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PLANT GROWTH-PROMOTING TRAITS OF ANTARCTIC ENDOPHYTIC BACTERIA

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Successful colonization of Antarctic lands by vascular plants *Deschampsia antarctica* and *Colobanthus quitensis* and their adaptation to stressful environments is associated not only with climate change but also with the functioning of microbial groups of phylo- and endosphere of these plants.

The aim of our study was to screen plant growth-promoting traits in endophytic bacteria of antarctic vascular plants.

Materials and methods. We have studied 8 bacterial cultures isolated from *D. antarctica* collected during the 25th Ukrainian Antarctic Expedition (January-April 2020) along the Western part of the Antarctic Peninsula.

Overnight liquid cultures were obtained on Nutrient Broth medium (HiMedia, Ltd.) in a shaking incubator (26 °C, 160 rpm). Bacterial isolates were grown on Ashby's combined-nitrogen-free medium with sucrose. Drop collapse assay for cyclic lipopeptide production (CLP), motility assay, exoprotease production and phosphate solubilizing ability were performed using generally accepted methods.

Results. All studied isolates have shown plant growth-promoting traits. The most abundant were nitrogen-fixing activity and motility. Both these play important role in plant colonization and promoting the growth of plants in harsh environments. The evidences of CLP were shown by two strains only. There was no notice of phosphate solubilizing ability and exoprotease production.

Conclusions. Endophytic bacteria of antarctic vascular plants could support the growth and nutrition needs of the plants.

Key words: PGPB, antarctic vascular plants, endophytes.

In both managed and natural ecosystems, beneficial plant-associated bacteria play a key role in supporting and/or increasing plant health and growth. Plant growth-promoting bacteria (PGPB) seem to function as a «normoflora» of a plant, can be applied in agricultural production or for the phytoremediation of pollutants [1].

Plant growth-promoting bacteria facilitate plant growth in two ways, either by direct stimulation or by biocontrol (i.e., suppressive activity against soil-borne diseases). The direct stimulation of plant growth may be a consequence of nitrogen fixation, phosphate solubilization, iron sequestration, synthesis of phytohormones (such as auxins, cytokinins, and gibberellins), or modulation of plant ethylene levels [2] helping plants to overcome stress and support cell metabolism. In fact, no single organism has the ability to make use of all the available mechanisms that could be used to promote plant growth. Various PGPB often possess one or more of the above mentioned traits [3].

Successful colonization of Antarctic lands by vascular plants *Deschampsia antarctica* and *Colobanthus quitensis* and their adaptation to stressful environments is associated not only with climate change but also with the functioning of microbial groups of phylo- and endosphere of these plants [4].

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Materials and Methods

We have studied 8 bacterial cultures isolated from *D. antarctica* collected during the 25th Ukrainian Antarctic Expedition (January-April 2020) along the Western part of the Antarctic Peninsula. Overnight liquid cultures were obtained on Nutrient Broth medium (HiMedia, Ltd.) in a shaking incubator (26 °C, 160 rpm). Nitrogen-fixing activity were checked on Ashby's combined-nitrogen-free medium with sucrose. Bacterial growth was determined by the change of optical density (OD_{600}) and evaluated as + (weak growth), ++ (moderate growth), +++ (abundant growth) [5].

Drop collapse assay for cyclic lipopeptides production (CLPs) was performed onto Parafilm. The reduction of the surface tension and the collapse of the droplet (10 µL aliquots of bacterial overnight culture) indicated the presence of surfactants [6].

Motility assay was performed onto 1/5 Nutrient agar (0.3%). 10 µL aliquots of bacterial overnight culture were spot in medium surface. Colony diameter was measured in 24, 48 and 72 h after inoculation on 0.3% 1/5 NA [7].

Exoprotease production was tested using skim milk agar [8]. A cleared zone surrounding bacterial growth after incubation for 48 and 72 h at 28°C was the evidence of exoprotease production.

Phosphate solubilizing ability was tested on Pikovskaya (PVK) medium [9] incorporated with $Ca_3(PO_4)_2$.

All experiments were performed in triplicates.

Table. **Plant growth-promoting traits of studied isolates***

Isolate No.	CLPs	N_2-fixing activity**	Motility
10.4	+	+++	–
15.6	–	+	+
24.4	+	+++	+
25.2	+	+++	+
26.2	+	+++	+
26.7	+	++	–
39.12	+	–	+
40.1	+	–	–

* Phosphate solubilizing ability and exoprotease production are no shown because it was not detected;

** + weak growth, ++ moderate growth, +++ abundant growth in Ashby's medium.

Results and Discussion

The mentioned set of screened plant growth-promoting characteristics was chosen based on the importance of adequate nutrition in low-temperature environment and defense system against numerous of pathogens [10].

All studied isolates have shown plant growth-promoting traits (Table).

The most abundant were nitrogen-fixing activity, cyclic lipopeptides production and motility. These traits play important role in plant colonization and promoting the growth of plants in harsh environments. Nitrogen is known as one of the limiting factors regarding plant growth [11]. Biological nitrogen fixation plays a great role in subsidizing plants with nitrogen in such limiting or low-mobility environments as Antarctic region. Phosphorus is one of the six elements essential for plant growth. The majority of phosphate solubilizing bacteria affiliates with *Paenibacillus, Bacillus, Pseudomonas, Lactococcus, Enterobacter* and *Alcaligenes* [12]. Although there were *Bacillus* and *Pseudomonas* among studied isolates there was no evidences of phosphate solubilizing ability as well as exoproteases synthesis.

The evidences of CLPs were shown by almost all isolates. Despite the fact that CLPs are known as biocontrol molecules, their role is believed more complicated than this [13]. CLPs exhibit interesting biological activities including interactions with biofilms [14] which affect not pathogens only but could manage colonization activity and the balance among endophytic community itself.

Conclusions

Antarctic endophytic bacteria seem affect plants directly through nutrition facilitating and various colonization mechanisms which needed to be studied deeper.

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CIRCADIAN VARIATION IN FUNCTIONAL POLARIZATION OF MURINE PERITONEAL MACROPHAGES

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Aim. This study aimed to investigate the circadian rhythm of the murine peritoneal macrophage (PM) metabolic profile.

Methods. The metabolic profile of PM was characterized by phagocytic activity, reactive oxygen species (ROS) generation, and by the expression of phenotypic markers, associated with a pro- and anti-inflammatory metabolic shift. Phagocytosis of FITC-labeled inactivated *Staphylococcus aureus*, ROS generation, CD80, CD86, and CD206 expression were estimated by flow cytometry at a regular 4h interval over the daily light-dark cycle.

Results. The phagocytic index and percentage of ROS-producing PM were found to be lower in the resting phase (ZT4) as compared to the active phase. In contrast, the level of CD86 expression was the highest in the inactive phase (ZT8). There was also a statistically significant peak in the proportion of ROS-producing PM, as well as in the level of ROS production per cell at the time of awakening (ZT12). As opposed to ROS generation, ZT12 was characterized by the lowest level of cell-surface CD206 expression.

Conclusions. Our results indicate that there is a circadian rhythm in functional polarization of murine PM with an anti-inflammatory activation state in the resting phase in comparison to the active phase.

Key words: circadian rhythm, peritoneal macrophages, phagocytosis, reactive oxygen species, phenotypic polarization.

It is known that circadian disruption plays a prominent role in the pathogenesis of many inflammatory disorders, such as obesity, diabetes mellitus, and others [1]. Peritoneal macrophages (PM) are important components of omentum-associated lymphoid tissue, and their involvement in the pathogenesis of different inflammatory diseases makes them a promising target for immunotherapy [2]. Despite a lot of research that demonstrated circadian rhythmicity of macrophage function [3], temporal variation in functional and phenotypic polarization of those cells remains understudied.

This study aimed to investigate the circadian rhythm in phagocytic activity, ROS production, and phenotype marker expression in murine PM.

Material and Methods

Male outbred mice were aged between 8–12 weeks. They were maintained on a 12 h light/12 h dark cycle (light: zeitgeber time (ZT) 0–12; dark: ZT 12–24). Murine PM were collected at 6 time points: ZT4, ZT8, ZT12, ZT16, ZT20, and ZT24. Phagocytosis of FITC-labeled inactivated *Staphylococcus aureus*, ROS generation, CD80, CD86, and CD206 expression were estimated by flow cytometry. Data statistical significance was determined using one-way ANOVA followed by the post-hoc Tukey-Kramer HSD test. The values of $P < 0.05$ were considered significant.

Results and Discussion

We have not detected circadian rhythm in percentages of $CD206^+$, $CD80^+$, $CD86^+$, and phagocytic PM (data not shown). Rhythmic patterns in explored metabolic characteristics of PM varied significantly. The phagocytic index value of PM was found to be significantly lower in the resting phase (at ZT4) as compared to ZT12 (Fig. *A*). Phagocytic activity peaked at ZT12, at the beginning of the dark phase, followed by a progressive decrease until the pre-dawn time.

There was an increase in the proportion of ROS-producing PM at ZT12 in comparison to time points representing the resting phase (Post-Hoc Tukey HSD: ZT4, $P = 0.035$; ZT8, $P = 0.094$) and ZT16 (Post-Hoc Tukey HSD: $P = 0.06$). Also, a trend for the elevated percentage of ROS-generating PM at ZT20 (Post-Hoc Tukey HSD: $P = 0.048$) and ZT24 (Post-Hoc Tukey HSD: $P = 0.091$) was observed as compared to ZT4 (data not shown).

Likewise, there was a pronounced spike in the level of ROS production per cell at ZT12 in comparison to ZT4, ZT8, ZT16, and ZT24 (Fig. *B*). High ROS generation is the feature of pro-inflammatory (M1) macrophages. ZT12 marks the start of the dark period, and thus, the beginning of the active phase of mice as nocturnal animals. There is a lot of data showing that the concentration of many proinflammatory cytokines and chemokines increases near the onset of organism activity [4], which corroborates our finding about the peak ROS production, and therefore, the proinflammatory metabolic skew of murine PM at ZT12. Conversely, a lower percentage of ROS-producing PM was observed at ZT4 and ZT8, indicating that PM are shifted into an anti-inflammatory state during the inactive phase, and perform regenerative functions.

As opposed to ROS generation, ZT12 was characterized by the lowest level of CD206 expression in PM (Fig. *C*). There is not enough data in the literature about a diurnal variation

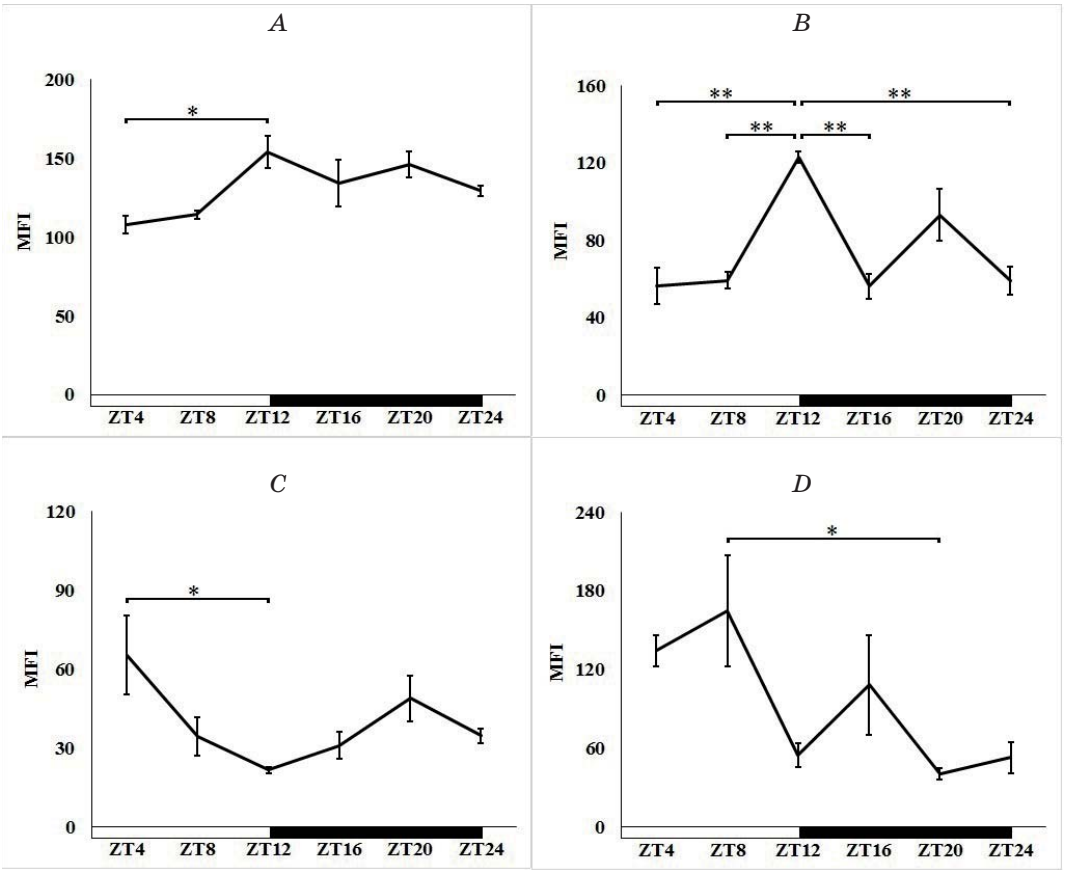

Fig. **Circadian variability in a functional state and phenotypic polarization of murine peritoneal macrophages:**

A — phagocytic index, *B* — level of ROS-production, *C* — CD206 expression level, *D* — CD86 expression level. MFI – mean fluorescence intensity.

$n = 3$ mice per time point. The data are presented as mean ± standard error of the mean.

$^*P < 0.05$; $^{**}P < 0.01$.

of CD206 expression in PMs. Kiessling et al. [5] discovered a higher percentage of $CD206^+$ PM in C57BL/6 J mice at CT9-CT15. The absence of such an effect in our experiment may be explained by the use of outbred mice in our study, which are characterized by higher genetic variability than inbred mice. Lower expression of CD206 per cell at ZT12 coincides with our data on ROS production described above, since CD206 is a marker of anti-inflammatory macrophages, and a decrease in its expression indicates pro-inflammatory phenotypic polarization of those cells.

There was no rhythmicity in the level of expression of CD80 on the PM cell surface at different time points. This observation coincides with the data obtained by Silver et al. [6], who found no significant difference in mRNA levels of this molecule in mice over the daily light-dark cycle. Conversely, the expression level of CD86 was higher in the resting phase (ZT8) in comparison to the active phase (Post-Hoc Tukey HSD: ZT12, $P = 0.069$; ZT20, $P = 0.035$; ZT24, $P = 0.063$) (Fig. *D*). In contrast to CD80, which is considered a marker of M1 macrophages, CD86 is used as a marker of both M1 and M2b macrophages [7]. Therefore, increased expression of CD86 in the resting phase in parallel with the absence of changes in the level of CD80 confirms our data regarding the anti-inflammatory phenotypic polarization of PM during that period since M2b is a subpopulation of alternatively activated macrophages with anti-inflammatory and immunoregulatory properties.

Conclusions

Our results indicated that there was a circadian rhythm in functional and phenotypic polarization of murine PM with an anti-inflammatory activation state in the resting phase as compared to the active phase, and a sharp proinflammatory shift of PM at the time of awakening (ZT12). This may be necessary for proper reparation of damaged tissues during sleep and fight against microorganisms amid activity when the risk of infection is more relevant.

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Conflicts of interest
Authors declare no conflict of interest.

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THE EFFECT OF *B. subtilis* IMV B-7724 LECTIN ON FUNCTIONAL ACTIVITY OF THE MAIN EFFECTORS OF ANTITUMOR IMMUNITY OF INTACT MICE

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The *purpose* of the study was to evaluate the effect of *B. subtilis* IMV B-7724 lectin on the functional activity of macrophages (Mph) and natural killer cells (NK) of intact Balb/c mice.

Materials and Methods. Balb/c mice were subjected to 10 consecutive administrations of the lectin in a dose of 1 mg/kg of body weight. The functional activity of peritoneal Mph and NK were studied. Statistical analysis of the results was performed according to the widely accepted methods of variational statistics.

Results. Administration of bacterial lectin increased Mph and NK cytotoxic activity; maximal increase was registered after the complete course of injections. A significant increase in the NO production indicates the polarization of peritoneal Mph into pro-inflammatory type M1. The transcription factors of IRF (at the early stage) and STAT (at the latter stages) signalling pathways were involved in the process of Mph polarization.

Conclusion. The ability of *B. subtillis* IMV B-7724 lectin to increase *in vivo* cytotoxic activity of innate immunity effectors and to maintain the long-term Mph M1 polarization urges further studies on the lectin effectiveness.

Key words: *B. subtilis* IMV B-7724 lectin, macrophages, natural killer cells, functional activity, STAT, IRF.

To date, active research into the effectiveness of new immunotherapy agents in the treatment of patients with malignant neoplasms of various nosologies continues, as well as the search for substances that can be used in the construction of such agents is going on. Among natural substances possessing immunomodulatory effects, lectins of various origins are especially interesting. Lectins are a unique group of proteins and glycoproteins possessing significant biological activity. Their antitumor activity has been proved both in *in vitro* and *in vivo* studies. While plant lectins are more investigated, much less information is found in the scientific literature about the properties and medical application of microbial lectins [1, 2]. At Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the National Academy of Sciences of Ukraine, the investigation of the extracellular lectin produced by microorganism *B. subtilis* IMV B-7724 is going on. The lectin's physicochemical and biological properties were characterized; moreover, its cytotoxic activity against tumor cells was demonstrated *in vitro* [3] pointing to the prospects of its application as a means of antitumor immunotherapy. On the other hand, its effects *in vivo* remain to be elucidated.

