УДК: 573.864.1:615.331

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EFFECT OF PROBIOTIC STRAINS OF LACTO- AND BIFIDOBACTERIA ON THE ACTIVITY OF MACROPHAGES AND OTHER PARAMETERS OF IMMUNITY IN CASES OF STAPHYLOCOCCOSIS

The immunomodulatory properties of Lactobacillus delbrueckii subsp. bulgaricus IMV B-7281, Lactobacillus casei IMV B-7280, Lactobacillus acidophilus IMV B-7279, Bifidobacterium animales VKL and B. animales VKB strains on the models of experimental staphylococcosis infection in mice were determined. It was found that after the mice, infected with staphylococcus, were treated by some probiotic strains of lacto- and bifidobacteria, a normalization of functional activity of phagocytic cells system and increase of the endogenous interferon production were observed. L. delbrueckii subsp. bulgaricus IMV B-7281, L. casei IMV B-7280, L. acidophilus IMV B-7279, B. animales VKB and B. animales VKL are promising for the development of probiotics, effective against staphylococci and for the immunity correction.

K e y w o r d s : Lactobacillus, Bifidobacterium, Staphylococcus, immunity, macrophages, interferon, mice.

The rapid growth of widespread infectious diseases, provoked primarily by the aggressive opportunistic commensal microorganisms, including staphylococcus, creates a complex set of problems connected with the necessity of finding new treatments that provide a general therapy of pathogen and balancing the immune status of the organism on the level of receptor-ligand interactions. It is urgent to search for a new agonists for receptors that form the immune response in cases of certain pathogen-associated molecular patterns (PAMPs) appearing in the body [1, 2].

It is known that some strains of probiotic bacteria have antibacterial properties against a wide range of pathogens, including causative agents of the most common infectious diseases. They can also act as adjuvants in the development of immune response in mucous membranes and at the system level [1, 3], activating the factors of innate and acquired immunity. At the same time there is a change in production of immunoregulatory cytokines after the interaction of their PAMPs with different types of Toll-like receptors (TLR-receptors) that are expressed on the surface of dendritic cells (DC), macrophages, intraepithelial T-lymphocytes, etc. [1]. Therefore, these strains are promising in creation of a highly efficient probiotic – imunobiotic – to correct the immune system in cases of inflammatory diseases.

It was previously shown that probiotic strains of lacto- and bifidobacteria: *Lactobacillus casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *Bifidobacterium animales* VKL and *B. animales* VKB, isolated from human biological material, suppressed the persistence of staphylococcus in the kidneys of the infected Balb/c line mice and normalized indicators of cellular immunity [4]. However, for complete understanding the mechanisms of protective action of these probiotic strains against staphylococcus it was necessary to examine their impact on the factors of innate immunity and the production of cytokines, which play an important role in defense against bacterial infection.

So, the aim of the work was to determine the immunomodulatory activity of *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKL and *B. animales* VKB on the model of experimental staphylococcosis in mice by examining their impact on functional activity of macrophages, the number of natural killer cells and interferon production.

Materials and Methods. Experimental studies were performed on six-week-old female BALB/c mice from the vivarium of the Institute of Molecular Biology and Genetics of NAS of Ukraine. All studies were performed taking into account the rules of the European Convention for the protection of vertebrate animals (09.20.1985) and the Law of Ukraine № 3447-IV "About the protection of animals from cruelty" [5].

The study was performed using lyophilized bacteria *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. casei* IMV B-7280, *B. animales* VKL and *B. animales* VKB. Before each experiment the viability of the probiotic cultures was tested by monitoring their growth on the Man-Rogosa-Sharpe (MRS) agar medium at 37 °C for 24-48 h.

Staphylococcosis was modeled through intraperitoneal administration of the *S. aureus* 8325-4 daily culture to mice, in a dose of 1 x 10^9 cells per animal. *S. aureus* 8325-4 was kindly provided to us by Professor V.S. Zuyeva, N.F. (Gamaleya Institute of Epidemiology and Microbiology, Russian Federation) and had plasmid-based resistance to gentamicin. The following clinical manifestations of the infection process were observed in the infected mice: elevation of body temperature, loss of inactivity and appetite.

