH 5.2, containing crystalline acetic acid, sodium acetate trihydrate and 15 mg EDTA), 2) buffered substrate solution (0.2% p-phenyldiamine (PPD) in acetic buffer), and 3) sodium azide solution (0.02% sodium azide solution in deionised water). 0.5 ml of buffered solution was added to each of the two 16 x 100 mm test tubes immersed in a water bath at a temperature of 37°C. One test tube served as the experimental sample, and the other one was the control. 50 μl of the serum was added to the experimental sample which was incubated for 15 min at 37°C. 2 ml of sodium azide solution was added to experimental and control samples. 50 μl of the serum was added to the control sample, and both samples were mixed. The absorbance of the experimental sample was measured at the wavelength of 540 nm, and the control served as a blind sample. Ceruloplasmin activity was determined with the use of a standard curve. The standard curve was plotted based on the optical density values for known ceruloplasmin concentrations.

Gammaglobulin levels. Whole blood samples were centrifuged for 5 min at 1,000 g to separate blood cells from the serum. The optical density of total protein was determined in the blood serum. 0.1 ml of the serum was placed in microplate wells, and 0.1 ml of 12% polyethylene glycol (Sigma Chemical Co.) suspended in deionised water was added. The microplates were incubated at room temperature for 2 h, and well contents were stirred continuously. The microplates were centrifuged for 10 min at 5,000 g to separate the γ-globulin fraction bound by polyethylene glycol (plate sediment) from the remaining total protein fraction that constituted the supernatant. The optical density of the supernatant was measured in a microplate reader at 620 nm. The optical density of the supernatant was subtracted from the optical density of total protein. γ-globulin levels were determined using a standard curve (plotted earlier for total protein) as the reference based on the ability of gamma globulins to bind with polyethylene glycol and precipitate.

Total protein levels. Whole blood samples were centrifuged for 5 min at 1,000 g to separate blood cells from the serum. 5 μl of the serum was placed in microplate wells, and 25 μl of reagent A and 200 μl of reagent B were added (Rio-Rad, Hercules, CA). The well contents were gently stirred with a pipette. The microplates were incubated at room temperature for 15 min. The optical density was measured in a microplate reader at 620 nm. Total protein levels were determined using a standard curve as the reference. The standard curve was plotted based on optical density values for known protein dilutions.

Evaluation of non-specific cellular immunity parameters. The metabolic activity of phagocytes was determined based on intracellular measurements:

Respiratory burst activity (RBA) was measured after stimulation with PMA (Phorobol Myristate Acetate, Sigma) as described by Chung and Secombes [37] and modified by Siwicki et al. [38]. The isolated cells were resuspended in RPMI-1640 medium (Sigma) at 10⁶ cells/ml. In 96-well U-shaped microplates, 100 μl of isolated blood leukocytes was mixed with 100 μl of 0.2% nitro blue tetrazolium (NBT, Sigma) solution in 0.2 M phosphate buffer at pH 7.2, and 1 μl of PMA with a concentration of 1 mg/ml in ethanol was added. After 30 min of incubation at 37°C, the supernatant was removed from each well. The cell pellet was washed with absolute ethanol and, three times, with 70% ethanol, then it was dried at room temperature. The amount of extracted reduced NBT after incubation with 2 M KOH and DMSO (dimethylsulfoxide, Sigma) was measured colourimetrically at 620 nm in a microplate reader (Tecan Sunrise). All samples were tested in triplicate, and the results are presented as mean values.

The potential killing activity (PKA) of mononuclear phagocytes and polymorphonuclear phagocytes was determined in isolated blood leukocytes stimulated with killed microorganisms, according to the method presented by Rook et al. [39] and adapted by Siwicki et al. (2004). In 96-well U-shaped microplates, 100 μl of leukocytes was mixed with 100 μl of 0.2% NBT in phosphate buffer at pH 7.2, and 10 μl of killed *Staphylococcus aureus* strain 209P (containing 10⁶ bacteria) was added. The mixture was incubated for 1 h at 37°C, and the supernatant was removed. The cell pellet was washed with absolute ethanol and, three times, with 70% ethanol, and it was dried at room temperature. 2 M KOH and DMSO were added to each well. The amount of extracted reduced NBT was measured at 620 nm in a microplate reader (Tecan Sunrise). All samples were tested in triplicate, and the results are presented as mean values.
The proliferative response of blood lymphocytes. The proliferative response of blood lymphocytes after stimulation with mitogens, concanavalin A (ConA) and lipopolysaccharide (LPS) was determined by MTT spectrophotometry (OD 570 nm) using (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), as described by Mosmann [40].