The effectors of innate immune response, especially macrophages (Mph) and natural killer cells (NK), play significant roles in cancer immune defense. Their

activity is significantly suppressed during tumor growth [4, 5]. The restoration or maintenance of the activity of innate immunity cells during tumor progression can lead to an antitumor and/or antimetastatic effect. Therefore, the aim of our study was to evaluate the effect of *B. subtilis* IMV B-7724 lectin on the functional activity of Mph and NK of intact Balb/c mice.

Materials and Methods

The study has been carried out on females Balb/c mice 2–2.5-month-old, weighting 19.0–21.0 g, bred at the vivarium of IEPOR. The use and care of experimental animals have been performed in accordance with the standard international rules on biologic ethics.

Strain *B. subtilis* IMV B-7724 was used as a source of the lectin. The lectin was isolated from the cultural fluid as described in [3] and was freeze dried at temperatures between +24 °C ...–32 °C. The lectin obtained looks like a brown colored powder, easily soluble in water, buffers (PBS, Tris-HCl); has the higherst sugar-binding specificity towards sialic (N-Acytylneuraminic and N-Glycolylneuraminic) acids. The hemagglutinating activity of lectin (1 mg/ml) is in the range within 1024–2048 titer^{-1} [3].

The lectin was administered to Balb/c mice (n = 15) subcutaneously in doses 1 mg/kg of body weight per 1 administration. The complete course consisted of 10 administrations. Mice in the control group (intact control (IC), n = 5) were injected with the 0.9% NaCl solution following the schedule described above.

The level of NK cytotoxic activity was determined as described in [6]. The functional state of peritoneal Mph was studied by nitric oxide (NO) production, arginase (Arg) and nonspecific cytotoxic activity, as well as by assessing the expression level of STAT-1, -6 and IRF-4, -5 mRNAs by qRT-PCR [6].

The statistical processing of the results was performed according to generally acsepted rules of the variation statistics using Prism software Version 8.0. The difference was concidered significant at $P < 0.05$.

Results and Discussion

The administration of *B. subtilis* IMV B-7724 lectin to intact mice had an activating effect on peritoneal Mph and NK which was the most evident after the completion of the full course of lectin administration. There was noted a statistically significant increase on the cytotoxic activity of peritoneal Mph and NK (by 1.1 and 1.2 times on day 21 and 28 respectively, $P < 0.05$) as compared to the IC (Fig. 1).

There is an evidence that some lectins (namely, GalNAc/Gal-specific lectin produced by sea mussel *Crenomytilus grayanus*) act as an immune modulatory reagent by inducing cytokines production in macrophages that leads to increased bactericidal activity of these cells [7]. We hypothesized that *B. subtilis* lectin can increase Mph and NK cytotoxic activity

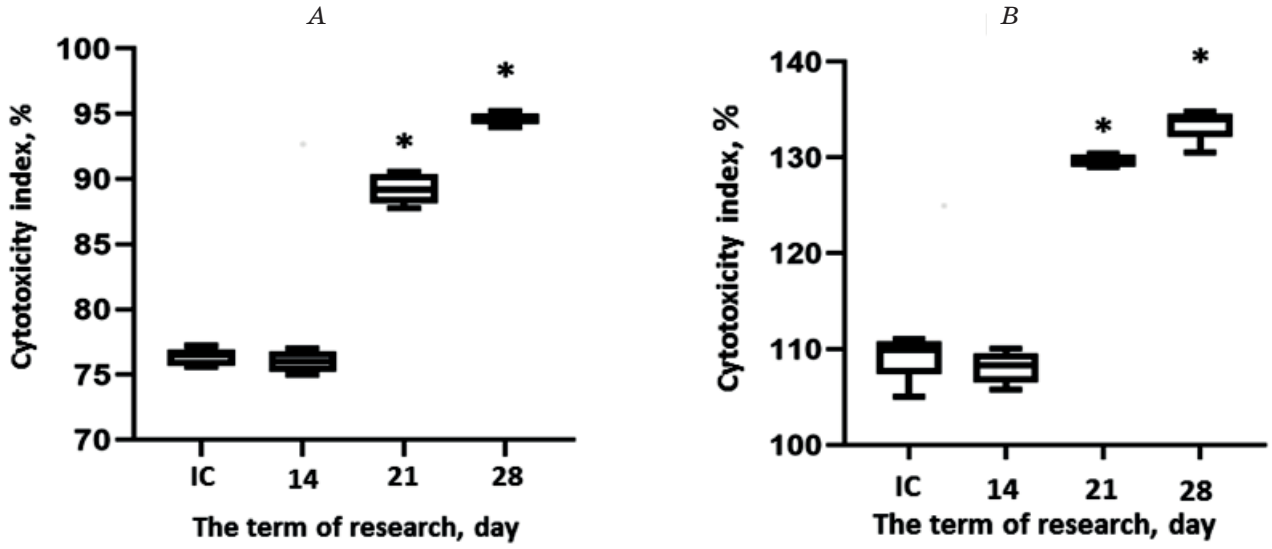

Fig. 1. **Cytotoxic activity (%) of peritoneal Mph (*A*) and NK cells (*B*)**
* — $P < 0.05$ compared with IC.

acting similarly — by inducing cytokines production after binding to certain surface carbohydrates.

Mph can perform opposite functions depending on the signals they receive from the microenvironment. In the antitumor response, the polarization state of Mph is important: M1 — possess antitumor properties, M2 — promote tumor progression. Such a property as plasticity enables Mph to change a polarization state (M1↔M2) in response to external stimuli making them interesting targets for antitumor therapy. Mph production of NO and Arg activity point to the Mph polarization state. After the last lectin administration (day 21), the NO/Arg ratio increased by 1.5 times compared to the control group ($P < 0.05$) and remined elevated on day 28. That is, after the full course of lectin injections, cells with the M1 phenotype prevailed in the peritoneal Mph.

Considering the interferonogenic properties of bacterial lectins [2], it can be assumed that the lectin of *B. subtilis* IMV B-7724 activates Mph indirectly through inducing IFNγ production by lymphocytes. In this process, the transcription factors (TF) of the STAT and IRF signaling pathways may be involved. STAT1 and IRF5 participate in M1 polarization; STAT6 and IRF4 — in M2 [8]. To test our hypothesis we evaluated the mRNA expression level of these TFs. Changes in STAT1/STAT6 and IRF5/IRF4 ratio in Mph coincide with changes in functional activities of these cells. A significant increase in mRNA expression of these TFs was noted after the complete course of lectin administration (Fig. 2). At the initial stages, the polarization of Mph M1 occurs due to the activation of TF of the IRF signaling pathway. Later, the polarization of M1 Mph was enabled probably due to the activation of the STAT signaling pathway.

Thus, the analysis of the obtained results allows us to conclude that the administration of *B. subtilis* IMV B-7724 lectin in a dose of 1 mg/kg (course dose of 10 mg/kg) to intact Balb/c mice lead to Mph and NK activation which both are potent players of anticancer immune response. The TFs of the STAT and IRF signaling pathways are involved in the polarization of peritoneal Mph M1 with antitumor properties. The ability of lectin to guide *in vivo* Mph polarization towards M1 type and to maintain the induction of a significant number of cells with pro-inflammatory properties for a long time can be used in designing a new antitumor immunotherapeutic agent.

Funding

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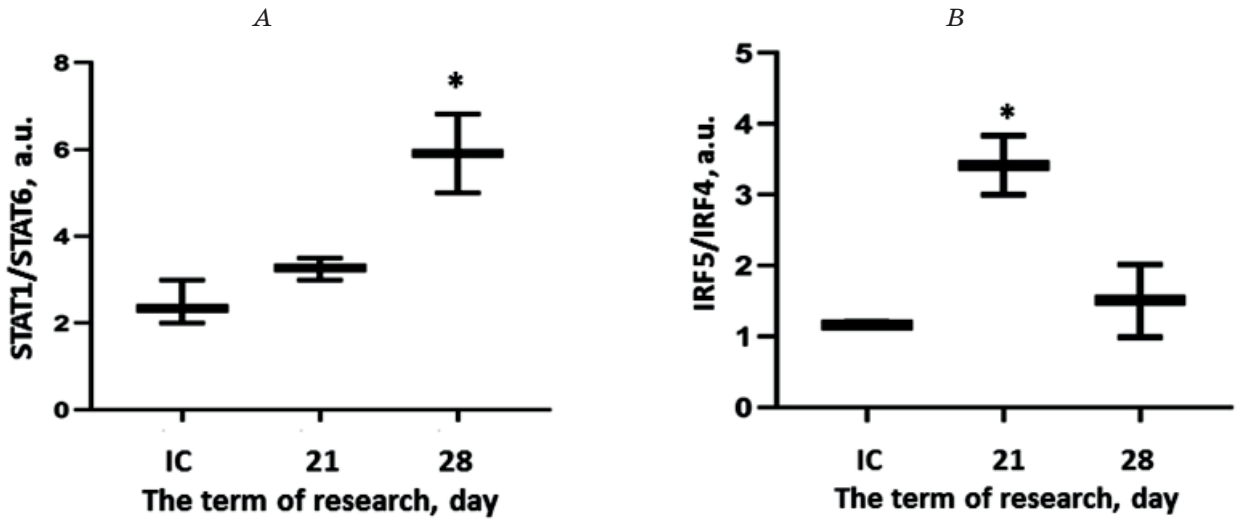

Fig. 2. **STAT1/STAT6 (*A*) and IRF5/IRF4 (*B*) mRNA expression levels ratio in peritoneal Mph**
* — $P < 0.05$ compared with IC.

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EFFECTS OF PROGENITOR CELL CONDITIONED MEDIA ON THE AMOUNT OF BRAIN CORTEX NEURONS IN A RAT MODEL OF TRAUMATIC BRAIN INJURY

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Aim. The purpose of the study was to examine beneficial effect of conditioned media (CM) of progenitor cells of different origin (neurogenic progenitor cells, or NPCs, and adipose-derived mononuclear cells, or AMCs) as a source of mesenchymal multipotent stromal cells (MMSCs) on brain cortex neurons in rats with traumatic brain injury (TBI).

Methods. TBI was reproduced in outbred sexually mature male rats by developing the model of free-falling load (50 g) with damage to the left hemisphere of the brain. The rats were injected 3 times with an interval of every other day intraperitoneally with NPCs CM and AMCs CM (dose 0.8 mg by total protein) that were obtained from cell cultures of fetal rat brain and adult rat adipose tissue. On the 5th day after TBI, the morphologic study of brain tissue was performed.

Results. The number of neurons in the cortex of rats on the 5th day after TBI in damaged hemisphere as well as in contralateral hemisphere compared to control group decreased by half. Three i.p. injections of NPCs CM or AMCs CM increased the number of neurons in the cortex in both hemispheres in rats of corresponding groups compared to the rats with TBI without additional treatment.

Conclusion. Obtained results indicate that CM obtained from NPCs and AMCs have noticeable neuroprotective effect on the damaged neurons and might be considered as an additional mode to treatment of TBI.

Key words: neurogenic progenitor cells, mesenchymal multipotent stromal cells, traumatic brain injury, conditioned medium, neuron viability.

The pathogenetic mechanism of traumatic brain injury (TBI) involves structural changes in nervous tissue as a result of primary mechanical damage as well as a cascade of secondary effects, which causes increased death of neurons and the emergence of functional deficits considered as neurological and functional consequences of trauma [1]. Significant number of ongoing studies has shown that stem cells and their metabolic products, such as anti-inflammatory and growth factors have potential in treatment of brain injury [2, 3]. While these findings, albeit tentatively, seem to confirming beneficial effects and usefulness of stem cells derived treatments, the question of their exact impact remains up for discussion [4].

The purpose of the study was to determine and register curative effect of conditioned media (CM) of neurogenic progenitor cells (NPCs) and adipose-derived mononuclear cells (AMCs) as a source of mesenchymal multipotent stromal cells (MMSCs) on brain cortex neurons in rats with TBI.

Materials and Methods

All procedures with experimental animals used in the work were carried out in compliance with legal norms and requirements of the Law of Ukraine no. 3447 IV "On protection of animals from cruel treatment", "European Convention for the protection of vertebrate animals used for research and other scientific purposes" (Strasbourg, 1986), principles of bioethics and biosafety standards. The study was approved by the Ethics and Bioethics Committee of the SI "INS NAMS" (protocol No. 26 of May 11, 2018).

The study was performed with outbred sexually mature male Wistar rats divided into 4 experimental groups (n = 6 in each group): 1) TBI; 2) TBI + NPCs CM; 3) TBI + AMCs CM; 4) control (intact rats). TBI was reproduced by developing the model of a free-falling load (50 g) with damage to the left hemisphere of the brain. The rats from groups 2 and 3 were injected three times i.p. with an interval of every other day NPCs CM or AMCs CM (dose 0.8 mg by total protein). NPCs CM or AMCs CM were obtained from cell cultures of fetal (E14) rat brain and adult rat adipose tissue [5]. On the 5th day after TBI, the brains were subsequently removed, fixed in 10% formalin solution, set in paraffin, cut (5–7 μm serial sections) and stained with hematoxylin-eosin and thionine. Microscopic examination and photo registration of tissue slices (×100) were performed with the use of Nikon Eclipse E200 microscope with ImageView software. Comparative histopathological and quantitative analysis of the samples was performed using QuPath and ImageJ software. Data are presented as M (25%; 75%), where M is the median, (25%; 75%) – the quartile interval between 25th and 75th percentiles.

Results and Discussion

On the 5th day after TBI the general number of neurons in the cortex of rats decreased almost two-fold. This decrease was observed in the damaged hemisphere and in the contralateral one (Fig). The medians were correspondingly 65 (59;76) and 72 (58;86) compared to 129 (125;147) in control (p = 0,000005, Mann-Whitney U-test).

Three i.p. injection of progenitor cells CM significantly increased the number of neurons in cortex of rats on 5th day after TBI compared to this value in rats with TBI with no additional treatment: the medians were

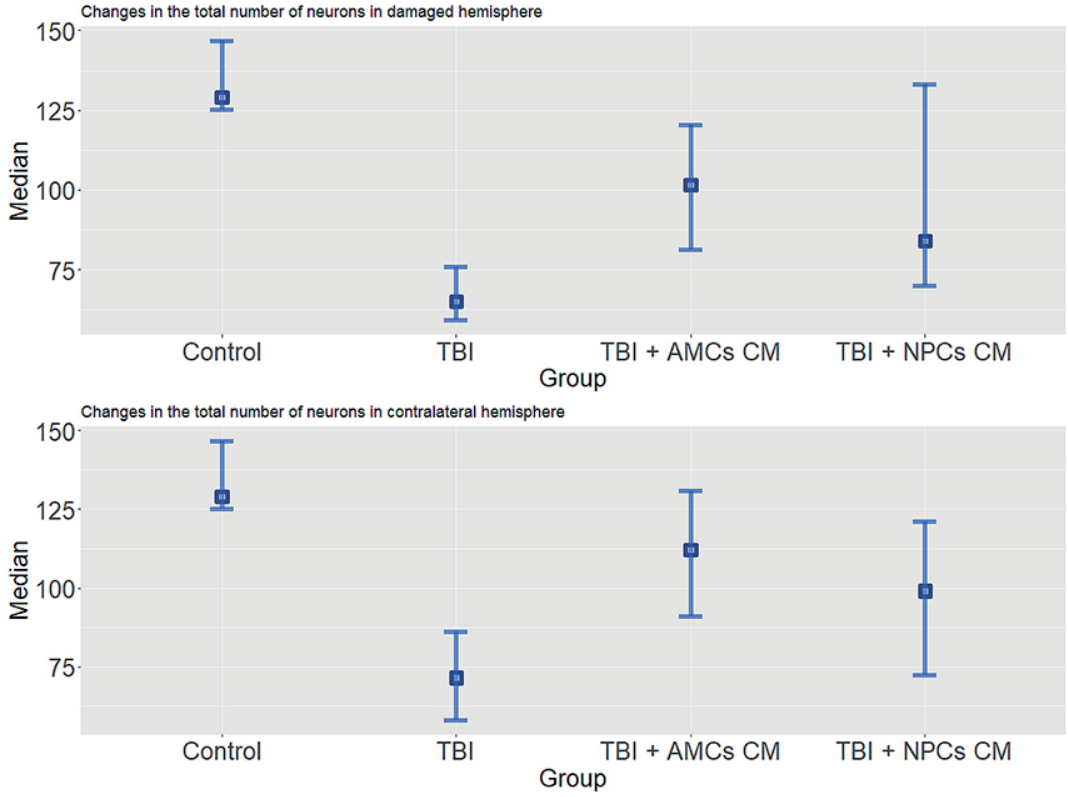

Fig. **The effect of conditioned media obtained from neurogenic progenitor cells and adipose-derived mononuclear cells on the total number of neurons in mice brain cortex (test area 2.55 mm^2),** $P \leq 0.05$

correspondingly 84 (70;133) and 99 (72;121) in damaged and contralateral hemisphere after NPCs CM impact ($P = 0{,}002$, $P = 0{,}02$, Mann-Whitney U-test) as well as 102 (81;120) and 112 (91;131) correspondingly after AMCs CM influence ($P = 0{,}000002$, $P = 0{,}00004$, Mann-Whitney U-test).

The increase of the neurons number in the brain cortex of rats treated with NPCs CM and AMCs CM after TBI comparing to untreated ones suggests the general neuroprotective effect of CM obtained from these cells on nervous tissue. The increase in total number of neurons in damaged as well as unaffected hemispheres could indicate anti-apoptotic and life-sustaining effect of the media. While comparing the action of CM of different origin slightly higher potential of AMCs CM should be noted. At the same time, the regenerative impact of CM of both origins should be further investigated.

Generally, obtained data confirm the concept regarding the paracrine mechanism of influence of the used progenitor cells due to their secretome [2, 3, 5]. In particular, such biologically active molecules — known components of the NPCs and MMSCs secretome as NGF, BDNF, CNTF, GDNF [2, 6], that prevent death and enhance the regeneration of target cell populations — can contribute to the neuroprotective effects of NPCs CM and AMCs CM.