Twenty-four hours after infection, mice were given a *per os* administration of a suspension of lyophilized lactobacillus and/or bifidobacteria cells in saline solution at a dose of 1 x 10^6 cells per animal, once a day for 7 days. Strains were injected individually or in the composition *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279 (in equal proportion). A separate group was formed by the infected mice that did not receive these strains or their composition, but received the saline intraperitoneally. The control group included intact mice. Experimental studies were conducted in three repetitions.

On the 1, 3, 6, 9 and 12th day after the injection of lactobacillus and/or bifidobacteria strains, alone or in composition, into the infected mice, peritoneal fluid, spleen and peripheral blood were taken from the test mice under anesthetic.

The functional activity of peritoneal macrophages (PM) – their ability to accumulate oxygen metabolites in the nitroblue tetrazolium recovery test (NBT-test) and the absorption of latex was studied. Absorptional activity was evaluated in terms of phagocytosis rate (PR) – the number of PM, that are able to absorb latex, and phagocytosis number (PN) – the average number of particles of latex, which were absorbed by PM [6].

The concentration of interferon (IFN) in the blood serum was determined by microtiter method in the culture of sensitive cells L-929. The results accounting was conducted by microscopy under the inverted microscope. Interferon activity was evaluated by inhibition of cytopathic vesicular stomatitis virus (VSV). The titer of interferon was that sample dilution in which 50 % protection from cytopathic action of VSV cells monolayer was observed. Reference α-interferon control (α-international standard B 69/19) with known activity was used at each titration [6].

Surface antigens of natural killer cells (NKC) were investigated with the help of the direct immunofluorescence method. Monoclonal antibodies against NK-antigens (MACS, Miltenyi Biotec, Germany) were used in the work. Calculation of NKC as well as analysis of the results was performed on a FACStar Plus cytofluorometer (Becton-Dickinson, USA).

All digital data obtained were processed with the help of the Origin Pro 8.5. software through analysis of variance. Numerical data were represented as arithmetic average and standard error (M ± m). The null hypothesis for the control and experimental comparative groups was checked using Wilcoxon-Mann-Whitney (U) and Kolmogorov-Smirnov nonparametric criteria. The differences between the groups were considered statistically meaningful at $P < 0.05$.

Results and Discussion. It was found that more rapid elimination of staphylococcus from the kidneys of the infected mice under the influence of *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. casei* IMV B-7280, *B. animales* VKL or *B. animales* VKB, each strain taken separately, or composition *L. delbrueckii subsp*. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279, as it was shown by us earlier [4], accompanied with the changes of innate immunity, including functional activity of PM (Table 1). It should be noted that the mice infected with staphylococcus had a partial violation of the phagocytic activity, which coincided with our previously obtained data [7]. In this study we observed a decrease in a PR of PM on the 2, 4 and 10th days after staphylococcus was administered to the mice, whereas their PN during the whole period of observation had no differences with the control group.

As it is clear from the data presented in Table 1, different probiotic strains of lactic acid bacteria have different effect on the functional activity of PM. So, an increase of PR of PM of the infected mice was detected under the influence of *B. animales* VKL or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, each strain taken separately, on the 1, 3 and 9th days as compared with the infected mice that did not receive probiotic strains. After *per os* administration of *L. acidophilus* IMV B-7279 (on the

3rd day) or *L. casei* IMV B-7280 (on the 3 and 6th days), each strain taken separately, to the infected mice PR was the same as in the mice that received no probiotic culture, but on the 9th day it increased. Instead, *B. animales* VKB activated PR of the PM of the infected mice only on the 1 and 6th days. After administration of the composition L. *acidophilus* IMV B-7279 - *L. delbrueckii subsp. bulgaricus* IMV B-7281 to the infected mice PR of the PM increased on the 1, 3 and 9th days.

Table 1

Changes of functional activity of the PM after an oral administration probiotic strains f lacto- and bifidobacteria to the staphylococcus infected mice, M ± m