MTT (Sigma) was dissolved in PBS at the concentration of 5 mg/ml and filtered. In 96-well culture plates (Sarstetd, USA), 100 ml of blood lymphocytes in RPMI 1640 containing 10% FCS, 2 mM L-glutamine, 0.02 mM 2-mercaptoethanol, 1% HEPES buffer and penicillin/streptomycin (100 U/100 mg/ml) were mixed with 100 ml of RPMI 1640 containing mitogens ConA (5 mg/ml) or LPS (20 mg/ml). After 72 h of incubation at 37°C in a 5% carbon dioxide atmosphere (Memmert Incubator), 50 ml of MTT solution was added to each well, and plates were incubated for 4 h at 37°C. After incubation, the plates were centrifuged (1,400 g, 15°C, 5 min). Supernatants were removed, and 100 ml of DMSO (Sigma) was added to each well and incubated for 15 min at room temperature. After incubation, the solubilised reduced MTT was measured colourimetrically at 620 nm in a microplate reader (Tecan Sunrise). All samples were tested in triplicate, and the results are presented as mean values. The final results are presented as the reactivity index (RI).

Statistical analysis

The results were verified statistically by one-way ANOVA (GraphPadPrism software package), and the significance of differences between the groups was evaluated with the Bonferroni test.

Results

The administration of cyclophosphamide (C) to rats led to a statistically significant (p ≤ 0.01) decrease (by 15%) in total serum protein in comparison with the control group (K) (Table 1). After the administration of β-glucan, total protein levels in cyclophosphamide-immunosuppressed rats (group C + G) increased significantly (by 21%) and reached a similar level to that noted in the control group (K) and in the group administered only β-glucan (G). No statistically significant differences were found between those three groups (C + G, K and G).

Significantly (P ≤ 0.001) lower values of humoral immunity indicators in rats, i.e. lysozyme activity, ceruloplasmin activity and serum concentrations of gamma globulins (Table 2), were noted in the group of cyclophosphamide-immunosuppressed animals. The administration of cyclophosphamide to rats (C) decreased lysozyme activity (by 33%), ceruloplasmin activity (by 21%) and γ-globulin levels (by 19%), in comparison with the control group. The stimulation of immunosuppressed rats (C + G) with β-glucan improved the values of the above parameters. A significant, more than two-fold increase in lysozyme activity was observed in comparison with cyclophosphamide-immunosuppressed rats (C), and lysozyme activity increased by more than 50% in comparison with the controls (K). Ceruloplasmin activity

| Parameter                  | Group                              | Control (K)   | Cyclophosphamide (C) | Cyclophosphamide + β-glucan (C+G) | β-glucan (G) |
|----------------------------|------------------------------------|---------------|----------------------|----------------------------------|-------------|
| lysozyme activity (mg/l)   |                                    | 9.06 ±0.56    | 6.10 ±0.24           | 12.90 ±1.07                      | 14.78 ±1.31 |
| ceruloplasmin activity (mg/l) |                                    | 101.90 ±1.90  | 80.30 ±2.66          | 102.70 ±2.97                     | 105.23 ±3.54 |
| γ-globulin level (g/l)     |                                    | 13.42 ±0.35   | 10.88 ±0.39          | 13.20 ±0.98                      | 16.54 ±1.07 |

**statistically significant difference at p < 0.001 in comparison with control (K)**

Table 1. The effect of Biolex-Beta HP on protein levels in cyclophosphamide-immunosuppressed rats

Table 2. The effect of Biolex-Beta HP on non-specific humoral immunity parameters in cyclophosphamide-immunosuppressed rats
The effect of Biolex-Beta HP on the parameters of specific and non-specific cellular immunity in cyclophosphamide-immunosuppressed rats