Conclusions

The study demonstrates that conditioned media obtained from neurogenic progenitor cells and adipose-derived mononuclear cells (as a source of mesenchymal multipotent stromal cells) have potential as a therapy for damaged nervous tissue. They also might be considered as an additional treatment mode of traumatic brain injury.

Funding source

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FUNCTIONAL AND PHENOTYPIC CHARACTERISTICS OF CIRCULATING PHAGOCYTES IN RATS WITH DIFFERENT MODELS OF ALZHEIMER'S DISEASE

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This study was *aimed* to evaluate manifestations of systemic inflammation in rats with Alzheimer disease (AD) induced by injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ by the assessment of functional polarization of circulating phagocytes.

Methods. AD was induced by intracerebral injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ Wistar male rats. Intact and sham-operated animals were used as a control. AD development was affirmed by the assessment of cognitive impairment in behavioral tests ('Open field' test, apomorphine test, Barnes maze test), as well as by the level of death of dopaminergic neurons. The functional polarization of circulating phagocytes was designated by phagocytic activity, oxidative metabolism, and the expression of phenotypic markers CD80 and CD206, which were examined by flow cytometry.

Results. Circulating phagocytes from rats with $A\beta_{1-40}$-induced AD were characterized by increased fraction of phagocytizing monocytes with decreased endocytic activity, moderately up-regulated granulocyte ROS generation along with temperate increase of $CD86^+$ mononuclear phagocyte fraction and high level of CD206 expression. Two widely accepted indices of systemic inflammation: NLR and SII were higher in these animals than those in control rats by 6,5 and 7,5 times respectively. In rats with $A\beta_{25-35}$-induced disease, significantly increased granulocyte ROS generation was registered. NLR and SII values in these animals were slightly higher than those in control rats.

Conclusion. Therefore, $A\beta_{1-40}$ AD model reproduces disease-associated systemic inflammation at the greater extent than $A\beta_{25-35}$-induced pathology, and is more appropriate for the study of inflammation in the disease pathophysiology.

Key words: Alzheimer's disease, animal models, systemic inflammation, circulating phagocytes.

Alzheimer's disease (AD) is an incurable devastating age-related neurodegenerative disorder with complex pathophysiology. According to the current hypothesis, inflammation is considered as one of the key nonamyloid components of the disease pathogenesis [1, 2]. Until recently, it was believed that neuroinflammation may be a central mechanism driving amyloid β (Aβ) pathology and progression. Genetic studies along with results from epidemiological and translational research indicate that inflammation outside of the central nervous system or systemic inflammation is another detrimental factor that can contribute to AD initiation and progression [3, 4]. In peripheral inflammation, circulating phagocytes (granulocytes and monocytes) are key players [5]. Sporadic late-onset AD is to a greater extent based on chronic neuro- and systemic inflammation than on mutations related to Aβ or tau generation. Consequently, appropriate *in vivo* models that reproduce the underlying inflammatory and AB-based mechanisms of the disease are needed for deep insight into AD pathophysiology and

developing pathogenetic treatment approaches [6]. $A\beta_{1-40}$ and $A\beta_{25-35}$ -induced AD animal models meet these requirements [7]. This study was aimed to evaluate manifestations of systemic inflammation in rats with AD induced by injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ by the assessment of functional polarization of circulating phagocytes.

Materials and Methods

AD was induced by intracerebral injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ Wistar male rats. Intact and sham-operated animals were used as a control. AD development was affirmed by the assessment of cognitive impairment in behavioral tests ('Open field' test, apomorphine test, Barnes maze test), as well as by the level of death of dopaminergic neurons. The functional polarization of circulating phagocytes was designated by phagocytic activity, oxidative metabolism, and the expression of phenotypic markers CD80 and CD206, which were examined by flow cytometry. All data are presented as mean ± SD. Statistical differences were determined using ANOVA with Tukey's post-hoc test. Differences were considered significant at $P \leq 0.05$.

Results and Discussion

Disturbances in eating behavior, spatial memory and cognitive flexibility, as well as death of dopaminergic neurons were observed in the animals with both AD models (data are not presented), and were much more pronounced in rats with $A\beta_{1-40}$-induced AD.

Table. **Systemic inflammation biomarkers in rats with different AD models**

Systemic inflammation biomarker	Intact animals, $n = 10$	Sham-operated animals, $n = 10$	$A\beta_{1-40}$-induced AD, $n = 10$	$A\beta_{25-35}$-induced AD, $n = 10$
Monocyte phagocytosis percentage[a]	7.60 ± 1.33	6.56 ± 0.45	42.53 ± 6.14$^{\#*}$	14.03 ± 3.31$^{\#*\&}$
Monocyte phagocytosis index[b], MFI	31.10 ± 9.58	31.15 ± 3.70	21.39 ± 4.13$^{\#*}$	92.00 ± 9.64$^{\#*\&}$
Monocyte ROS generation, MFI	35.16 ± 7.00	101.38 ± 17.60$^{\#}$	44.77 ± 9.73$^{\#*}$	132.91 ± 35.50$^{\#*\&}$
Granulocyte phagocytosis percentage	49.29 ± 9.22	34.38 ± 16.39	68.05 ± 11.37$^{\#*}$	65.85 ± 7.07$^{\#*\&}$
Granulocyte phagocytosis index, MFI	31.54 ± 2.40	54.68 ± 12.19$^{\#}$	73.51 ± 14.15$^{\#*}$	55.32 ± 2.54$^{*\&}$
Granulocyte ROS generation, MFI	123.84 ± 30.00	359.03 ± 77.58$^{\#}$	147.60 ± 21.69*	656.95 ± 99.10$^{\#*\&}$
Phagocyte fraction expressing CD86	10.03 ± 2.70	97.50 ± 19.59$^{\#}$	51.67 ± 5.11$^{\#*}$	39.74 ± 9.51$^{\#*\&}$
CD86 expression level in phagocytes, MFI	41.19 ± 0.53	167.29 ± 42.78$^{\#}$	30.20 ± 2.00$^{\#*}$	66.28 ± 8.67$^{\#*\&}$
Phagocyte fraction expressing CD206	10.48 ± 0.96	1.33 ± 0.38$^{\#}$	12.48 ± 2.83*	23.79 ± 7.28$^{\#*\&}$
CD206 expression level in phagocytes, MFI	20.13 ± 2.62	45.32 ± 10.04$^{\#}$	96.12 ± 12.25$^{\#*}$	20.69 ± 0.48$^{*\&}$
Neutrophil to lymphocyte ratio	0.64 ± 0.19	0.68 ± 0.13	4.42 ± 0.13$^{\#*}$	0.88 ± 0.27$^{\&}$
Systemic immune inflammation index (SII)	139.65 ± 31.07	233.06 ± 40.73$^{\#}$	1760.89 ± 125.36$^{\#*}$	272.39 ± 65.47$^{\#\&}$

Notes: [a] — percentage of cells emitting fluorescence; [b] — the mean fluorescence per phagocytic cell; $^{\#}$ — $P \leq 0.05$ as compared to intact animals;

* — $P \leq 0.05$ as compared to sham-operated animals; $^{\&}$ — $P \leq 0.05$ as compared to animals with $A\beta_{1-40}$-induced AD.

Circulating phagocytes from rats with $A\beta_{1-40}$-induced AD were characterized by increased fraction of phagocytizing monocytes with decreased endocytic activity, moderately up-regulated granulocyte ROS generation along with temperate increase of $CD86^+$ mononuclear phagocyte fraction and high level of CD206 expression (Table). Two widely accepted indices of systemic inflammation: NLR and SII were higher in these animals than those in control rats by 6,5 and 7,5 times respectively. In rats with $A\beta_{25-35}$-induced disease, significantly increased granulocyte ROS generation was registered. NLR and SII values in these animals were slightly higher than those in control rats.

In this study, two most commonly used interventional models of AD were used. $A\beta_{1-40}$ represents full-length peptide which is predominantly present in amyloid plaques in AD patients. $A\beta_{25-35}$ represents the shortest fragment of full-length Aβ peptide with neurotoxic properties [8]. In both models, recapitulating of cognitive impairment, which is characteristic for AD patients, were observed in lesioned rats. Both models are considered as most appropriate for the study of AD-associated inflammation. Nevertheless, patterns of systemic inflammation manifestation differed significantly in animals with different models. Canonical indices of systemic inflammation (NLR and SII) were drastically increased in rats with $A\beta_{1-40}$ AD model. Cognitive impairment was also more prominent in these animals as compared to $A\beta_{25-35}$-induced model (data are not shown). Moreover, functional characteristics of circulating phagocytes (decreased monocyte phagocytic activity along with moderate increase in the number of $CD86^+$ cells) indicate appearance of circulating monocytic-myeloid-derived regulatory cells, that is typical in the course of prolonged systemic inflammation [9]. In rats with $A\beta_{25-35}$-induced model, NLR and SII values evidence low-grade systemic inflammation, of a rather reparative nature, considering augmented ROS generation by granulocytes, which was found to promote tissue reparative processes [10]. Literature data and our previous experiments indicate that pronounced peripheral inflammation is associated with aggravation of neuroinflammation and neurodegenerative disease progression [11, 12]. Therefore, $A\beta_{1-40}$ AD model reproduces disease-associated inflammation at the greater extent.

Conclusion

Taken together, our findings indicate that AD model in rats, based on intracerebral injection of full-length Aβ peptide ($A\beta_{1-40}$) is more appropriate for the study of inflammation in the disease pathophysiology, considering the association of significant cognitive impairment with prominent systemic inflammation in lesioned animals.

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ELECTROLYTIC AGGREGATION IN SOLUTIONS WITH QUANTUM DOTS AND GOLD NANOPARTICLES MODIFIED WITH OLIGONUCLEOTIDES

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Aim. To investigate electrolytic aggregation of different nano-objects in solutions with quantum dots (QDs) and Au nanoparticles (NPs) modified by oligonucleotides as well as the effect of aggregates on the photoluminescence (PL) of QDs.

Methods. Au NPs and AgInS2/ZnS QDs were modified by oligonucleotides. Two types of QDs that differ in size and stabilizing ligand were used. PL and optical absorption of nano-objects in water and SSC buffer solutions were studied.

Results. The transfer of modified by oligonucleotides QDs from water to a buffer solution and the addition of Au NP modified by oligonucleotides to the solution caused quenching of the QD PL intensity. The PL quenching was observed for the QDs of two types and increased during the incubation of solutions, but didn't depend on its multiplicity. An aggregation of Au-DP occurred only in buffer solutions with QDs of one type and increased with multiplicity of the buffer solution.

Conclusion. It is found that the electrolytic aggregation of Au NPs modified by oligonucleotides in buffer solutions with QDs depends on the QD type and didn't affect the quenching of the PL intensity of the QDs.

Key words: quantum dots, Au nanoparticles, photoluminescence, electrolytic aggregation.

Recently, gold nanoparticles (Au NPs) have become an important component widely used in optical bio-sensors due to their unique chemical and physical properties, as well as high biocompatibility [1]. In particular, when Au NPs are irradiated with visible light, the phenomenon of surface plasmon resonance (SPR) can be observed. It is caused by the resonant excitation of oscillations of free electrons on the surface of Au NPs (surface plasmon) when absorbing the energy of an incident electromagnetic wave [2]. In the optical absorption spectra of Au NPs, an SPR peak is observed. The intensity, spectral position, and half-width of SPR peak depend on the size and shape of the nanoparticle, as well as on its local dielectric environment. The interaction between metal NPs and luminescent semiconductor crystals of nanometer size, the so-called quantum dots (QDs), attracts considerable attention of researchers due to wide range of their potential applications extending from sensors to quantum information processing [3]. This interaction can lead to the enhancement of the QD photoluminescence (PL) intensity as well as to its reduction. PL enhancement occurs due to the excitation of surface plasmons in metal NPs and energy transfer to QDs [4], and quenching of PL is caused by the non-radiative transfer of energy from QDs to NPs [5]. In the last case, the so-called fluorescence (Foster) resonance energy transfer (FRET) takes place — the process of energy transfer from a fluorescent donor to a lower energy acceptor through a distance-dependent dipole-dipole

interaction [6, 7]. It is believed that quenching of QD PL in the presence of Au NP is an effect that acts at a short distance and weakens with distance much faster than the electromagnetic field of the SPR [4]. In the vast majority of fluorescent biosensors, the same effect of PL quenching by Au NPs is used, and the detection of oligonucleotides in vitro or in vivo occurs by recording the change in the PL intensity of QDs during formation the QD-Au NP conjugate or its decay [3]. However, it is known that Au NPs are inherent in electrolytic aggregation in salt buffer solutions [8], which can affect the PL of QDs. Therefore, in this paper the effect of electrolytic aggregation of Au NPs on the optical characteristics of QDs modified by thiolated oligonucleotides was investigated.

Materials and Methods

In this paper, water-soluble Au NPs with a diameter of 13 nm, stabilized by citrate, and aqueous solutions of QDs of two types: (1) QD-88 — a fraction of $AgInS_2/ZnS$ QDs with a diameter of ≈3 nm, stabilized with mercaptoacetic acid, (2) QD-92 – unfractionated preparation with QD $AgInS_2/ZnS$ with a diameter of 2–3 nm, stabilized with glutathione were used. In both types, the stabilizing ligands formed a negative charge on the QD surface. QDs and Au NPs were modified by oligonucleotides. The 23-base oligonucleotide GCTGAAGGGCTTTTGAACT (hereafter MP-SH), in which a sequence of four thymidines, 6 methylene groups, and a sulfhydryl group covalently connected to the 3′-end, was used for QD modification; and the 26-base oligonucleotide TGGCTGAGTGGACGATGA (hereinafter HS-DP), in which a sequence with eight thymidines, 6 methylene groups, and a sulfhydryl group covalently connected to the 5′-end, was used to modify Au NPs. The surface of Au NPs was additionally covered with lipoic acid (LA) and mercaptohexanol (MCH). Citrate on the surface of Au NPs is replaced by sulphurous compounds (LA, MCH, HS-DP), what makes the NPs more stable in a saline solution, preserving their water-soluble properties, and creating a negative charge on their surface [8, 9]. All solutions were prepared in deionized water and in SSC buffer solutions with different multiplicities (0.1×SSC, 0.25×SSC, 0.5×SSC).

PL spectra were excited by the 411 nm line of a continuous laser and recorded by a BLACK-Comet C-SR-50 spectrometer. PL intensity was also measured at a fixed wavelength of ~590 nm using a scanning spectrofluorometer "Synergy HT". Optical absorption spectra were recorded with a NanoDrop 2000 microvolume spectrophotometer, and optical absorption intensity at fixed wavelengths (~540 nm, ~620 nm, ~690 nm) was measured using a TitertekMultiskan MCC/340 photometric reader.

Results and Discussion

In the PL spectra of aqueous solutions of QDs of both types (Fig. 1, *a*, *b*), a broad PL band is observed in the yellow-red spectral region. The band is due to emission of defects in QDs [10]. QD88 are characterized by a longer wavelength position of the PL band maximum (~630 nm) compared to QD92 (~587 nm), which is consistent with bigger QD

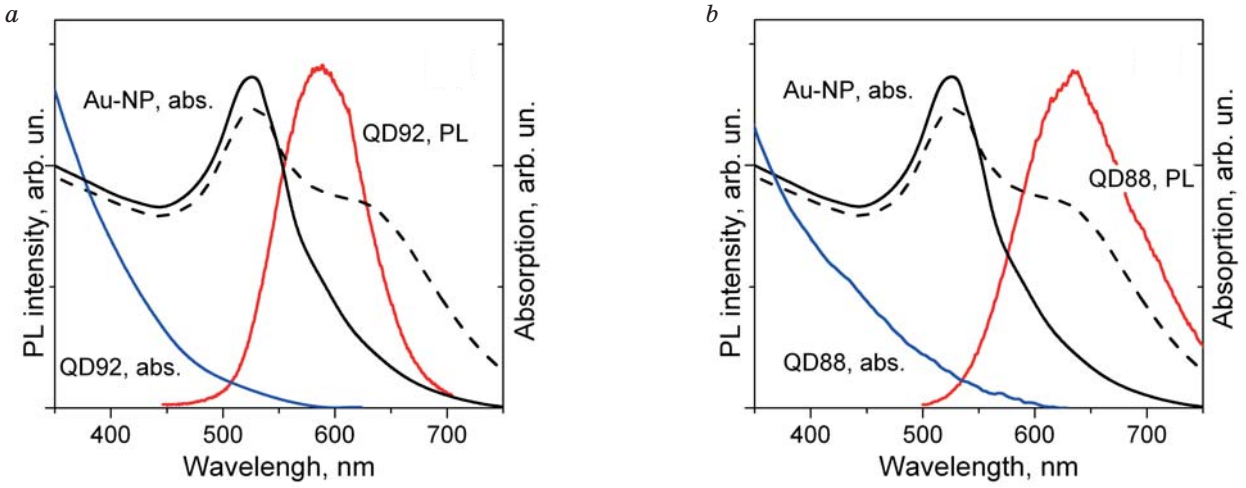

Fig. 1. **Optical absorption and photoluminescence spectra of QD92 (*a*) and QD88 (*b*) in water and optical absorption spectra of Au NPs in water (solid lines) and partially aggregated Au NPs (dashed lines)**

size in the case of QD88. The optical absorption spectra of QD solutions show an edge extended into the long-wavelength region without a well-defined exciton absorption peak. The extended edge in the QD absorption spectra of $A_1A_3B_6$ compounds is usually explained by scatter of QDs in size and shape, and/or optical transitions via the levels of defects in the QD band gap [11]. In the absorption spectra of solutions with Au NPs, a clear SPR maximum at ~510 nm is observed (Fig. 1). In the case of Au NP aggregation, the intensity of the peak at 510 nm decreased and a new peak at 640 nm appeared, due to SPR in the aggregated NPs.