Groups of mice	Time of observation, day	Rate of phagocytosis, %	Phagocytic number, standard unit	Stimulated NBT-test, %	Spontaneous NBT-test, %	Functional reserve, standard unit
Intact mice	-	70.0 ± 3.2 •	2.1 ± 0.3	17.0 ± 2.5	11.0 ± 2.6	6.0 ± 1.1
Mice infected with *S. aureus* 8325-4	1	24.0 ± 2.7 *	1.7 ± 0.2	68.0 ± 3.8 *	56.0 ± 3.8 *	12.0 ± 2.5 *
	3	50.0 ± 2.9 *	2.2 ± 0.2	44.5 ± 4.2 *	40.2 ± 3.6 *	4.4 ± 1.7
	6	82.9 ± 2.4 *	2.5 ± 0.3	38.9 ± 3.3 *	35.0 ± 4.8 *	3.9 ± 2.0
	9	50.0 ± 3.1 *	2.4 ± 0.1	50.0 ± 2.4 *	21.7 ± 4.1 *	28.3 ± 3.1 *
Infected mice, that received *B. animales* VKL	1	72.0 ± 1.9 •	2.3 ± 0.1 •	46.3 ± 5.3 * •	36.0 ± 4.7 * •	10.3 ± 2.6
	3	62.7 ± 2.6 •	1.8 ± 0.3	36.4 ± 3.4 *	25.5 ± 2.8 * •	10.9 ± 2.1 •
	6	87.1 ± 3.1 *	2.5 ± 0.3	30.3 ± 2.1 * •	25.8 ± 3.9 *	4.6 ± 2.0
	9	68.0 ± 2.8 •	2.9 ± 0.2•	35.0 ± 3.4 * •	25.0 ± 5.1 *	10.0 ± 2.6 •
Infected mice, that received *L. acidophilus* IMV B-7279	1	36.1 ± 2.6 *•	2.3 ± 0.1 •	35.2 ± 3.8 * •	30.2 ± 4.3 * •	5.0 ± 1.1 •
	3	44.0 ± 3.0 *	2.2 ± 0.2	30.0 ± 3.5 * •	20.0 ±2.9 * •	10.0 ± 1.8 * •
	6	50.0 ± 3.7 *•	2.1 ± 0.2	30.0 ± 2.8 *	14.0 ± 4.8 •	16.0 ± 1.7 * •
	9	70.0 ± 2.4 •	2.6 ± 0.1	48.0 ± 5.1 *	29.0 ± 5.6 *	19.0 ± 3.1 * •
Infected mice, that received *L. casei* IMV B-7280	1	48.0 ± 3.2 *•	1.9 ± 0.2	50.0 ± 4.1 * •	32.0 ± 2.5 * •	18.0 ± 2.4 * •
	3	45.5 ± 2.2 *	2.1 ± 0.3	34.0 ± 3.4 *	30.0 ± 3.9 * •	4.0 ± 1.0
	6	83.8 ± 3.3 *	3.5 ± 0.3 * •	33.8 ± 3.6 *	31.7 ± 4.2 *	2.2 ± 1.2 *
	9	70.0 ± 3.7 •	2.3 ± 0.1	42.0 ± 4.7 *	31.0 ± 3.8 * •	11.0 ± 1.8 * •
Infected mice, that received *B. animales* VKB	1	74.0 ± 2.6 •	2.5 ± 0.2 •	40.0 ± 4.0 * •	36.0 ± 3.7 * •	4.0 ± 1.6 •
	3	52.0 ± 3.2 *	2.8 ± 0.1•	33.9 ± 2.4 * •	32.3 ± 3.4 *	1.6 ± 1.0 *
	6	59.0 ± 7.8 •	3.5 ± 0.2 * •	22.0 ± 1.8 * •	20.3 ± 2.6 * •	1.7 ± 1.2 *
	9	54.0 ± 1.9 *	2.0 ± 0.1	40.0 ± 4.3 * •	34.0 ± 3.8 * •	6.0 ± 1.6 •
Infected mice, that received *L. delbrueckii subsp. bulgaricus* IMV B-7281	1	74.0 ± 2.4 •	2.8 ± 0.3 •	40.0 ± 4.9 * •	20.0 ± 5.6 * •	20.0 ± 2.4 * •
	3	62.0 ± 1.9 •	3.3 ± 0.4 * •	43.1 ± 3.3 *	23.1 ± 3.1 * •	20.1 ± 1.7 * •
	6	85.0 ± 3.4 *	1.9 ± 0.2	27.5 ± 2.4 * •	25.4 ± 3.1 * •	2.2 ± 0.9 *
	9	61.7 ± 3.9 •	2.5 ± 0.3	34.0 ± 3.5 * •	16.0 ± 5.1 * •	8.0 ± 1.4 •
Infected mice, that received *L. acidophilus* IMV B-7279 - *L. delbrueckii subsp. bulgaricus* IMV B-7281	1	79.0 ± 2.6 •	2.3 ± 0.3	38.8 ± 3.5 * •	28.3 ± 2.3 * •	10.4 ± 3.1 *
	3	64.0 ± 2.4 •	2.6 ± 0.2	30.0 ± 1.9 * •	21.7 ± 2.9 * •	8.3 ± 2.3
	6	76.5 ± 3.4	2.6 ± 0.3	28.0 ± 2.6 * •	20.4 ± 4.8 * •	7.6 ± 1.6
	9	64.0 ± 3.1 •	2.5 ± 0.1	35.0 ± 3.4 * •	12.5 ± 3.4 •	22.5 ± 3.3 *