**Table 3.**

| Parameter | Group                        | Control (K) | Cyclophosphamide (C) | Cyclophosphamide + β-glucan (C + G) | β-glucan (G) |
|-----------|------------------------------|-------------|----------------------|-------------------------------------|-------------|
| RBA (OD 620 nm) |                              | 0.42 ±0.03  | 0.24 ±0.02***        | 0.51 ±0.08†                          | 0.62 ±0.12***±‡ |
| PKA (OD 620 nm)  |                              | 0.43 ±0.04  | 0.29 ±0.01***        | 0.49 ±0.07†                          | 0.51 ±0.06**±§ |
| MTT-ConA (RI)    |                              | 1.03 ±0.12  | 0.54 ±0.17***        | 1.01 ±0.05†                          | 1.23 ±1.14***±§ |
| MTT-LPS (RI)     |                              | 1.04 ±0.04  | 0.51 ±0.13***        | 1.09 ±0.11†                          | 1.19 ±0.19†   |

**Statistical significance:**
- **†** statistically significant difference at p < 0.001 in comparison with control (K)
- **‡** statistically significant difference at p < 0.001 in comparison with control (K)
- **§** statistically significant difference at p < 0.001 between groups C and C + G
- **** statistically significant difference at p < 0.001 between groups C and G
- *** statistically significant difference at p < 0.001 in comparison with control (K)
- ± statistically significant difference at p < 0.001 in comparison with control (K)
- ±‡ statistically significant difference at p < 0.001 between groups C and G and G
- ±§ statistically significant difference at p < 0.001 between groups C + G and G

...and γ-globulin concentrations increased by 28% and 21%, respectively, in comparison with group C and reached a similar level to that noted in the control group (K). Statistically significant differences between the group of rats fed β-glucan (G) and the control group (K) were not reported only with regard to ceruloplasmin activity.

The indicators of non-specific cell-mediated immunity, i.e. respiratory burst activity (RBA) and potential killing activity (PKA) of phagocytes, and the parameters of specific cell-mediated immunity, i.e. proliferation rates of lymphocytes (MTT assay) stimulated with LPS and ConA (Table 3), were significantly (p ≤ 0.001) lower in the group of rats immunosuppressed with cyclophosphamide (C) in comparison with control (K). The respiratory burst activity (RBA) and potential killing activity (PKA) of phagocytes in immunosuppressed rats fed β-glucan (G) were significantly higher (by 113% and 69%, respectively) than in the group of cyclophosphamide-immunosuppressed animals (C). The above parameters did not differ significantly from those noted in the control group. The respiratory burst activity (RBA) and the potential killing activity (PKA) of phagocytes in rats fed β-glucan (G) were significantly higher (by 48% and 19%, respectively) than in the control group (K). The proliferative capacity of T cells (ConA) and B cells (LPS) increased in immunosuppressed rats fed β-glucan (C + G) to the level noted in the control group. Statistically significant differences between the group of rats fed β-glucan (G) and the control group (K) were reported only with respect to the proliferative capacity of T cells (ConA).

**Discussion**

In the present study, cyclophosphamide inhibited protein production and decreased total protein and protein fractions. The administration of cyclophosphamide led to a decrease in γ-globulin levels and ceruloplasmin activity. The noted decrease could be attributed to cyclophosphamide’s direct and/or indirect effect on hepatocytes that produce various protein fractions: albumins, α-globulins (including ceruloplasmin) and C-reactive protein (CRP) – γ-globulin. Cyclophosphamide could exert a direct influence on hepatocytes by inhibiting the activity of liver enzymes responsible for protein synthesis. Its indirect effect, which is of particular importance for acute phase proteins, including ceruloplasmin, could result from impaired synthesis of proinflammatory cytokines (IL-1, IL-6 and TNF-α), which are produced mainly by monocytes/macrophages that stimulate the synthesis of acute phase proteins in the liver. The mechanism responsible for cyclophosphamide’s hepatotoxicity has never been described in literature. The results reported by King and Perry [41] seem to confirm cyclophosphamide’s inhibitory effect on selected liver enzymes. The cited authors suggested that cyclophosphamide does not exert direct hepatotoxic effects, but that its action is mediated by cytochrome P450, which converts the drug to active metabolites. In the above study, intensive changes that accompany the administration of large doses of cyclophosphamide led to disrupted hepatocyte function and synthesis of selected proteins. Cyclophosphamide’s hepatotoxic effects and the resulting death of hepatocytes were also noted by Aubrey [42] in a breast cancer patient and by Snyder et al. [43] in a granuloma patient undergoing cyclophosphamide therapy. The observed drop in the concentrations of immunoglobulins, the γ-globulin fraction of total protein, can also be attributed to cyclophosphamide’s direct effect on plasma cells, which involves the inhibition of protein synthesis, and its indirect effect on B cell proliferation. A drop in the number of proliferating cells could imply a decrease in plasma cell...
counts and the produced antibodies. The above hypothesis seems to be validated by the findings of Čejka et al. [44], who observed a decrease in immunoglobulin and β2-microglobulin levels in a patient with acute leukemia after cyclophosphamide treatment. Zhu et al. [45] noted a drop in the ability of B cells to differentiate into plasma cells and secrete immunoglobulins in patients subjected to long-term treatment with low doses of cyclophosphamide. The effect of cyclophosphamide on ceruloplasmin levels was investigated by Abreu and Abreu [46], who administered the drug to rats with syngeneic sarcomas. In the above study, the physiological levels of ceruloplasmin were restored already after just a single dose of cyclophosphamide (100 mg/kg BW).