Modification of the QDs with MP-SH oligonucleotides didn't change the spectral position of the PL peak and led to a slight increase of PL intensity in the case of QD88. At the same time, transfer of QDs modified with oligonucleotides (MP-QD) into a buffer solution resulted in a slight decrease in PL intensity (Fig. 2). It turned out that the addition of Au NPs modified with oligonucleotides (Au-DP) to the solutions with MP-QD88 or MP-QD92 also results in a decrease in the PL intensity of QD. The degree of PL intensity quenching with Au NPs depended on the type of QD solutions and increased with an increase of incubation time (Fig. 2).

The PL intensity decreased in all QD solutions after 24 h of incubation compared to 1 hour in the same solution. In particular, for MP-QD88 the PL intensity of solutions without Au-DP (Fig. 2a,c) decreased by 11%, 22.0%, 29.6% and 27.8% in water, 0.1×, 0.25× and 0.5×SSC, respectively; and for solutions with Au-DP by 27.9%, 43.0%, 39.5% and 40.7%, respectively. So, the decrease of MP-QD88 PL occurs more intensively in the presence of Au-DP. Similarly, the PL decrease in MP-QD92 solutions (Fig. 2, *b, d*) was also more noticeable in the presence of Au-DP, although the absolute indexes were smaller. In particular, the PL intensity of MP-QD92 without Au-DP

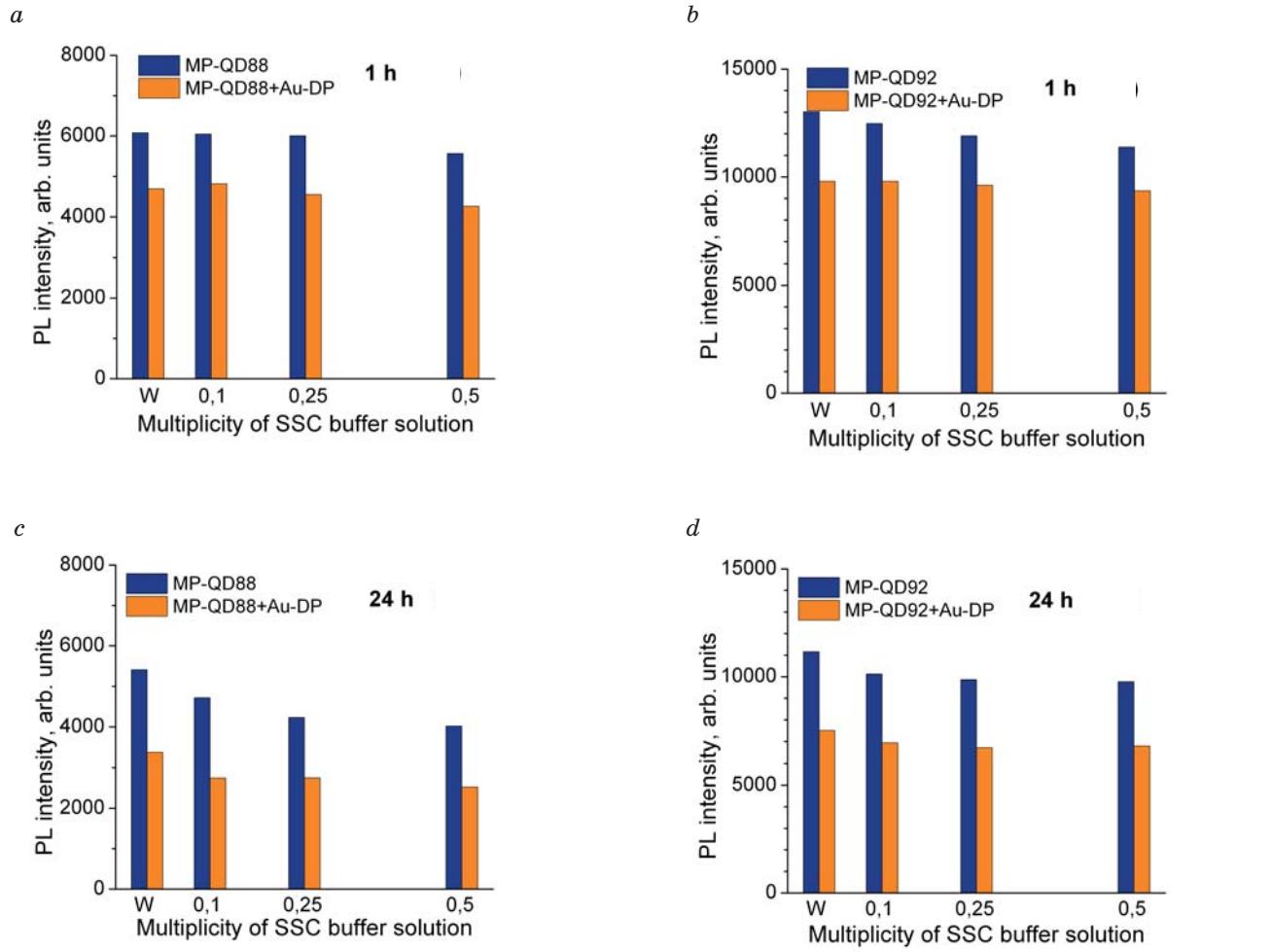

Fig. 2. **PL intensity of mixture of MP-QD88 (*a, c*) and MP-QD92 (*b, d*) with Au-DP in water (w) and in SSC buffer solutions of different multiplicity (0.1, 0.25, 0.5) detected 1 h (*a, b*) and 24 h (*c, d*) after preparation**

decreases in water and 0.1×, 0.25× and 0.5× SSC by 14.3%, 18.9%, 17.2% and 14.1%, respectively, and in the presence of Au-DP by 23.3%, 29.2%, 30.2% and 27.5%, respectively. As can be seen, the decrease in PL intensity during 24 h for a mixture of QDs and Au NPs is bigger in buffer solutions as compared to water. At the same time, there is no clear dependence of the degree of PL quenching the on the multiplicity of the buffer solution.

To detect the electrolytic aggregation of Au NPs and to study its influence on the PL of QDs, the optical absorption of QD and Au NP solutions was measured at the wavelengths corresponding to the SPR peak of unaggregated (~540 nm) and aggregated Au NPs (~620 and 690 nm) (Fig. 3). It has been turned out that the formation of aggregates depends on the type of QDs and the multiplicity of the buffer solution. In particular, in samples with MP-QD88 and Au-DP, an increase in absorbance at 620 nm and 690 nm, due to the appearance of Au NPs aggregates, was observed in the solutions with a multiplicity of 0.25× and 0.5×SSC one hour after the preparation and continued to increase during the 24 h of incubation (Fig. 3, *a, c*). Aggregation of Au NPs was not observed in water and in 0.1×SSC solution. Therefore, for MP-QD88, an increase in the concentration of the buffer solution promotes formation of Au NP aggregates. At the same time, in all solutions with MP-QD92 and Au-DP, the aggregation of Au NPs during 24 h was not recorded (Fig. 3, *b, d*).

As the analysis of the obtained results shows, in the buffer solutions of 0.5×SSC an obvious aggregation of Au NPs takes place for MP-QD88 and no sign of aggregation is found for MP-QD92. At the same time, for both types of QDs, the PL intensity decreases during 24 h. Similarly, for MP-QD88, a comparable decrease in PL intensity occurs in all buffer solutions, although aggregation is recorded

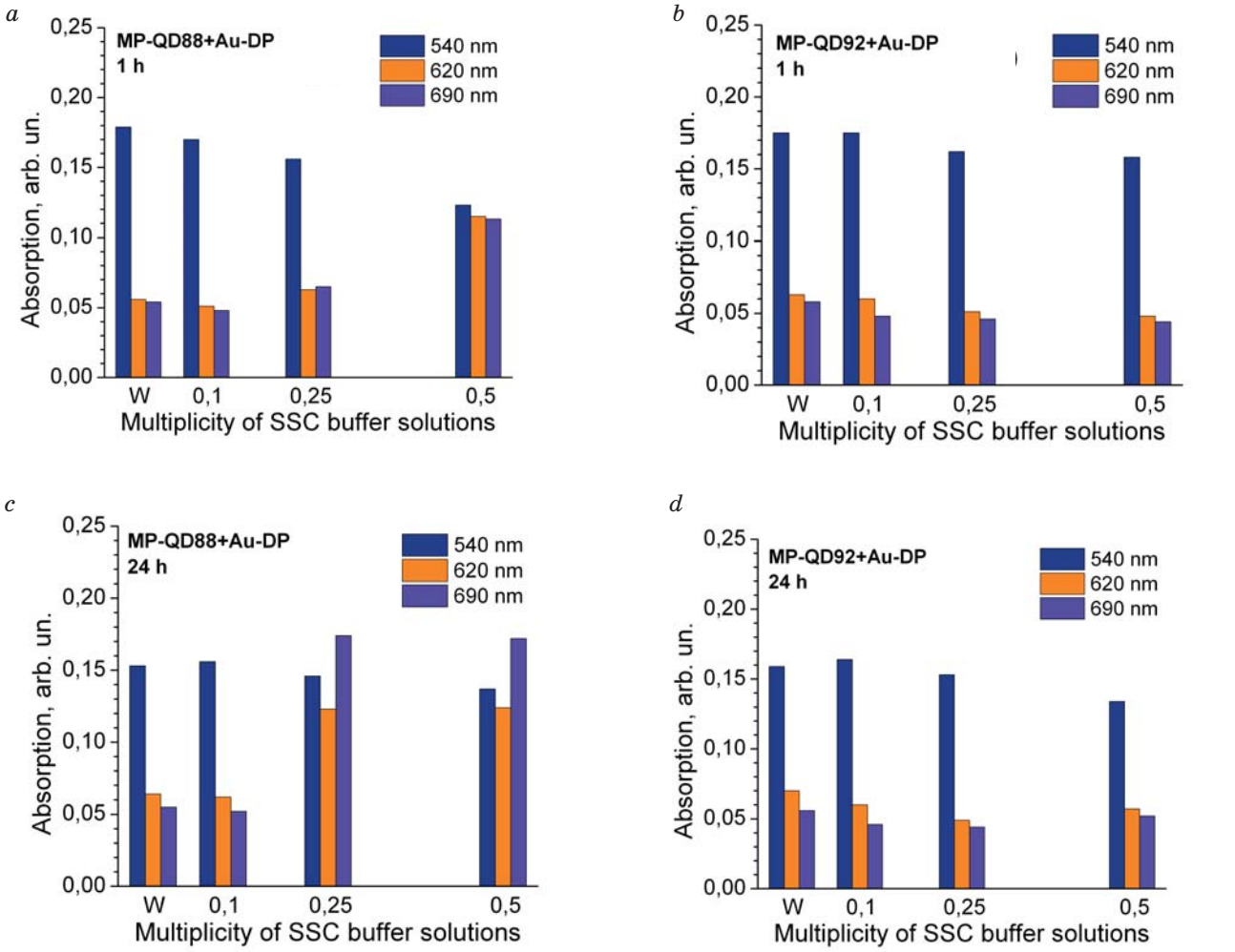

Fig. 3. **Optical absorption of MP-QD88 (*a, c*) and MP-QD92 (*b, d*) with Au-DP in water (W) and in SSC buffer solutions of different multiplicity 1 h (*a, b*) and 24 h (*c, d*) after preparation**

only for solutions with a multiplicity of 0.25× and 0.5×SSC. It can be concluded that the formation of electrolytic aggregates of Au NPs in solutions of QDs and Au NPs modified by oligonucleotides does not affect the decrease of PL intensity.

Conclusion

It is found that the transfer of modified by oligonucleotides $AgInS_2$/ZnS QDs from water to SSC buffer solution results in a decrease in PL intensity. The addition of Au NP modified by oligonucleotides to QD buffer solutions also causes a decrease in the PL intensity of QDs, but the magnitude of the effect is larger. It is found that the degree of PL intensity quenching depends on the type of QDs' stabilizing ligand (glutathione and mercaptocytic acid) and increases with the incubation time of the solutions from 1 h to 24 h. At the same time, the multiplicity of the buffer solution in the range from 0.1 to 0.5 has weak effect on the change in PL intensity.

It is found that the electrolytic aggregation of Au NPs modified by oligonucleotides in buffer solutions with QDs depends on the QD type. In particular, no signs of aggregation of Au NPs were observed within 24 h in buffer solutions with QDs (∅~2-3 nm) stabilized with glutathione and modified by oligonucleotides. At the same time, the aggregation of Au NPs in solutions with QDs (∅~3 nm) stabilized by mercaptoacetic acid and modified by oligonucleotides, was observed already 1 h after solution incubation. In this case, the degree of aggregation increased with the increase of the concentration (multiplicity) of buffer solution. It is found that the changes in the aggregate state of modified by oligonucleotides Au NPs in SSC buffer solution do not affect the decrease in PL intensity of QDs.

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CYTOKININ FRACTION OF THE *Hericium coralloides* INCREASES OXIDATIVE METABOLISM OF MURINE PERITONEAL MACROPHAGIES

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This study was *aimed* to examine influence of cytokinin fraction of basidiomycete *Hericium coralloides* on the spontaneous and induced phagocytic activity of murine peritoneal macrophages

Materials and methods. Mononuclear phagocyte (macrophage) fraction of peritoneal exudate of C57BL/6 mice was used. Macrophages were incubated under standard conditions at 37 °C, 100% humidity and 5% CO_2 for 4 hours. Phorbol 12-myristate 13-acetate (PMA) was added to part of the samples to activate oxidative metabolism. *Hericium coralloides* was added in two concentrations that were 5 and 10 times lower than the IC50, defined as an antiproliferative effect on colon cancer cells. Incubation with samples was carried out for 2 hours.

Result. When adding PMA, *Hericium coralloides* (0.017 and 0.035 μg/ml) and in the combination of PMA with *Hericium coralloides*, activation of reactive oxygen species (ROS) in peritoneal macrophages was revealed by 1.37–1.7 times, compared to the spontaneous activity of phagocytes.

Conclusions. Thus, the effect of the cytokinin extract of the basidial fungus *Hericium coralloides* was manifested by an increase in the phagocytic activity of peritoneal macrophages as one of the possible mechanisms of immunomodulatory action.

Key words: *Hericium coralloides*, NBT test, oxidative metabolism, peritoneal macrophages.

Fruiting bodies and cultivated mycelium of many basidial fungi contain biologically active substances that enhance innate and acquired immune responses and demonstrate antitumor activity in animal and human cell culture [1]. Growing mushrooms in culture is a convenient way to obtain a significant amount of mushroom mycelial biomass, which has all the valuable properties inherent in mushroom fruiting bodies. Previously, we found an antiproliferative, proapoptotic effect and modification of the metabolism of tumor cells *in vitro* for a number of medicinal basidiomycetes (crude extracts and cytokinin fractions) [2, 3].

The most active mushroom was *Hericium coralloides* (Scop.) Pers., strain 2332. Since the enhancement of the antiproliferative effect may be associated with immunomodulatory activity, it was important to determine the effectiveness of the cytokinin fraction of this mushroom on the primary link of innate immunity.

To examine the effect of cytokinin fraction of *Hericium coralloides* on the spontaneous and induced phagocytic activity of murine peritoneal macrophages.

Materials and Methods

Peritoneal macrophages were isolated from C57BL/6 mice by standard procedure according to Pietrangeli [4]. Spontaneous and induced oxidative metabolism was examined in the nitroblue tetrasolium (NBT) test. Briefly, 0.1 ml of NBT in a phosphate-buffered solution was added to the test samples to determine spontaneous activity.

To determine the stimulating activity, 0.1 ml of NBT and 10 ng/ml of phorbol 12-meristat-13-acetate (PMA) were added as an additional stimulus under standard conditions. Cytokinin fraction of *Hericium coralloides* was used at concentrations that were lower than IC_{50} (0.17 μg/ml) by 5 and 10 times (0.035 μg/ml and 0.017 μg/ml, respectively). Only 0.1 ml of phosphate buffer was added to the control wells. The cells were incubated for 1 hours at a temperature of 37 °C in a CO_2 incubator. After incubation, the 96-well plate was centrifuged for 10 min at 400 g. The supernatant was removed, and 0.2 ml of ethanol was added to the sediment. Centrifugation was repeated under the same conditions. After removing the supernatant, 0.1 ml of 0.1M KOH and 0.1 ml of DMSO were added to all wells, and the contents were carefully resuspended. The results were calculated using the spectrophotometric method at a wavelength of 540 nm. Spontaneous activity of peritoneal macrophages was expressed in conventional units. The percentage of stimulation of the activity of peritoneal macrophages was calculated according to the formula:

$$(St - Sp) / 0\ Sp \times 100\%,$$

where Sp — value of the optical density of a spontaneous sample; St — value of the optical density of the sample stimulated with PMA.

Results and Discussion

Spontaneous value of ROS generation in peritoneal macrophages was 0.43±0.035 a.u. (Fig. *A*). Incubation of macrophages with PMA led to an increase in this indicator to 0.63±0.021, which was almost 1.5 times more than spontaneous activity ($P < 0.05$). The addition of *Hericium coralloides* at a concentration of 0.017 μg/ml slightly increased the activity of NBT absorption by macrophages, which was 0.51±0.03 and was not significantly different from the control, while the concentration of *Hericium coralloides* of 0.035 μg/ml led to an increase in phagocytic activity by 37%, compared to the control and was 0.58±0.029 (Fig. *D*). Cell treatment with cytokinin fraction (concentration 0.035) μg/ml along with PMA resulted in the increase of ROS generation by 1.7 times.

It is known that cytokinins exhibit cytotoxic and immunomodulatory effects, they are considered as promising modern biologically active compounds with high therapeutic potential [5].