Significant differences with the control are represented by * ($P < 0.05$) while differences with the indicators of the infected mice who did not receive probiotic strains or their composition are represented by • ($P < 0.05$).

Some of these probiotic strains of lactic acid bacteria enhanced the intensity of the phagocytic function of PM of the staphylococcus infected mice. In particular, we found the increasing of PN under the influence of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (on the 1 and 3rd days), *B. animales* VKB (on the 1, 3 and 6th days), *B. animales* VKL (on the 1 and 9th days), *L. casei* IMV B-7280 (on the 6th day), *L. acidophilus* IMV B-7279 (on the 1st day), each strain taken separately, as compared with the infected mice that did not receive probiotic cultures. After the injection of the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 to the infected mice, this indicator did not change during the entire period of observation.

When analyzing the obtained data, we can make a conclusion that only *B. animales* VKL and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, each strain taken separately, and the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 completely normalized PR of the PM, affected by staphylococcal infection. However, *B. animales* VKL, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKB or *L. casei* IMV B-7280 activated the intensity of their phagocytic function in different periods of observation.

A significant increase of the PM ability to accumulate oxygen metabolites indicates the dysfunction of phagocytes under the influence of staphylococcosis. The activation of oxygen-dependent metabolism of PM (in spontaneous and stimulated NBT-test) was detected during the period of observation. It should be noted that the inverse correlation between PR and indices of spontaneous NBT-test ($R = 0.92$; $P < 0.05$) was found. Therefore it is possible to suppose that the decrease in phagocytic activity of PM from the mice with staphylococcosis, at least for PR, is due in part to the excessive accumulation of these oxygen metabolites. The latter, as known [8], may damage phagocytes and influence the genetic apparatus of bacteria, causing, in particular, the emergence of strains with multiple resistances to antibiotics.

It was established a lowering of spontaneous NBT-test indicators of the PM from mice with staphylococcosis under the influence of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 during the entire period of observation as compared with the infected mice that did not receive probiotic strains. The number of NBT-positive PM in spontaneous test also decreased after administration to the infected mice of *L. acidophilus* IMV B-7279 – on the 1, 3 and 6th days; *L. casei* IMV B-7280 – on the 1, 3 and 9th days; *B. animales* VKB – on the 1, 6 and 9th days; *B. animales* VKL – on the 1 and 3rd days.

The indicators of stimulated NBT-test of staphylococcus infected mice PM were decreasing during the entire period of observation under the influence of *B. animales* VKB or the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281. Other probiotic strains also cause the reduction of NBT-positive PM in a simulated test – *B. animales* VKL or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, each strain taken separately, – on the 1, 6 and 9th days; *L. acidophilus* IMV B-7279 – on the 1 and 3rd days; *L. casei* IMV B-7280 – on the 1st day.

The oxygen-dependent bactericidal activity of PM (defined in NBT-test) of staphylococcus infected mice treated with these probiotic cultures of lactic acid bacteria or their composition, in most cases remained higher than in control (intact mice). Normalization of spontaneous NBT-test indicators was observed only under the influence of *L. acidophilus* IMV B-7279 on the 6th day or the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 9th day.

Staphylococcosis does not result in violation of functional reserve of PM. In contrast, this indicator was higher than in the control on the 2 and 10th days. Functional reserve of macrophages increased in relation to the infected mice which did not receive probiotic cultures, under the influence of *L. acidophilus* IMV B-7279 on the 3, 6 and 9th days, *L. casei* IMV B-7280 – on the 1, 6 and 9th days, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 1, 3 and 6th days, and the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 1 and 9th days. Functional reserve decreased to the control level on the 1st day under the influence of *B. animales* VKL, *L. acidophilus* IMV B-7279 and *B. animales* VKB, on the 9th day – under the influence of all probiotic bacteria (separately).