In the present experiment, total protein and ceruloplasmin activity were restored to their initial levels after the administration of β-glucan to cyclophosphamide-immunosuppressed rats, whereas gamma globulin levels were higher than before immunosuppression. β-glucans had a stimulating effect on γ-globulin and ceruloplasmin levels in our earlier studies of non-immunosuppressed rats administered Biolex Beta-HP [47], non-immunosuppressed lambs fed Biolex MB-40 [48], and 28-, 50- and 70-day-old lambs whose mothers had been administered Saccharomyces cerevisiae dry brewer’s yeast (Inter Yeast) from the fourth month of pregnancy or after lambing [13]. The above effect can be attributed to β-glucan binding to β-glucan receptors on macrophages and/or granulocytes, which activates the nuclear transcription factor (NF-κB) for genes encoding inflammatory proteins, such as cytokines and chemokines, and induces free radical production [49]. Estrada et al. [50] demonstrated in vitro an increase in IL-1α production by murine macrophages in the presence of oat β-glucans and enhanced secretion of interleukin (IL)-2, IFN-γ and IL-4 by spleen cells. Interleukin 4 stimulates Th2 cell development to produce IL-5, IL-6, IL-10 and IL-13, which are involved in the humoral immune response, and it stimulates the production of antibodies. In a study by Suzuki et al. [51], isolated splenocytes from mice administered SSG increased IgG2a production and decreased IgG1 production. Since IgG2a responses are induced by IFN-γ and suppressed by IL-4, whereas immunoglobulin (Ig) G1 production is inhibited by IFN-γ and stimulated by IL-4, those responses were identified as Th1 and Th2 antibody responses, respectively. Estrada et al. [52] reported on the stimulating effects of oat β-glucans in dexamethasone-immunosuppressed beef steers, which led to an increase in serum levels of specific (after simulation with leech ovalbumin and hemocyanin) and non-specific IgG. It should be noted, however, that gamma globulin levels account not only for specific antibodies, but also for haemagglutinin and haemolysin – antibodies of the first class that play a very important role in non-specific humoral immune response. β-glucans can also stimulate phagocytes to produce IL-1, IL-6 and TNF-α,

ulate the synthesis of acute phase proteins in hepatocytes. The above processes could be responsible for the observed increase in ceruloplasmin activity [53]. Guzdek and Rokita [54] demonstrated the stimulating effects of curdlan sulphate (a sulphate derivative of curdlan – 1,3-β-glucan) on the levels of selected blood proteins, including ceruloplasmin. The noted increase is of significance because ceruloplasmin, an acute phase protein that is implicated in the non-specific humoral immune response, contributes to the quick restoration of homeostasis.

In the present study, cyclophosphamide contributed to a decrease in the activity of lysozyme (which is also a γ-globulin) produced by phagocytes. Zhao et al. [55] administered this drug to rats and reported a decrease in serum lysozyme levels, the percentage phagocytosis and the macrophage phagocytic index. According to Yang [56], the above could be attributed to a drop in the number of phagocytes, impairment of phagocytosis and the release of lysozyme into the plasma by macrophages [57-59]. However, after the administration of β-glucan to immunosuppressed rats, lysozyme activity increased and considerably exceeded the levels noted in the control group. A similar increase in lysozyme activity was noted by Zabek et al. [60] in the blood serum of non-immunosuppressed sheep fed Biolex®-Beta S (β-1,3/1,6-D-glucan). Paulsen et al. [61] have suggested that β-glucan directly activates the transcription of lysozyme genes (much higher expression of lysozyme mRNA was noted in macrophages stimulated with β-glucan). According to Kokoshis et al. [62], the increase in lysozyme activity after stimulation with β-glucan is proportional to the phagocytic efficiency of macrophages, which is also determined by β-glucan stimulation. Activated macrophages containing more lysosomes and lysosomal enzymes expresses more histocompatibility complex class II proteins [63]. A study of mice [64] also demonstrated that serum levels of lysozyme do not always increase in response to the administration of β-glucans.