The content of the following cytokinins trans-zeatin, zeatin riboside and isopentenyladenine in *Hericium coralloides* most likely determines their ability individually and together with PMA to stimulate phagocytic activity. Therefore, the issue of

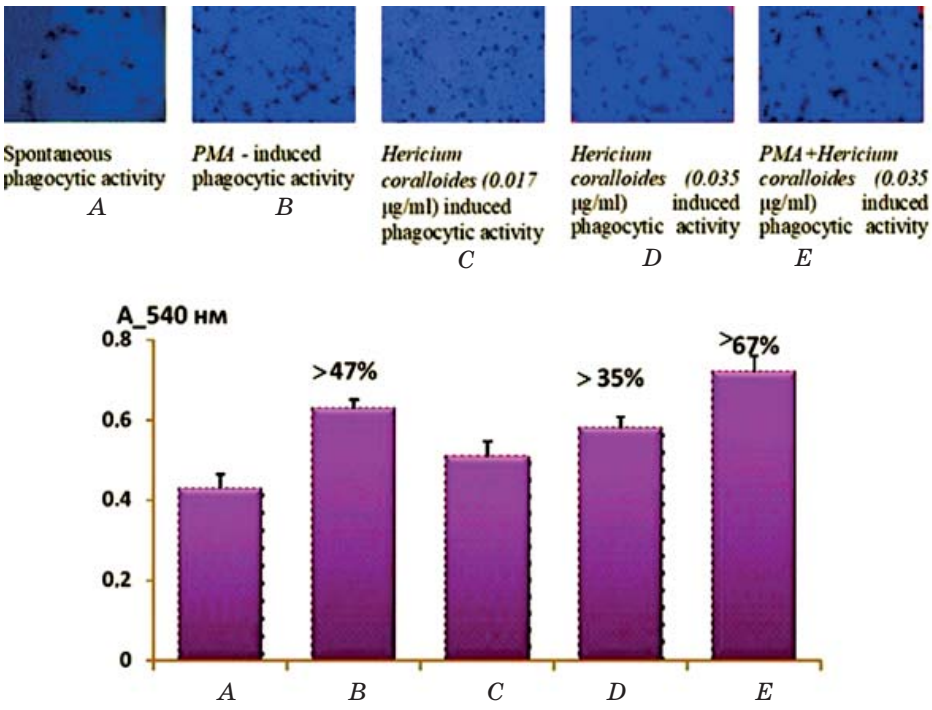

Fig. **Levels of the spontaneous and induced (PMA, *Hericium coralloides* and *Hericium coralloides*+PMA) oxidative metabolism of the peritoneal macrophages showed by the nitroblue tetrasolium (NBT) test**

pharmacological properties of phytohormones produced by mushrooms is extremely relevant and requires further research.

Conclusions

Thus, the effect of the cytokinin extract of the basidial fungus *Hericium coralloides* was manifested by an increase in the phagocytic activity of peritoneal macrophages as one of the possible mechanisms of immunomodulatory action.

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The authors state that they have no conflict of interest.

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IMMUNOGLOBULIN ISOTYPES AND BLOOD MONOCYTE SUBPOPULATIONS IN COVID-19 FEMALE PATIENTS WITH DIFFERENT DISEASE SEVERITY

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COVID-19, an acute respiratory infection caused by the SARS-CoV-2 virus, manifests itself in various severity forms - mild, moderate and severe, caused by the reactions of the patient's immune response.

Aim. To evaluate the serum levels of immunoglobulins G, M, and A and the number of circulating monocytes of different phenotypes in female patients with the abovementioned forms of COVID-19 severity.

Methods. Blood samples of 53 women with SARS-CoV-2 infection were studied. Flow cytofluorimetry was used to estimate monocyte subpopulations by the expression of CD14 and CD16. Concentrations of IgM, IgG, and IgA in the serum were determined in radial immunodiffusion test according to Mancini.

Results. The relative number of non-classical monocytes with CD14+-CD16++ phenotype was significantly decreased in the blood of COVID-19 patients from all 3 clinical severity groups, while changes in the number of classical and intermediate monocytes were insignificant. The levels of IgA in COVID-19 patients significantly decreased after recovery as compared to the acute phase of the infection.

Conclusion. The results emphasize the importance of monocyte subpopulation analysis in COVID-19 diagnosis and indicate dynamic changes in IgA levels depending on disease severity. The research data may help in the development of new diagnosis methods and therapy for SARS-CoV-2 infection.

Key words: COVID-19, circulating monocytes, immunoglobulin isotypes M, G and A.

SARS-CoV-2 infection involves various forms of human diseases, namely mild, moderate and severe ones depending on the patient's immune response [1]. The humoral immune response to SARS-CoV-2 is mediated by antibodies directed against the surface glycoproteins of the virus, mainly the S-glycoprotein and the nucleocapsid protein [2]. Monocytes are the main effector cells of the cytokine syndrome: they produce inflammatory cytokines and regulate the recruitment and activation of new tissue-damaging cells from the blood [3]. However, the connection between the disease's clinical forms and the functioning level of humoral and cellular immunity is still poorly explored. Coronavirus infection diagnosis is often based on the assessment of blood immunoglobulin content [4]. The evaluation of innate cellular immunity might enable the development of new approaches both for diagnosis and therapy [5].

This research was targeted to assess humoral and cellular immune response in patients suffering from COVID-19 of different disease severity, namely, to determine the number of different sub-populations of monocytes and the levels of IgG, IgM and IgA

in the COVID-19 female patients' blood before and after treatment.

Methods

Flow cytofluorimetry of heparinized blood samples was applied to determine monocyte populations by CD14 and CD16 expression. The method of radial immunodiffusion in agarose gel according to Mancini was used for the quantitative estimation of immunoglobulins G, A, and M in the patient's blood serum.

Participants and settings. 53 women took part in the experiment. The average age was 35 years (the youngest was 26 and the oldest was 44). All participants were divided into 3 groups depending on the severity of the coronavirus disease. Group 1 (mild course) consisted of 12 women; group 2 (moderate course) was the largest and included 30 women, and group 3 (severe course) was the least numerous and covered 11 female patients. The recommendations for treatment and taking medications depending on the symptoms and severity of the disease were provided by the family doctor listed in each participant's healthcare declaration. But additionally, as part of the experiment, each participant took vitamins C (500 mg), D3 (800 IU) and zinc (10 mg) daily during the illness.

Results and Discussion

The first step was to determine the level of immunoglobulins G, M and A in the blood serum of patients with COVID-19 of different disease severity. The obtained data indicate a normal concentration of immunoglobulin M (IgM) in all experimental groups before and after treatment. In patients with a severe form of infection at the time of recovery, a slight decrease in the concentration of IgM was observed, while in the other two groups, an increase in the concentration of this immunoglobulin was recorded after the disease (Fig. 1, *A*).

Although a decrease in IgG was found after recovery in each of the three groups, its concentration was also within the normal range. Dynamics of IgA concentration showed a decrease in patients' blood serum of all three groups after treatment, namely by 15.8% within the mild group, and by 21.2% within the other two groups, compared to the levels measured in the acute phase of the disease (Fig. 1, *A*, *B*, *C*).

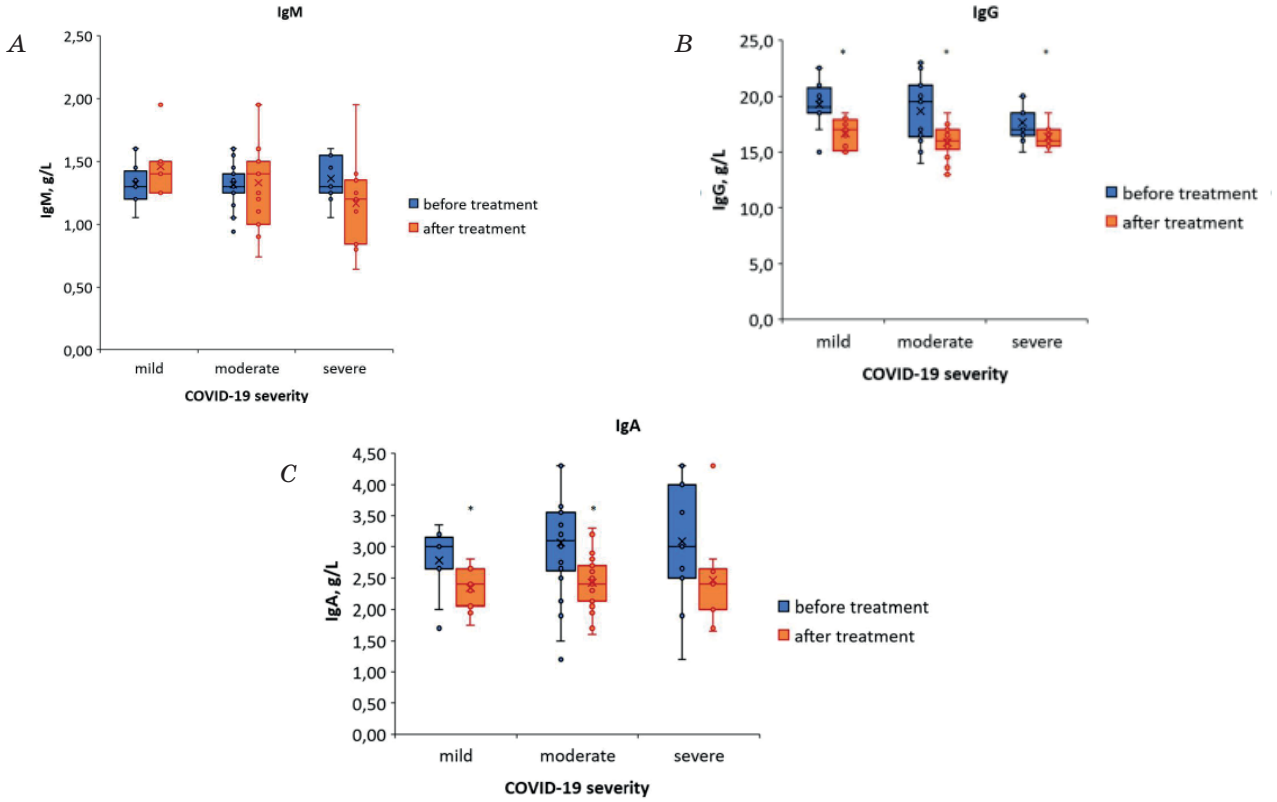

Fig. 1. **Levels of IgM (*A*), IgG (*B*), IgA (*C*) in blood serum of the COVID-19 female patients with different disease severity: mild, *n* = 12; moderate, *n* =30; severe, *n* =11**
* — $P < 0,05$ compared to the values before treatment.

The next step was to determine the number of monocyte populations with classical, intermediate and non-classical phenotypes based on the expression of CD14 and CD16 markers. In the group of patients with a moderate form of the disease, the greatest variability of the classical CD14+CD16- monocytes relative number was revealed: an equal number of patients (13%) had an excess number of this cell population in blood or this index decrease compared to the reference values. A decrease in the number of CD14+CD16- occurred in a third of patients with a mild form of Coronavirus infection. After the treatment, these cells were within the normal values in almost all the examined patients. As for the severe form of the disease, no significant deviations from the reference values were recorded (Fig. 2, *A*).

An increased number of intermediate monocytes was observed in all groups after treatment compared with those registered in the acute phase of the infection, but they did not exceed the reference range of values in both time points of the survey. In particular, the number of CD14+CD16+ monocytes increased almost twice and was at the upper limit of the reference range among women with moderate disease severity. Among the patients of the third group, who suffered from severe COVID-19, the number of cells with intermediate phenotype increased statistically insignificantly (Fig. 2, *B*). The relative number of non-classical monocytes with CD14+-CD16++ phenotype in the majority of patients from all examined groups, both before and after treatment, was lower than the reference values. Also, a decrease in these cells' index after treatment was registered in patients from each group: by 3.3 times — in the mild severity group, by twice — in the moderate severity group, and by 1.6 times – in the third group with the highest severity level. It is important to note that the number of CD14+-CD16++ cells was less than 1% in about 90% of those patients examined after treatment, which may indicate a persistent dependence of this subpopulation on the COVID-19 course and severity (Fig. 2, ***C***).

Conclusions

The results indicate that the SARS-CoV-2 infection is associated with prominent quantitative changes in the monocyte-macrophage lineage in female patients

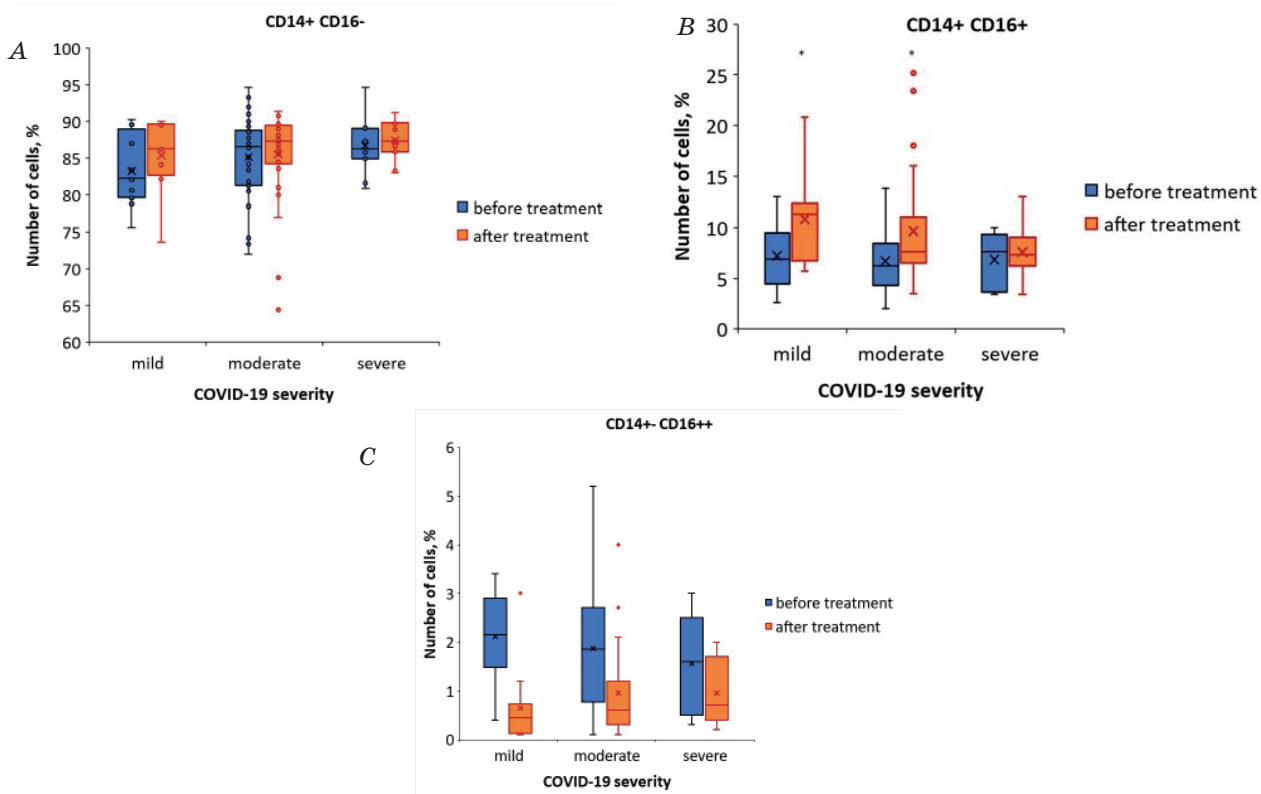

Fig. 2. **The relative number of classical CD14+CD16- monocytes (*A*), intermediate CD14+CD16+ monocytes (*B*), and non-classical CD14+-CD16++ monocytes (*C*) in female patients with different COVID-19 severity: mild, $n = 12$; moderate, $n = 30$; severe, $n = 11$**
*— $P < 0.05$ compared to the values before treatment.

with different disease severity. Significant severity-related decrease of the non-classical monocyte number in patient blood can indicate their important role in the resolution of inflammation during COVID-19. A noticeable decrease in the levels of immunoglobulins after recovery was typical for IgG and IgA, however, it was statistically significant for the female patients with mild and moderate COVID-19.

Based on the obtained results, the tendency to decrease the concentrations of immunoglobulins of all classes after the illness may indicate that after recovery, the level of virus-specific antibodies decreases over time. Also, a decrease may prove a deterioration of the functional properties of the immunity humoral link, which is expressed in a violation of their synthesis or an increase in catabolism and adsorption on immune complexes. Additional studies of the functional characteristics of monocytes are needed for their use as diagnostic markers of disease severity.

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GRAIN-RESIDING ENDOPHYTIC BACTERIUM *Paenibacillus polymyxa* P 6.3 POSSESSES GROWTH-PROMOTING ACTIVITY AND PROTECT WHEAT GRAIN FROM PATHOGENIC EFFECT OF PSEUDOMONAS SYRINGAE

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Aim. To examine the effect of endophytic bacteria *Paenibacillus polymyxa* P 6.3, which was isolated from grains of winter wheat variety Podolyanka, on the germination of wheat grains after the exposure *Pseudomonas syringae*.

Methods. Growth-promoting and biocontrol activity of *P. polymyxa* P 6.3 were examined using roll method. Standardized wheat grains were soaked in a suspension of 24 h culture of *P. polymyxa* P 6.3 for 12 h, control grains were soaked in sterile distilled H_2O. After soaking, both pre-treated and control grains (of 25 pcs) were put into rolls. In three days, half of both pre-treated and control grains were exposed to phytopathogen *P. syringae*, and germination was continued. The lengths of coleoptile and main root were measured on the 7th day of the experiment. Results were expressed as $M \pm m$. Differences were considered significant at $P \leq 0.05$.

Results. Treatment wheat grains with *P. polymyxa* P 6.3 resulted in increased growth of coleoptile and main root in all three varieties. Most prominent effect was registered in Favorytka variety. After the exposure to phytopathogenic pseudomonads, slowing down of the growth of coleoptile and main root occurred in all wheat varieties. Highest susceptibility to *P. syringae* pathogenic effect was registered in Holikovs'ka variety. Pre-treatment of wheat grains with endophytic bacteria abrogated growth-inhibiting effects of *P.syringae.*

Conclusion. Endophytic bacteria *P. polymyxa* P 6.3 exerts a growth-stimulating effect on wheat germination and a protective effect against *P. syringae*. The plant growth promoting potential and antagonistic activity make strain P 6.3 a promising biocontrol agent and growth stimulator as a biofertilizer.