The obtained data (Figure) indicate that staphylococcosis causes a reduction of NKC in the experimental animals for long periods (from 1st to 6th day). The injection of probiotic strains of lactic acid bacteria alone or in composition to the staphylococcus infected mice resulted in changes of NKC number in the spleen. Thus, an oral administration of *B. animales* VKB to the animals with staphylococcosis resulted in the increase of NKC in the spleen during the entire period of observation as compared with the indicators of staphylococcus infected animals that did not receive probiotic strains. The number of NKC also increased after the *per os* administration of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 on the 1, 6 and 9th days; *L. casei* IMV B-7280 – on the 1, 3 and 9th days; *L. acidophilus* IMV B-7279 – on the 1st day; *B. animales* VKL – on the 3rd day. After the injection of the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 to the infected animals, the number of NKC remained at the level of intact animals on the 1, 3 and 6th days. Thus, only the composition of two probiotic strains – *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animales* VKB alone for the whole period of observation increased the number of NKC to the level of control group (intact animals).

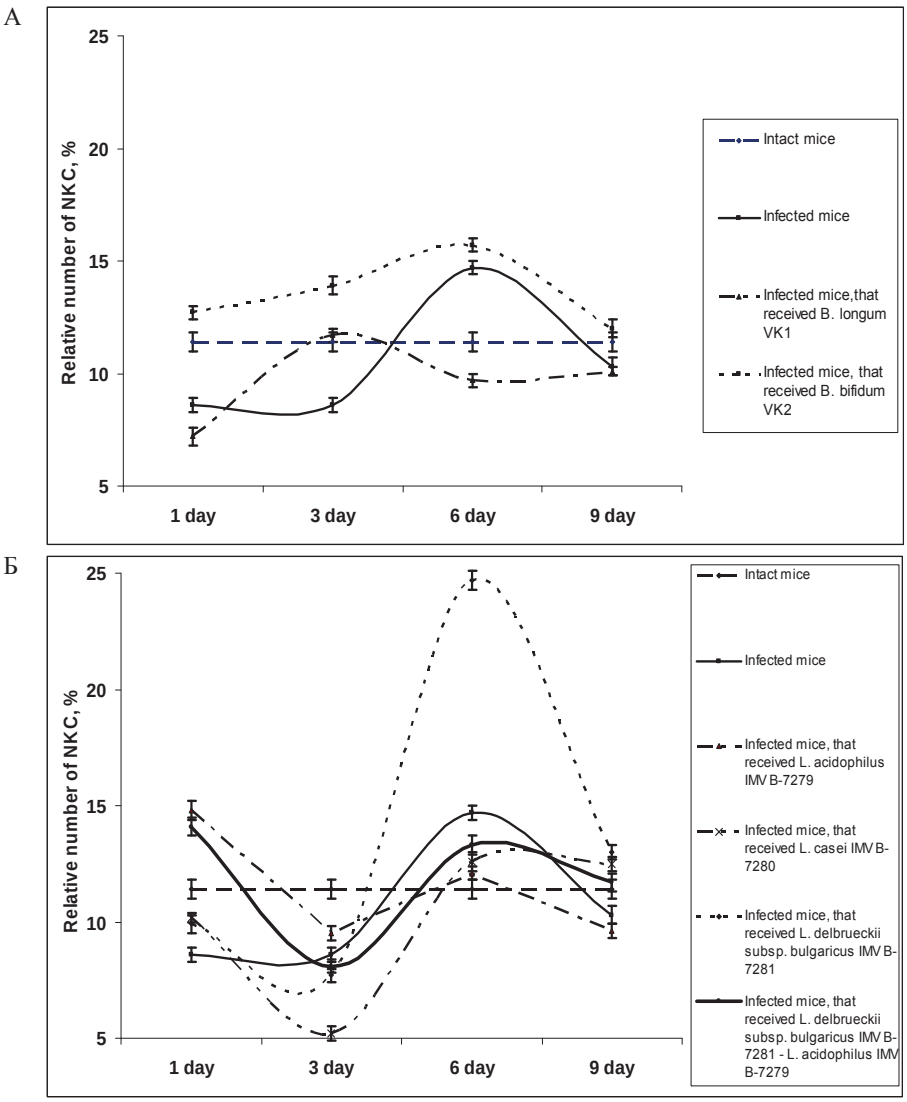

Fig.1. **The number of NKC in the spleen of infected mice that received probiotic cultures**

Probiotic strains had a considerable effect on the endogenous IFN production, which is known [9] to have a wide range of immunomodulatory actions and play an important physiological role, because it is necessary for the maturation of some immune cells, such as dendritic cells, and control their cellular proliferation on the intestinal level.