The results of our experiment indicate that cyclophosphamide had a negative effect on the respiratory burst activity (RBA) and the potential killing activity (PKA) of phagocytes. The values of the investigated parameters decreased in immunosuppressed rats. The negative effect cyclophosphamide could result from its indirect or direct influence on phagocytes. The analysed drug exerts a direct effect by alkylating DNA, RNA and enzymes, which impairs phagocytic function at different stages of phagocytosis. The above can inhibit chemotaxis, synthesis of cytokines, enzymes, substances present in macrophage lysozymes and granulocyte granules, receptors for complement protein C3 (CR3), Fc antibody fragments (FcR) or toll-like receptors that directly bind microbial products (TLR). Cyclophosphamide exerts a similar effect by influencing the production of cytokines released by lymphocytes [65-71].

In this study, dietary supplementation with β-glucan enhanced the activity of monocytes and granulocytes in

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The activation of lactosylceramide and scavenger receptors on the phagocytes is mediated when they are bound to TLR-2 (toll-like receptor 2), which was manifested by increased phagocytosis, bactericidal killing and oxidative burst of isolated heterophiles. This effect was observed in neutrophil-like cells in chickens.

Cyclophosphamide’s negative influence could result from its direct or indirect effect on cells. The analysed drug directly contributes to the formation of cross-links between nitrogen bases in DNA, which negatively affects daughter cells. Indirectly, cyclophosphamide exerts a negative effect on monocytes/macrophages that control lymphocyte function.

In this study, dietary supplementation with β-glucan stimulated the proliferative activity of T and B cells. β-glucan exerts a direct effect by binding with glucan receptors on the surface of B cells (human and mouse B cells express Dectin-1) and T cells (αβ T cell receptors – αβ TCRs) to induce a cascade reaction and activate the NF-κB transcription factor. NF-κB induces the expression of cytokines, mostly IL-2 and IL-4, which stimulate the proliferation of B and T cells.

The immunosuppressive effects of cyclophosphamide lead to an increase in the respiratory burst activity of lymphocytes, leading to an increase in the respiratory burst activity of lymphocytes, leading to an increase in the respiratory burst activity of lymphocytes, leading to an increase in the respiratory burst activity of lymphocytes. Indirectly, cyclophosphamide exerts a negative effect on monocytes/macrophages that control lymphocyte function.

The reported results clearly indicate that β-1,3/1,6-D-glucan has a positive effect on the analysed parameters of non-specific cellular and humoral immunity after immunosuppression with cyclophosphamide. Owing to its high efficacy, β-1,3/1,6-D-glucan contributed to an improvement in all immune system functions that had been impaired by cyclophosphamide. Nevertheless, the observed effect only marked a return to the norm, as most of the analysed parameters were merely restored to their initial levels, with the exception of lysozyme activity, which considerably exceeded the level noted before immunosuppression. This experiment demonstrated that β-glucan does not directly reverse the changes induced by cyclophosphamide, but it mobilises the remaining functional cells to compensate for the resulting deficiencies. The functions of immunocompetent cells were analysed individually, and further work is needed to investigate the studied polysaccharides.
charide’s effect on the entire immune system. All elements of the immune system are interconnected therefore, every substance that delivers a negative or a positive effect on a given type of cells will, to a varied degree, affect other cells. For this reason, studies that investigate specific immune functions should make a reference to the immune system as a whole.

The results of this study suggest that Biolex-Beta HP (β-1,3/1,6-D-glucan) could effectively stimulate the immune system of immunosuppressed subjects, including patients who have undergone cyclophosphamide chemotherapy. Subjects exposed to chronic stress, patients with chronic conditions and victims of pesticide poisoning.

Author declares no conflict of interest.

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