Key words: endophytes, wheat, *Paenibacillus polymyxa*, *Pseudomonas syringae*, roll method.

Plant Probiotic Microorganisms (PPM) are useful, plant-associated microorganisms that provide a promising alternative to chemical fertilizers and pesticides as biofertilizers and biostimulants [1]. Particular attention is focused on endophytic bacteria as a group of PPM. A wide variety of endophytes with probiotic properties makes them a powerful tool in agroindustry. It is known that endophytic bacteria promote plant growth and nutrient uptake, and also have a biocontrol activity [2, 3]. Their employment as bioinoculants is a promising practice in multitudinous parts of the world [4], but the best strategy for their application in agriculture is still unknown [5]. Since wheat is a significant food crop in Ukraine, the selection of endophytic composition and development of agrobiotechnological approaches based on them could be a new ecological way to achieve a high-quality wheat crop.

To examine the effect of endophytic bacteria *Paenibacillus polymyxa* P 6.3, which was isolated from grains of winter wheat

variety Podolyanka, on the germination of wheat grains after the exposure *Pseudomonas syringae*.

Material and Methods

Three wheat varieties of Ukrainian selection were used in the study: two winter wheat varieties (*Triticum aestivum* L.) — Favorytka and Chyhyrynka, and one spring wheat variety (*Triticum dicoccum* Schuebl) — Holikovs'ka. Growth-promoting and biocontrol activity of *P. polymyxa* P 6.3 were examined using roll method [6]. Standardized wheat grains were soaked in a suspension of 24 h culture of *P. polymyxa* P 6.3 for 12 h, control grains were soaked in sterile distilled H_2O. After soaking, both pre-treated and control grains (of 25 pcs) were put into rolls. In three days, half of both pre-treated and control grains were exposed to phytopathogen *P. syringae*, and germination was continued. The lengths of coleoptile and main root were measured on the 7^{th} day of the experiment. Results were expressed as M ± m. Differences were considered significant at $P \le 0.05$.

Results and Discussion

Treatment wheat grains with *P. polymyxa* P 6.3 resulted in increased growth of coleoptile and main root in all three varieties (Fig. *A, B*). Most prominent effect was registered in Favorytka variety: length of coleoptile has risen by 24%, and length of main root — by 40% as compared to untreated control. Less pronounced effect was observed in Chyhyrynka variety. After the exposure to phytopathogenic pseudomonads, slowing down of the growth of coleoptile and main root occurred in all wheat varieties. Highest receptivity to *P. syringae* pathogenic effect was registered in Holikovs'ka variety: the length of coleoptile was 12% lower, and the length of main root — 30% lower than those in control untreated grains. Surprisingly, treatment with phytopathogenic bacteria led to the increase of main root length in Favorytka grains, and did not affect germination in Chyhyrynka variety. Pre-treatment of wheat grains with endophytic bacteria abrogated growth-inhibiting effects of *P. syringae*: values of lengths of coleoptile and main root in exposed grains did not differ significantly from these values in corresponding untreated control.

In this study, we found that treatment of wheat grains with endophytic bacteria, which we previously isolated from wheat variety with high resistance to causative agent of basal glume rot and leaf blight — *P. syringae* [7], stimulates growth of coleoptile and main root in three other wheat varieties of domestic selection, and abolishes negative effect of phytopathogen on grain germination. These growth-promoting and preventive effects slightly differed between examined varieties. According to literature data, wheat resistance to bacterial diseases is reached when frost

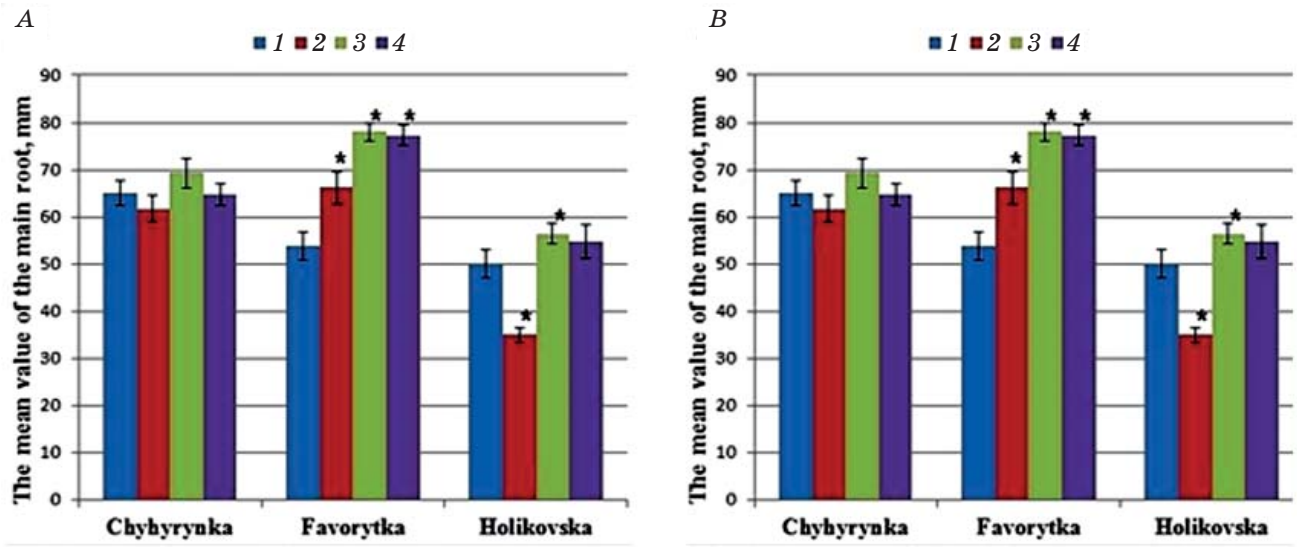

Fig. **Effect of *P. polymyxa* P 6.3 on coleoptile (*A*) and main root growth (*B*) in wheat grain:** *1* — control; *2* — seedlings infected with phytopathogen; *3* — grains pre-treated with *P. polymyxa* P 6.3; *4* — grains pre-treated with the investigated endophyte followed by infection of seedlings with phytopathogens, $M \pm m$, $n = 25$, $^*P \le 0.05$ compared to control

tolerance and disease-resistance genes are combined in the same genetic background [8]. All three varieties are characterized by high winter hardiness and high resistance to fungal diseases. Nevertheless, high growth-promoting and phytopathogen-protecting effects of endophytic bacteria were observed in winter wheat variety Favorytka and spring wheat variety Holikovs'ka with different vulnerability to *P. syringae*. One can suppose the existence of additional determinant of high receptivity of wheat grains to positive effects of external endophytic bacteria - their own endophytic community, which governs and maintains defense responses in host plant, and probably positively perceives foreign endophytic bacterium. However, our assumption needs in further experimental validation.

Conclusion

Endophytic bacteria *P. polymyxa* P 6.3, depending on the individual characteristics of varieties, have a growth-stimulating effect on wheat germination and a protective effect against *P. syringae*. The plant growth promoting potential and antagonistic activity make strain P 6.3 a promising biocontrol agent and growth stimulator as a biofertilizer.

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EPITOPES IDENTIFICATION OF BROADLY NEUTRALIZING MONOCLONAL ANTIBODIES AGAINST *Corynebacterium diphtheriae* EXOTOXIN

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Better and high-potency vaccines against diphtheria are urgently needed to provide broader protection against diverse strains and subtypes. Identification of novel broadly neutralizing epitopes targeted by protective antibodies could aid in such efforts.

Aim. In this study we focused on the search of binding sites identification of anti diphtheria toxin monoclonal antibodies and their neutralizing activity to block binding of recombinant exotoxin derivates with host receptors.

Methods. *Vero* cells were cultured in the complete RPMI-1640 medium under standard conditions and used for flow cytometry assay. Recombinant antigens and products of tryptic hydrolysis of CRM197 and SbB were characterized by Ni^{2+}-NTA affinity chromatography and SDS-PAGE under reducing conditions with following ECL Western-Blot using several hybridomas clones of anti-diphtheria toxin monoclonal antibodies.

Results. ECL western blot film results for clone 9.1-E11 showed the specific binding both to whole CRM197 molecule, and to almost all fragments of CRM197 formed as a result of limited proteolysis. In particular, a band corresponding to SbB in molecular weight can be identified. Thus, epitope region of the CRM197 molecule specific to 9.1-E1 mAbs is located within the structure of SbB. At the same time 16.4-E9 clone antibodies had high specificity to R-domain of SbB. In addition, both hybridoma clones antibodies have neutralizing activity against the DT binding subunit, which is a key factor in blocking between cell receptor and it ligand, *C. diphtheriae* exotoxin.

Conclusions. The results obtained indicate that produced antibodies are prospective for improving new diagnostic tools and therapeutic agents, which are used for treatment and understanding of the molecular mechanisms of diphtheria pathogenesis.

Key words: diphtheria toxin, CRM197, SbB, monoclonal antibodies, proHB-EGF, epitopes mapping, neutralizing antibody.

Diphtheria is a potentially fatal infection caused by toxigenic *Corynebacterium diphtheria* strains. The causative agent of diphtheria and diphtheria toxin (DT) are well studied, but thousands of diphtheria cases are still reported annually from several countries in Asia and Africa, along with many outbreaks worldwide [1]. Diphtheria is characterized by the formation of a pseudomembrane in the throat, but cutaneous infections are possible together with high mortality rate, which highlights the need for the development new diagnostic and therapeutic tools. CRM197 (Cross-Reacting-Material 197) is a variant of most studied non-toxic analogue of DT featuring a single site-specific substitution (G52E) which does not affect the deoxyribonuclease activity [2] but suppresses the ADP-ribosylating activity and the toxicity of the parental protein [3]. CRM197 is a commonly used glycoconjugate carrier that improves the immunogenicity of vaccines, particularly in infants [4]. Considering the attention that fusion vaccines are gaining, we thought it of interest to develop and analyze monoclonal

antibodies (mAbs) against DT (or CRM197) that will reveal the key sites of the effect of the toxin on cell receptors and become therapeutic agents for the prevention of the *C. diphtheriae* disease.

Methods

Cell cultures. Vero cell line was obtained from the Bank of Cell Lines of R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine, Kyiv. Cells were cultured in RPMI-1640 medium (Sigma Aldrich, USA) supplemented with 2 g/l sodium bicarbonate and 10% fetal bovine serum (FBS) with the addition of the antibiotic-antimycotic solution to prevent bacterial and fungal contamination.

Production of recombinant proteins by E. coli cells and their purification. Protocols for CRM197 and recombinant fragments of DT expression in *E. coli* Rosetta BL21 (DE3) cells on LB media by IPTG induction were elaborated earlier and published in previous publications [5]. Purification of proteins by Ni-NTA agarose column chromatography with imidazole elution gradient and determination of protein concentration by tricine SDS-PAGE analysis with TotalLab TL120 software were also performed according to [6]. *Enhanced chemiluminescence western blot* (ECL) protocol was described in [7]. *Limited proteolysis (trypsinolysis)* of CRM197 and SbB was carried out as described in [8] with modification.

Flow cytometry. The protocol of sample preparation for flow cytometry, including cell detachment from culture flasks surface, assessment of cell density in a counting chamber, endocytosis suppression by NaN_3 and low temperature (4 °C), adjusting conditions of incubation medium to reduce a cell surface non-specific protein adsorption by 1% BSA, were described in detail in [5].

Results and Discussions

For evaluation of the selected mAbs samples specificity to fragments of the CRM197, limited proteolytic cleavage by trypsin (EC: 3.4.21.4) of CRM197 and SbB was carried out. Epitope mapping of the CRM197 molecule was conducted by the western blot method (ECL) individually for each of the three selected samples of mAbs (9.2-H4, 9.1-E11, 16.4-E9 clones). The binding specificity of mAbs was determined for whole molecules of CRM197, DT recombinant fragments SbB, SbA, R-domain, as well as cleaved fragments obtained as a result of limited trypsinolysis of CRM197 and SbB. The blotogram for clone 9.1-E11 demonstrated that the chosen antibodies show specific binding not only to the whole CRM197 molecule, but also to almost all fragments of CRM197 formed as a result of limited proteolysis (Fig. 1, *A, B*). In particular, a band corresponding to SbB in molecular weight can be identified. Result indicates that the epitope region of the CRM197 molecule specific to mAbs obtained from clone 9.1-E11 is located within the structure of SbB. Analyzing the blotogram obtained during testing of clone 16.4-E9, high specificity for SbB and R-domain was observed (Fig. 1, *C, D*). Since the R-domain is part of SbB, it can be concluded that the R-domain contains an epitope region specific to mAbs expressed by clone 16.4-E9. We hypothesize that these mAbs can block the binding of DT to cellular receptors.

The natural receptor for DT on the cell surface is the membrane-anchored form of HB-EGF, proHB-EGF, is biologically active, providing mitogenic stimulation to neighboring cells in a juxtacrine mode [9]. Significant amount of proHB-EGF is present on the surface of the *Vero* cell line. To determine the neutralizing activity of the studied mAbs samples, a fluorescent derivative of DT - EGFP-SbB (SbB fused with green fluorescent protein in one open reading frame), which specifically interacts with the above-mentioned receptors was used. Among the tested mAbs, were identified two samples (clones 16.4-E9 and 9.1-E11) the neutralization efficiency of which was almost 100% compared to the control. Thus, mAbs are able to specifically bind to SbB of DT, while preventing its interaction with partner molecules on cell surface (Fig. 2). Our experiments confirm assumption regarding the ability of mAbs samples 16.4-E9 and 9.1-E11 to block the binding of DT with host cell receptors.

Conclusions

Among mAb-producing clones obtained in our laboratory three clones were characterized (9.1-E11, 16.4-E9 and 9.2-H4), which have the highest specificity to CRM197 and to individual target antigens (SbB, and R-domain). Moreover, it was found that clones 9.1-E11, 16.4-E9 have a neutralizing activity to preventing binding of DT to the proHB-EGF

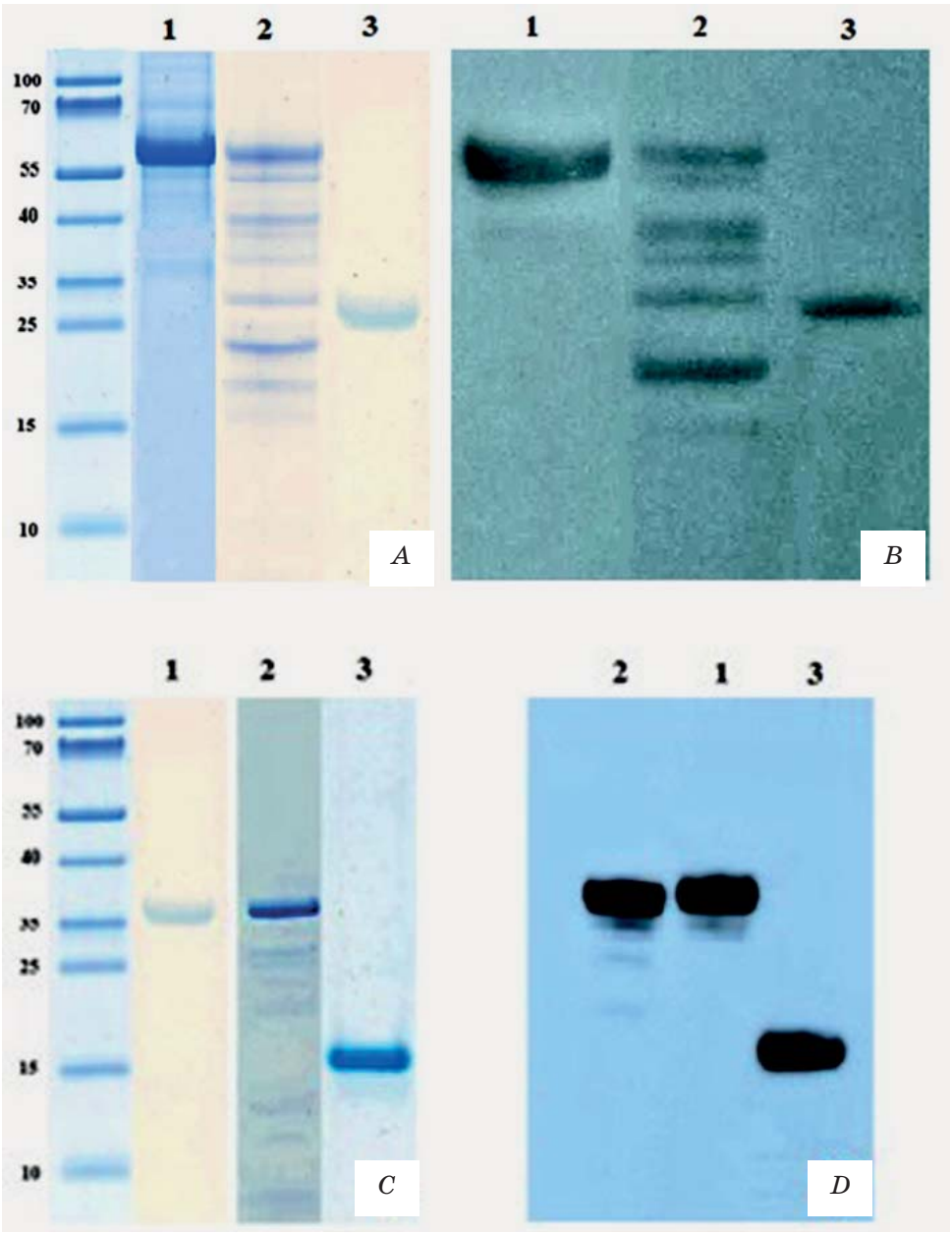

Fig. 1. **Epitope mapping of CRM197 and SbB by mAbs of clones 9.1-E11 (*A, B*) and 16.4-E9 (*C, D*):** *A* — SDS-PAGE of entire CRM197 molecule (1), after trypsinolysis (2), SbB (3); *B* — ECL blotting of corresponding electropherogram by mAbs; *C* — SDS-PAGE of SbB molecule (1), SbB after trypsinolysis (2), R-domain (3); *D* — ECL blotting of corresponding electropherogram by mAbs.

receptor, which is a perspective for improving existing and developing new diagnostic and therapeutic agents based on mAbs, including for prevention, treatment and understanding of the molecular mechanisms of diphtheria pathogenesis.