The titers of serum IFN, which decrease was observed on the 3th day after the mice were infected with staphylococcus, substantially increased under the influence of *B. animales* VKL, *L. casei* IMV B-7280, *B. animales* VKB, each strain taken separately – on the 1, 3 and 9th days; *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 1 and 3th days; *L. acidophilus* IMV B-7279 – only on the 3th day in comparison with the rates of the infected mice that did not receive probiotic bacteria (Table 2). After the mice, infected with staphylococcus, received the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, the concentration of IFN in the blood serum increased on the 1 and 9th days. These data suggest that the injection of probiotic strains to staphylococcus infected mice at different times of observation led to the activation of IFN production. *L. casei* IMV B-7280, *B. animales* VKB, or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and a composition of two probiotic bacteria caused an activation of interferon genesis on the 1st day. All strains of lacto- and bifidobacteria, which were studied, and the composition raise the concentration of IFN in the blood serum on the 3rd day to the level of control as compared with the infected mice that did not receive these probiotic strains.

Table 2

Interferon titers in blood serum under the influence of lacto- and bifidobacteria in cases of staphylococcosis in mice

Group of mice	Interferon titers (log_2) /the day of the experiment after injection of probiotic strains			
	1 day	3 day	6 day	9 day
Intact mice (control)	5.3 ± 0.4	5.3 ± 0.4•	5.3 ± 0,4	5.3 ± 0,4
Mice infected with *S. aureus* 8325-4	4.3 ± 0.4	2.6 ± 0.4*	4.3 ± 0.4	7.3 ± 0.4
Infected mice, that received *B. animales* VKL	7.3 ± 0.4*	7.0 ± 0.0*•	5.0 ± 0.0	9.0 ± 0.0*•
Infected mice, that received *L. acidophilus* IMV B-7279	5.0 ± 0.6	8.3 ± 0.4*•	5.3 ± 0.4	8.0 ± 0.6*
Infected mice, that received *L. casei* IMV B-7280	8.3 ± 0.4*•	7.7 ± 0.4*•	4.7 ± 0.4	4.7 ± 0.4•
Infected mice, that received *B. animales* VKB	5.7 ± 0.4•	4.0 ± 0.0*•	4.7 ± 0.4	5.0 ± 0.0•
Infected mice, that received *L. delbrueckii* subsp. *bulgaricus* IMV B-7281	6.7 ± 0.4*•	4.3 ± 0.4*•	4.3 ± 0.4	4.3 ± 0.4•
Infected mice, that received *L. acidophilus* IMV B-7279 – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281	7.3 ± 0.4*•	4.3 ± 0.4*•	5.3 ± 0.4	4.7 ± 0.4•

Significant differences with the control are represented by * ($P < 0.05$) while differences with the indicators of the infected mice who did not receive probiotic strains or their composition are represented by • ($P < 0.05$).

Thus it was found that *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKL and *B. animales* VKB strains and the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 on the model of experimental staphylococcosis in mice had immunomodulatory action pointed to the induction of endogenous interferongenesis, increasing the number of NKC and activation of PM in different periods of observation. We can assume that these probiotic strains after interaction with immune and epithelial cells of intestinal mucosa of mice infected with staphylococcus activate them and cause the increased production of a number of immunoregulatory cytokines, including IFN. This leads to changes of body immunoreactivity on the local and systemic levels. Presumably, the changes of the PM functional activity after the probiotic strains injection is a consequence of the increasing IFN production, as phagocytes are known [7, 9] to be the main target of these cytokines.