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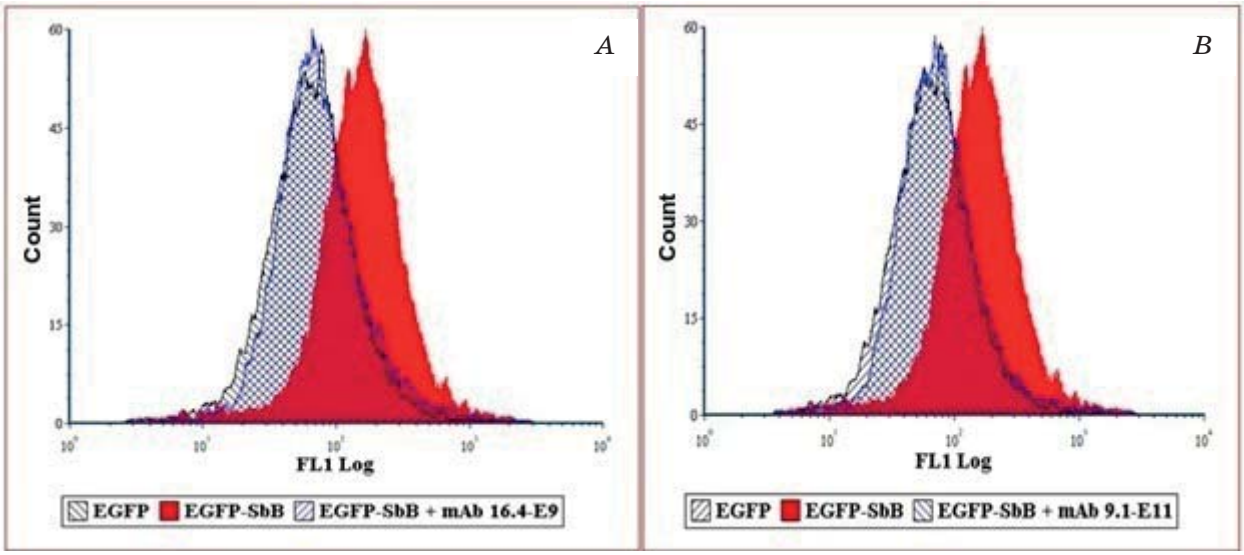

Fig. 2. **Fluorescence intensity of *Vero* cells incubated with SbB-EGFP. Detection of the ability of mAbs from clones 16.4-E9 (*A*) and 9.1-E11 (*B*) to block the interaction of SbB-EGFP with proHB-EGF**

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INDICATORS OF THE SKIN MICROBIOTA AND THE PHAGOCYTIC ACTIVITY IN MEAT AND EGG PRODUCTION WORKERS

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Aim. The analysis of the indicators of skin microbiota and phagocytic activity of neutrophils and monocytes in employees of the "Peremoga Nova" poultry farm.

Methods. The presence of sanitary and epidemiologically important groups of bacteria and the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) on the skin surface, leukogram parameters and phagocytic activity of professional phagocytes were determined. The indicators of students of the Bohdan Khmelnytsky National University of Cherkasy were used as a control.

Results. It was found that the MAFAnM index in the experimental group (3.2×10^3 CFU/cm^3) is significantly lower than in the control group (2.7×10^3 CFU/cm^3), however, the percentage of *Staphylococcus spp*. carriers is higher (67.5% versus 40.0%). In the experimental group, the relative and total number of monocytes is significantly higher compared to the control group. There is a positive correlation between the phagocytic number and the phagocytic index of monocytes and the value of MAFAnM.

Conclusions. There was an increase of the level of monocytes in meat and egg products workers against the background of the presence of bacteria of the *Staphylococcus spp*. group on the skin. It may indicate the activation of pro-inflammatory factors at the level of peripheral blood. An increased percentage of staphylococcal carriers is a sign of adaptation of *Staphylococcus spp*. bacteria to the antibiotics used in the manufacturing process.

Key words: skin microbiota, phagocytic activity, poultry farm, sanitary condition.

The modern industrial production of food products requires compliance with strict requirements for the sanitary condition of working areas and compliance with the hygiene standards by personnel. One of the important areas of control is microbiological monitoring. Microbial contamination can cause a deterioration of the employees' health and the development of occupational diseases in addition to affecting product quality [1]. An important biomarker of negative exogenous effects on the human body is the indicator of natural resistance [2]. A normal microbiota of the human body evolved in parallel with the formation of an immune system [3]. The question of the relationship between the microbiota of the body and factors of the human immune system in the conditions of specific production activities, in particular, remains largely open.

The analysis of the indicators of the skin microbiota and the phagocytic activity of neutrophils and monocytes in employees of the SOE "Peremoga Nova" poultry farm.

Materials and Methods

The microbiota biomaterial was obtained by rinsing from hands with a disposable applicator in a transport tube onto sterile nutrient media. The presence of bacteria of the *Escherichia coli* group

(ECG) was determined; *Staphylococcus spp.* (Staphylococcus); *Enterococcus spp*. (group D Streptococcus b-hemolytic); the total microbial count was assessed (an indicator of mesophilic aerobic and facultative anaerobic microorganisms, *MAFAnM*). Peripheral blood samples were obtained at the Municipal Non-Commercial Enterprise "Cherkasy Central District Hospital", where the examined people underwent a professional examination. The leukogram parameters were determined on the basis of a stained smear and the phagocytic activity of neutrophils and monocytes was determined by the ability to absorb latex particles. Control indicators were obtained from students of the Bohdan Khmelnytsky National University of Cherkasy. The number of examined people in both groups was 15 people. After testing for the normality of the distribution, the samples were compared using the Student's t-test. Relationships between indicators were analyzed by determining the Pearson correlation coefficient.

Results and Discussion

It was found that in the control group, bacteria of the *Escherichia coli* group were present in 22% of samples, in the experimental group in 20%; *Staphylococcus spp*. was present in 40% of the examined participants from the control group and 67.5% of the experimental one, *Enterococcus spp*. in 8% of the control and 5% of the experimental group, the average value of the MAFAnM index was 3.2×10^3 CFU/cm^3 in the control group and 2.7×10^3 CFU/cm^3 in the experimental group; the employees of the SOE "Peremoga Nova" had a significantly higher relative and total number of monocytes compared to the control (Table 1).

Within the groups, no significant difference was found between those examined with the presence of a sanitary-important microbiota and its absence. The employees of the SOE "Peremoga Nova" have a statistically significant positive correlation between the phagocytic number, phagocytic index of monocytes and the value of MAFAnM (Table 1).

Recent studies show an important role of the body's normal microbiota in immunological processes. There must be constant effective contact between the immune system and the microbiome. Sufficient exposure to a large number of microbes is critical for the development of a normal, functioning immune system. However, the resident microbiota can become pathogenic in case of injury, wound, or weakening of the host's immunity [3, 4].

Complex relationships with the immune system are observed not only in endosymbionts but also in bacterial groups on the body surface [5]. The mechanisms of interaction between the commensal skin microbiota and the immune system, in particular, during the development of inflammatory processes, are still not well understood [6]. An increased level of monocytes in workers of the SOE "Peremoga Nova" is an indirect sign of the activation of pro-inflammatory processes [7], which may be due to a constant contact with biological objects, antibiotics, premix feed, allergenic factors, etc. The maximum indicators of the phagocytic activity of monocytes were found in the carriers of *Staphylococcus spp*; apparently, they determined the statistically significant correlation between the phagocytic index of monocytes and the MAFAnM value. The highest percentage of *Staphylococcus spp.* in the experimental group may be a sign of adaptation of staphylococci to antibiotics. This is the only indicator of sanitary important forms that was higher than in the control. In general, the level of MAFAnM in the experimental group is lower than in the control, which indicates the proper sanitary condition of the working premises and the observance of hygiene standards by the personnel.

Conclusions

The indicators of MAFAnM among the workers of the meat and dairy products of the SOE "Peremoga Nova" are within the sanitary and hygienic norm, however, an increase of the monocytes' level against the background of the presence of *Staphylococcus spp*. group's bacteria on the skin may indicate the activation of pro-inflammatory factors at the level of peripheral blood. An increased percentage of carriers of staphylococci in the experimental group is a sign of adaptation of *Staphylococcus spp*. to antibiotics used in the production process.

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Table 1. **Indicators of professional phagocytes and MAFAnM in control and experimental groups**

Components	Control (n = 15) M ± SE	Experimental group (n = 15) M ± SE
Monocytes, %	6.21 ± 0.34	11.01 ± 0.67 ***
Monocytes, $\times 10^9$ /l	0.42 ± 0.06	0.65 ± 0.08 *
Neutrophils, %	59.11 ± 2.37	53.55 ± 1.72
Neutrophils, $\times 10^9$ /l	4.54 ± 0.74	3.01 ± 0,26
Phagocytic index of neutrophils	6.01 ± 0.57	6.75 ± 0.42
Phagocytic number of neutrophils	75.35 ± 0.95	75.22 ± 0.67
Phagocytic index of monocytes	5.44 ± 0.71	6.81 ± 0.51
Phagocytic number of monocytes	74.96 ± 0.82	76.12 ± 0.74
MAFAnM, $\times 10^3$ CFU/cm^3	3.20 ± 0,04	2.72 ± 0,08 ***
Correlation coefficients$_{MAFAnM/PhIN}$	0.35	0.46
Correlation coefficients$_{MAFAnM/PhNN}$	0.37	0.41
Correlation coefficients$_{MAFAnM/PhIM}$	0.44	0.86#
Correlation coefficients$_{MAFAnM/PhNM}$	0.42	0.65#

Note: * — $P < 0.05$; ** — $P < 0.01$; *** — $P < 0.001$: indicates significant differences in comparison with control; MAFAnM/PhIN — correlation between MAFAnM and the neutrophil phagocytic index; MAFAnM/PhNN — correlation between MAFAnM and the phagocytic number of neutrophils; MAFAnM/PhIM — correlation between MAFAnM and the phagocytic index of monocytes; MAFAnM/PhNM – correlation between MAFAnM and the phagocytic number of monocytes; # — significant correlation, $\boldsymbol{P} < 0.05$.

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THE IMPACT OF BILIARY DRAINAGE MODE ON BACTERIOBILIA OCCURRENCE IN PATIENTS WITH HILAR MALIGNANT OBSTRUCTION

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The *purpose* of this study was comparative assessment of the frequency of bacterial colonization of the bile in patients with hilar malignant biliary obstruction after the palliative biliary decompression using different methodological approaches.

Methods. 50 patients with proximal mechanical jaundice of tumor origin aged of ~62 years (25 males and 25 females), who were on steady-state treatment in Main military clinical hospital (Kyiv, Ukraine) were recruited in this prospective study. All patients underwent cholangiostomy using percutaneous transhepatic (PTBD) and external-internal suprapapillary (EISBD) approaches. Bile specimens were taken right after the biliary drainage. Identification of bacterial isolates was performed using standard cultural and biochemical methods.

Results. The incidence of cholangitis was almost twice lower in EISBD group (n = 26) than in PTBD group (n = 24): 25.6% vs 49.1%. The rates of bacteriobilia did not differ significantly in patients from different groups: 23.1% in EISBD group and 25.0% in PTBD group. However, the frequency of biliary bacterial colonization coupled with cholangitis was also 2 times lower in EISBD group in comparison with patients underwent PTBD: 7.7% vs 16.7%. *Escherichia coli* predominated in bile specimens from patients with bacteriobilia associated with cholangitis in both groups.

Conclusions. The use of EISBD for palliative biliary decompression in patients with proximal mechanical jaundice of tumor origin is associated with lower risk of bacterial colonization of the bile as compared to PTBD approach, and as a result with less risk of the development of infectious complications.

Key words: hilar malignant biliary obstruction, biliary decompression methodology, bacteriobilia.

Palliation of jaundice is therapeutic purpose in majority patients with malignant hilar obstruction [1]. Malignant obstruction to outflow of bile predisposes to bacteriobilia, in part as a result of high frequency of the use of biliary interventions and immunocompromised state in these patients [2, 3]. Biliary tree is usually the aseptic system. Obstruction in biliary tree magnifies ductal pressure, causes biliary stasis and multiplication of bacteria (bacteriobilia), and, as a result, the development of cholangitis. There are three main pathways of the infection of bile system: ascending from ampulla, hematogenous, and lymphatic. Numerous experimental studies evidences that malignant biliary obstruction impairs already compromised local and systemic innate immunity due to metabolic exhausting of phagocytic cells, that in turn facilitates further bacterial translocation and can adversely affect the clinical outcome [4]. Biliary decompression methodology is one of the common factors crucially affecting bacteriobilia occurrence. The purpose of this study was comparative assessment of the frequency of bacterial colonization of the bile in patients with hilar malignant biliary obstruction after the palliative biliary

decompression using different methodological approaches.

Materials and Methods

50 patients with proximal mechanical jaundice of tumor origin aged of ~62 years (25 males and 25 females), who were on steady-state treatment in Main military clinical hospital (Kyiv, Ukraine) were recruited in this prospective study. After providing informed consent, all patients underwent cholangiostomy under ultrasound and X-ray control using percutaneous transhepatic (PTBD) and external-internal suprapapillary (EISBD) approaches. Bile specimens were taken right after the biliary drainage. Identification of bacterial isolates was performed using standard cultural and biochemical methods according to EUCAST. The clinical diagnosis of cholangitis was established on the basis of the following criteria: body temperature above 38.5 °C, white blood cell count > 10×10^9/L, and neutrophil granulocytes percentage > 70. Statistical processing of the obtained data was performed using the IBMSPPS Statistics 22 package. Descriptive statistics were carried out. The normality of the distribution of variables was assessed using the Shapiro-Wilk test.

Results and Discussion

The incidence of cholangitis was almost 2 folds lower in EISBD group (***n*** = 26) than in PTBD group (***n*** = 24): 25.6% vs 49.1%. The rates of bacteriobilia did not differ significantly in patients from different groups: 23.1% in EISBD group and 25.0% in PTBD group. However, the frequency of biliary bacterial colonization coupled with cholangitis was also 2 times lower in EISBD group in comparison with patients underwent PTBD: 7.7% vs 16.7%. *Escherichia coli* predominated in bile specimens from patients with bacteriobilia associated with cholangitis in both groups. *Klebsiella* spp. was also isolated from infected bile specimens from both groups. *Enterococcus* spp. was isolated in patients from PTBD group but not from EISBD group (Table).

All isolated bacteria were sensitive to ceftriaxone. Regular sanitation of the drainage along with the appointment of ceftriaxone allowed to eliminate the clinical manifestations of cholangitis and achieve bile sterility within 5–6 days in both groups.

In this study we observed advantages of ESBD approach for the biliary decompression in patients with hilar malignant biliary obstruction in context of the occurrence of bacteriobilia and cholangitis. One of the shortcomings of PTBD approach is the loss of bile. It is well known that bile acids and cholates participate in maintaining intestinal barrier integrity and commensal microbiota balance. The lack of cholates leads to an imbalance of intestinal microbiota with a predominance of gram-negative bacteria, increases intestinal permeability, and as a result bacterial translocation and endotoxemia followed by the development of septic complications and renal dysfunction [5]. In addition, disturbances in bile outflow are associated with metabolic disorders of Kupffer cells [6, 7], that might be one of the reasons for increased risk of bacteriobilia.

Conclusion

We believe, that one of the reasons of moderately lower rates of bacteriobilia in patients from EISBD group is the nature of external and internal drainage, which can be of at least two types: those that come into contact with the contents of the duodenum, i.e. pass through Fater's nipple, and those that do not come into contact with it and are located subpapillary. This is a very important fact, because, as was mentioned above, among

Table. **Microorganisms in bile specimens of patients with malignant biliary obstruction and concomitant cholangitis who underwent different approaches for biliary decompression**

Biliary decompression approach	*Escherichia coli*		*Enterobacter* spp.		*Klebsiella* spp.	
	The number of infected specimens	%	The number of infected specimens	%	The number of infected specimens	%
PTBD (n = 4)	2	66.7	1	25	1	25
EISBD (n = 3)	2	50.0	0	0	1	33.5

the pathways of drainage infection: antegrade (from the outside), retrograde (from the intestinal flora) and hematogenous, the most important one is retrograde, and in the case of EISBD, this way of infection is excluded.

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EFFECTS OF *Bacillus subtilis* IMV B-7724 LECTIN ON MALIGNANT AND NORMAL CELLS *in vitro*

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Cancer cells upregulate surface expression of N-glycolyl-neuraminic acid (Neu5Gc). At R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of NAS of Ukraine, *B. subtilis* IMV B-7724 lectin specific for Neu5Gc was obtained.

Aim. The scope of the research was to study *in vitro B. subtilis* IMV B-7724 lectin activity towards malignant and normal cells.

Materials and methods. Cytotoxic and mitogenic activities was studied by, respectively, MTT-assay and *in vitro* lymphocytes proliferation assay.

Results. The lectin possesses cytotoxic activity towards human (A549, HL60) and murine (Ehrlich carcinoma, L1210) cancer cell lines. The most sensitive were L1210 and Ehrlich carcinoma cell lines. IC_{50} was 0.16 mg/ml in both cases. The lectin was less cytotoxic to murine peritoneal macrophages, lymphocytes and thymocytes: IC_{50} was 0.47, 2.02 and 3.49 mg/ml respectively. In a dose of 25 µg/ml the lectin induced lymphocytes proliferation.