After the injection of probiotic bacteria to the staphylococcus infected mice an increase of IFN production with intensification of absorption activity of PM and a decline in their ability to accumulate oxygen metabolites were observed. So, *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKL and *B. animales* VKB had an immunomodulatory and antioxidant effects on mice with staphylococcosis. Under the influence of *L. acidophilus* IMV B-7279 and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, each strain taken separately, or the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, the functional reserve of PM increased. This points not only to the increase of the reserve capacity of phagocytosis, but also to the immunity reserve at all. Presumably, therefore these two probiotic strains and their composition effectively suppressed *Staphylococcus* persistence in the kidneys of infected mice as determined by us earlier [4]. It should be noted that *L. casei* IMV B-7280 and *L. acidophilus* IMV B-7279 also had the antistaphylococcal and immunomodulatory effect on the intravaginal staphylococcosis of mice, as was shown by us recently [10].

According to results, the effect of *L. bulgaricus* CRL 423 [11] and *L. bulgaricus* LB51 [12] strains on the phagocytic activity of macrophages is similar. Such strains as *L. delbrueckii* UFV-H2b20 [13], *L. fermentum* AF7, *L. acidophilus* GG5, and *L. plantarum* BB9 [14], *L. plantarum* YU [15], *L. casei Shirota* [16, 17], *L. paracasei* KB28 [18] activate different aspects of the functional activity of macrophages *in vitro* and *in vivo*. However, *L. casei* 3260, on the contrary, inhibits the production of TNF and prostaglandin E2 in macrophage Raw 264.7 cells [19]. Some strains of bifidobacteria – *B. longum* [20], *B. longum* BCRC 14634 [21], *B. adolescentis* BBMN23 and *B. longum* BBMN68 showed the ability to activate the phagocytic system cells [22].

We have not determined the mechanisms of lactic acid bacteria action on macrophages. However, it was recently shown that macrophages *in vivo* and *in vitro* become activated by live and heat-, acid- or trypsin-killed *Lactobacteria*, but not by ultrasound-killed *Lactobacteria* [14]. So, the cell wall components of lactobacilli (polysaccharides [16], lipoteichoic acid [23] and exopolysaccharide

[18]) are critical in the activation of phagocytes. Immunomodulatory properties of bifidobacteria are associated with the presence of unmethylated CpG sequences in DNA [24, 25], as well as peptidoglycan [26].

Because of the macrophages activation, the probiotic strains of lactic acid bacteria can influence the development of congenital and acquired immunity [27]. Previously we have shown that the injection of these probiotic strains of lacto- and bifidobacteria to the staphylococcus infected mice resulted in changes of the acquired immunity parameters. In particular, under the influence of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *B. animales* VKB alone or the in composition *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279 immunoregulatory index CD_4/CD_8 became normal as compared with the infected mice that did not receive probiotic cultures. *B. animales* VKL, *L. acidophilus* IMV B-7279, *B. animales* VKB, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 alone, or the composition *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279 induced the increase of CD_{19}^+ B-lymphocytes number in spleen in different periods of observation. However, *B. animales* VKB, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, and the composition *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279 (on the 6 and 9th days) increased the number of CD_{25}^+ cells in the spleen of infected mice. It should be noted that this composition had a wider range of immunomodulatory actions in cases of experimental staphylococcosis, since under its influence in different periods of observation the activity of PM and their phagocytic reserve increased, endogenous interferongenesis activated, and immunoregulatory index CD_4/CD_8 became normal (because of the increasing of CD_4^+ cells number)[4]. The number of CD_{19}^+ B-lymphocytes and CD_{25}^+ cells also increased.

In this study was have found that the infected mice after the injection of lacto- or bifidobacteria probiotic strains alone or in composition increased the number of NKC in the spleen. Some other strains of lacto- and bifidobacteria (*L. casei* Shirota, *L. rhamnosus* GG, *L. plantarum* NCIMB 8826, *L. reuteri* NCIMB 11951, *B. longum* SP 07/3 or *B. bifidum* MF 20/5) [28] also induce the increase of the number of NKC and activate them. So, the immunomodulatory properties of different cultures of lacto- and bifidobacteria are their individual characteristics that should be considered during the development of probiotics for treatment and prevention of infectious diseases.

Thus, when analyzing the obtained data, we can conclude that *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKL, *B. animales* VKB and the composition *L. acidophilus* IMV B-7279 - *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 after *per os* injection to the mice infected with staphylococcus changed the indicators of body immunoreactivity at the system level. Thus, the activation of PM, higher production of interferon and increase of the number of NKC, is an important protective mechanism of probiotic bacteria action in cases of experimental staphylococcosis in mice. So these probiotic strains and their composition can be used to create a highly-effective immunobiotic, able to inhibit the persistence of Staphylococcus and influence the development of the immune response to the pathogen, but more additional studies are needed.