Conclusion. Depending on the target cells type and applied dose, *B. subtilis* IMV B-7724 lectin shows cytotoxic or mitogenic activities. Both of lectin's activities can be applied in cancer treatment and thus deserve further investigation.

Key words: *B. subtilis* IMV B-7724 lectin, cytotoxic activity, mitogenic activity.

The antigenic landscape of cancerous cells differs from the one of normal cells. Among the other changed characteristic of the cancer cells, the upregulation of sialylated glycans on cell surfaces is commonly described. These changes can be traced and applied in cancer diagnostic and targeted treatment [1]. Carbohydrates can be specifically bound by proteins called lectins. Lectins are found in vast majority of organisms and are engaged in pleura processes in natural and pathological conditions. Based on their ability to induce apoptosis, influence immune reactions or cell signaling, many lectins are considered as prospective option in cancer treatment [2]. At Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR), *B. subtilis* IMV B-7724 lectin was obtained. It was shown that the lectin has specificity towards N-acetylneuraminic (Neu5Ac) and N-glycolylneuraminic (Neu5Gc) acids and D-glucuronic acid and fructose-1,6-diphosphate and expresses high cytotoxic activity (CTA) toward Ehrlich carcinoma cells [3]. Considering the above mentioned and the fact that lectin produced by other Bacillus strain — *B. subtilis 7025* — possess anticancer activity and is utilized in cancer treatment [4] the obtained lectin was subjected for further investigations. The scope of this research was to determine *in vitro B. subtilis* IMV B-7724 lectin cytotoxic activity towards different types of malignant and normal cells.

Materials and Methods

The lectin was isolated as described in [3] and was freeze dried at temperatures between +24 °C … −32 °C. The lectin obtained looks like a brown colored powder, easily soluble in water, buffers (PBS, Tris-HCl); has the higherst sugar-binding specificity

towards sialic (N-Acytilneuraminic and N-Glycolylneuraminic) acids. The hemagglutinating activity of lectin (1 mg/ml) is in the range within 1024–2048 titer^{-1} [3]. The lectin's CTA was tested towards Ehrlich ascites undifferentiated murine mammary adenocarcinoma (EC), L1210 (murine lymphocytic leukemia), A549 (human pulmonary adenocarcinoma), and HL60 (human acute promyelocytic leukemia) cells, freshly isolated mouse peritoneal macrophages (Mph), lymphocytes and thymocytes. All cell lines were acquired from Bank of Cell Lines from Human and Animal Tissues, IEPOR.

The lectin's CTA was measured with the MTT-assay [5]. Target cells were used at concentration of 1×10^5 cell/well. The lectin was added in concentration of 0.25; 0.5; 1.0; 1.5 mg/ml. Incubation time was 2 and 24 h.

Mitogenic activity was studied in standard *in vitro* lymphocyte proliferation assay. Either different concentration of the lectin (25; 50; 100 μg/ml), or standard mitogens Concanavalin A (ConA, 50 μg/ml) and lipopolysaccharides (LPS, 100 μg/ml) were added to the cell-containing (4×10^5 per well) wells. Some lymphocytes were left without stimulation (spontaneous blast-transformation). Incubation time was 48 h. The amount of life cells was assessed based on MTT oxidation [5]. The proliferation index was calculated.

The data are expressed as the means ± standard errors of three replicated determinations. The differences were considered as statistically significant if $P < 0.05$. The IC_{50} values were calculated using on-line calculator available on http://www.ic50.tk/

Results and Discussion

The cytotoxic activity of *B. subtilis* IMV B-7724 lectin was evaluated *in vitro* using murine and human cell lines. The resulting IC_{50} values are shown in the Table 1.

The lectin cytotoxic effect depended on the cells' origin more than on cells' histotype. The most sensitive to the lectin were murine EC and L1210 cell lines. Independently of histotype, cell lines of the same origin demonstrated almost the same sensitivity to the lectin: IC_{50} values of the L1210 and EC cells did not differ significantly, and the same for the human cell lines HL60 and A549. The lesser sensitivity of human-originated cell lines may be due to the mutation of the enzyme hydroxylating Neu5Ac and Neu5Gc in humans [6]. In human, Neu5Gc cannot be endogenously synthesized but it can be acquired from dietary sources and metabolically processed and expressed on epithelial cell surfaces [7, 8]. Thus, it is tempting to conclude that the murine cancerous cells are more susceptible to the lectin because of expressing both Neu5Ac and Neu5Gc. Considering that cancer cells can acquire Neu5Gc from dietary sources [7, 8] and are prone to incorporate Neu5Gc more intensively than their normal counterparts [1] lectin's effect on freshly isolated human malignant cells remains to be elucidated. It is possible that incorporated Neu5Gc presented on the cells surface renders freshly isolated cancer cells more sensitive to the lectin cytotoxic action.

To test whether the lectin exert CTA towards non-malignant cells, freshly isolated murine immune cells were used as targets (Table 2). The lectin showed moderate CTA towards peritoneal Mph. However, the IC_{50} values for Mph were significantly higher than that of IC_{50} for the most sensitive cancer cell lines (L1210 and EC) and comparable with those of moderately sensitive HL60 and A549.

On the contrary, lymphocytes and thymocytes demonstrated the lowest sensitivity to cytotoxic effect of the lectin. Contrary to the expected, IC_{50} value increased as incubation continued. We hypothesized that the lectin could stimulate these cells' proliferation as it is for the plant lectin ConA or some bacterial LPS. We conducted an *in vitro* lymphocytes blast-transformation assay comparing effects of the lectin with that of ConA and LPS.

It was shown (Table 3) that the lectin in concentration of 25 μg/ml has mitogenic effect comparable with that of LPS.

Table 1. **The cytotoxic activity (IC_{50}, mg/ml) of lectin against cancer cell lines**

Duration of exposure, h	IC_{50}, (mg/ml)			
	L1210	HL60	EC	A549
2	0.21±0.02*	0.7±0.02	0.17±0.01*	0.61±0.02
24	0.16±0.01*	0.5±0.01[1]	0.16±0.01*	0.47±0.01[1]

* — $P < 0.05$ as compared to the human cell line; 1 — $P < 0.05$ as compared to the 2 h incubation

Table 2. **The cytotoxic activity (IC_{50}, mg/ml) of lectin against freshly isolated murine immune cells**

Duration of exposure, h	IC_{50}, (mg/ml)		
	Mph	Lympho-cytes	Thymo-cytes
2	0.58±0.03	1.65±0.13	0.84±0.05
24	0.47±0.02	2.02±0.06	3.49±0.48

probably is based on its specificity to Neu5Gc which was shown to have a highly tumor-restricted expression [9]. Therefore, the lectin can be applied almost as a target agent.

Moreover, the lectin has mitogenic effect on immune cells. Considering that other *B. subtilis* strains possess interferonogenic activity [4], *B. subtilis* IMV B-7724 lectin should be subjected to further investigation as an immunomodulating substance. Thus, *B. subtilis* IMV B-7724 lectin worth further investigation both as a cytotoxic and immunomodulating agent.

Table 3. **Lymphocytes blast-transformation on response to lectin stimulation**

Parameters	Spontaneous	Known mitogens, (μg/ml)		Lectin, (μg/ml)		
		ConA, (50)	LPS, (100)	100	50	25
Optical units	0.07±0.01	0.339±0.05*	0.089±0.01*	0.059±0.01	0.057±0.01	0.084±0.01*
Stimulation index	–	4.8	1.3	0.8	0.8	1.2

* — $P < 0.05$ as compared to the spontaneous proliferation.

Conclusion

So, the lectin demonstrated different activities depending on the cell type and application dose. The lectin possesses CTA towards cancer cell lines but is less aggressive towards freshly isolated nonmalignant cells. The lectin's specificity towards cancer cells

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THE INFLUENCE OF PHOTOSENSITIVE PEPTIDOMIMETICS ON WEIGHT INDICES OF IMMUNE ORGANS OF EXPERIMENTAL ANIMALS WITH TRANSPLANTABLE LEWIS LUNG CARCINOMA

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The use of photodynamic therapy of drugs capable of selective accumulation in the tumor or in affected cells, in particular photosensitive peptidomimetics, increases its effectiveness in various treatment schemes.

Aim. Determination of weight indices (WI) of thymus and spleen in animals with carcinoma after photodynamic therapy with peptidomimetics: LMB002 and LMB033.

Methods. Studies of WI of immune organs were carried out in mice of the C57 Black line on the 28th day after transplantation of Lewis lung carcinoma. The therapeutic effect was studied using photosensitive peptidomimetics: LMB002 and LMB033. The natural antibiotic gramicidin C was used as a control.

Results. Comparison of the WI of the spleen of intact animals and animals with tumors showed a twofold ($P < 0.05$) increase in animals with tumors. As for the WI of the thymus, a tendency to its increase was observed in animals with tumors, compared to intact ones, but no significant difference was found. The following results were obtained: a decrease in the WI of the spleen in animals with tumors and the introduction of placebo and an increase in the WI of the spleen in animals treated with gramicidin C; an increase in spleen WI and a slight decrease in thymus WI under the influence of LMB002, an increase in spleen WI under the influence of LMB033, while the thymus WI did not change.

Conclusions. It was established that the WI of the spleen during therapy with photosensitive peptidomimetics LMB033 and LMB002 increased in all groups of experimental animals compared to intact ones. The most significant effect on the spleen index was observed for the schemes of double administration of LMB033 and double phototherapy. No significant changes in the weight index of the thymus during therapy with photosensitive peptidomimetics LMB033 and LMB002 were found.

Key words: peptidomimetics, photodynamic therapy, cell culture, Lewis lung carcinoma.

Today, photodynamic therapy (PDT) is a highly effective method for cancer treatment. PDT induces acute inflammation, expression of heat shock proteins, tumor invasion, and infiltration by leukocytes, and can enhance the presentation of tumor antigens to T cells [1, 2].

To increase the effectiveness of non-invasive treatment methods, photo-controlled peptidomimetics are used, which are prescribed in a non-cytotoxic concentration range. Peptidomimetics are capable of selective accumulation in a tumor or affected cells [3, 4]. They are usually administered in an inactive, less toxic isomeric form, which is subsequently activated under the influence of light with a high level of spatiotemporal precision at the desired site of action. Thus, after reaching the target of the action, light-induced thermal inactivation of peptidomimetics contributes to their wide application, as safe medicines [5, 6].

Materials and Methods

Studies were conducted on a transplantable model of LLC in mice of the C57 Black line [7]. Inoculation of tumor cells was carried out in the femoral muscle in a concentration of 0.8×10^6 cells of the primary LLC culture, which was grown in an *invitro* system in a culture medium DMEM (Sigma, USA) containing 10% FBS (Sigma, USA) in standard CO_2 incubator conditions at 37 °C and 100% humidity. After reaching a complete monolayer, cells were washed from the culture medium, counted, dissolved in physiological buffer, and inoculated into experimental animals.

To determine the weight index (WI) of the spleen and thymus in animals with tumors and animals with tumors and therapy, mice were weighed, after euthanasia, the immune organs were removed, weighed, and according to the given formula, the weight indices of the immune organs were determined.

$$I_{spleen}\,(\%) = \text{spleen weight} / \text{the weight of the animal} \times 100$$

$$I_{thymus}\,(\%) = \text{thymus weight} / \text{the weight of the animal} \times 100$$

The obtained data were averaged by groups of experimental animals and presented in the form of histograms.

The drugs for phototherapy were photo-controlled peptidomimetics: LMB002 and LMB033. The natural antibiotic gramicidin C was used as a control.

7 groups of animals were used in the study ($n = 5$).

I group — intact control group of animals;

II group — a group of animals with a transplanted tumor (control without treatment), these animals were kept in a dimmed light/dark regime during the experimental period;

III group — a group of animals injected with Gramicidin C (GS), two procedures (intratumoral injection of GS, no irradiation), an interval of two days between procedures;

IV group — a group of animals that were injected with the peptidomimetic LMB033(closed form), according to the scheme: two intratumoral injections of the compound, no radiation, and an interval between injections of two days.

V group — a group of animals injected with the peptidomimetic LMB002(closed form), according to the scheme: two intratumoral injections of the compound, no radiation, and an interval of two days between injections.

VI group — a group of animals administered peptidomimetic LMB033 (closed form), according to the scheme: two intratumoral injections of the compound, with double irradiation, the interval between injections is two days.

VII group — a group of animals that were administered peptidomimetic LMB002 (closed form), according to the scheme: two intratumoral injections of the compound, with double irradiation, the interval between injections is two days.

Results and Discussions

Comparison of the weight index of the spleen of intact animals and animals with tumors showed a 2-fold ($p < 0.05$) increase in animals with tumors (Fig.).

As for the WI of the thymus, a tendency to increase was observed in animals with tumors compared to intact ones, but no significant difference was recorded. The increase in splenic WI in tumor-bearing animals is a consequence of immunosuppression and inflammation.

Weight indicators of the spleen and thymus differed from those in the untreated control group: in animals with transplanted Lewis lung carcinoma, a decrease in the WI of the spleen was observed by the administration of a placebo, and in animals treated with gramicidin C — an increase in the WI of the spleen and a decrease in the thymus.

Therapy with peptidomimetics according to the scheme of double administration of LMB033 in the absence of irradiation did not lead to changes in the weight index of the spleen and thymus compared to the control; therapy with a double injection of the peptidomimetic LMB033 and irradiation led to an increase in the weight index of the spleen in the VI group of animals relative to the group of untreated animals, while the weight index of the thymus did not change.

An increase in the weight index of the spleen and a decrease in the weight index of the thymus in comparison with the control was observed, with the double administration of the peptidomimetic LMB002 to the animals and the absence of irradiation. The weight indices of the spleen and thymus in the group of animals treated with peptidomimetic therapy and exposed to radiation differ from the control: WI of the spleen increases and WI of the thymus decreases.

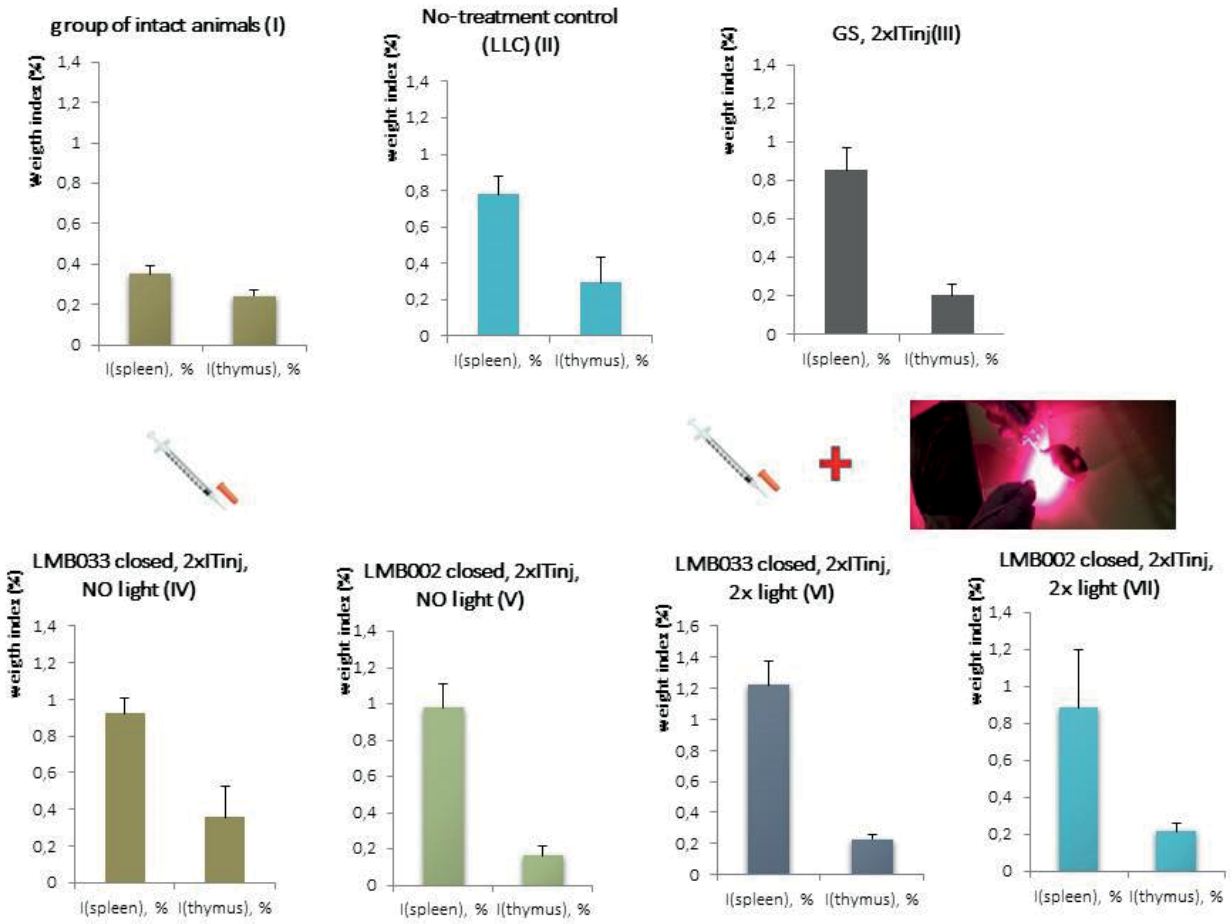

Fig. **Weight index of the spleen and thymus of C57 Black mice under the influence of controlled photopeptidomimetics**

Conclusions

It was established that the WI of the spleen during therapy with photosensitive peptidomimetics LMB033 and LMB002 increased in all groups of experimental animals compared to intact ones. The most significant effect on the spleen index was observed for the schemes of double administration of LMB033 and double phototherapy. No significant changes in the weight index of the thymus during therapy with photosensitive peptidomimetics LMB033 and LMB002 were found.

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