Conclusions. Probiotic bacteria *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKL, *B. animales* VKB each strain taken separately, and the composition *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279 in different periods of observation increased an absorptional activity of PM of staphylococcus infected mice and reduced their ability to accumulate oxygen metabolites. *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and the composition *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279 increased the reserve capacity of phagocytes.

The number of NKC in the spleen of staphylococcus infected mice and the production of interferon, that is confirmed by the high titers of this cytokine in serum, increased in different periods of observation under the influence of *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKL, *B. animales* VKB, each strain taken separately, and the composition *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 - *L. acidophilus* IMV B-7279.

Probiotic strains *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animales* VKB and *B. animales* VKL are promising for the creation on their basis of immunobiotic drugs with antistaphylococcal activity and for immunity correction by phagocytosis activation, increasing the number of NKC in the spleen and interferon titers in the blood serum, in cases of infectious-inflammatory diseases.

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ВПЛИВ ПРОБІОТИЧНИХ ШТАМІВ ЛАКТО- ТА БІФІДОБАКТЕРІЙ НА АКТИВНІСТЬ МАКРОФАГІВ ТА ІНШІ ПОКАЗНИКИ ІМУНІТЕТУ ПРИ СТАФІЛОКОКОВІЙ ІНФЕКЦІЇ

Р е з ю м е

Визначено імуномодулювальні властивості штамів *Lactobacillus delbrueckii* subsp. *bulgaricus* IMV B-7281, *Lactobacillus casei* IMV B-7280, *Lactobacillus acidophilus* IMV B-7279, *Bifidobacterium animales* VKL та *Bifidobacterium animales* VKB на моделі експериментальної стафілококової інфекції у мишей. Встановлено, що після введення інфікованим стафілококом мишам окремих пробіотичних штамів лакто- та біфідобактерій спостерігали нормалізацію функціональної активності клітин фагоцитарної системи, а також підвищення продукції ендогенного інтерферону. Штами *Lactobacillus delbrueckii* subsp. *bulgaricus* IMV B-7281, *Lactobacillus casei* IMV B-7280, *Lactobacillus acidophilus* IMV B-7279, *Bifidobacterium animales* VKB та *Bifidobacterium animales* VKL є перспективними для створення пробіотичних препаратів, ефективних проти стафілококу, та для корекції імунітету.

К л ю ч о в і с л о в а: *Lactobacillus, Bifidobacterium,* імунітет, макрофаги, інтерферон, стафілокок, миші

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ВЛИЯНИЕ ПРОБИОТИЧЕСКИХ ШТАММОВ ЛАКТО- И БИФИДОБАКТЕРИЙ НА АКТИВНОСТЬ МАКРОФАГОВ И ДРУГИЕ ПОКАЗАТЕЛИ ИММУНИТЕТА ПРИ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ

Р е з ю м е

Определены иммуномодулирующие свойства штаммов *Lactobacillus delbrueckii* subsp. *bulgaricus* IMV B-7281, *Lactobacillus casei* IMV B-7280, *Lactobacillus acidophilus* IMV B-7279, *Bifidobacterium animales* VKL и *Bifidobacterium animales* VKB на модели экспериментальной стафилококковой инфекции у мышей. Установлено, что после введения инфицированным стафилококком мышам отдельных пробиотических штаммов лакто- и бифидобактерий наблюдали нормализацию функциональной активности клеток фагоцитарной системы, а также повышение продукции эндогенного интерферона и количества природных киллеров в селезенке. Штаммы *Lactobacillus delbrueckii* subsp. *bulgaricus* IMV B-7281, *Lactobacillus casei* IMV B-7280, *Lactobacillus acidophilus* IMV B-7279, *Bifidobacterium animales* VKB и *Bifidobacterium animales* VKL являются перспективными для создания пробиотических препаратов, эффективных против стафилококка, и для коррекции иммунитета.

К л ю ч е в ы е с л о в а: *Lactobacillus, Bifidobacterium,* иммунитет, макрофаги, интерферон, стафилококк, мыши.

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Отримано 10.06.2011