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THE EFFECT OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA ON THE NUMBER OF NATURAL KILLER CELLS IN NORMAL CONDITIONS AND IN CASES OF INTRAVAGINAL STAPHYLOCOCCOSIS IN MICE

Background. Development of new immunobiotics based on commensal nonpathogenic probiotic bacteria such as lactic acid bacteria and bifidobacteria with antibacterial and immunomodulatory effects is an important area of modern biotechnology.
Objective. The aim of this study was to determine the effect of *Lactobacillus acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *Bifidobacterium animalis* VKL and *B. animalis* VKB (individually) or their different compositions on the number of natural killer cells (NKC) in the spleen of BALB/c mice at normal conditions and in the case of the experimental intravaginal staphylococcosis.
Methods. The number of NKC in the spleen was studied using monoclonal phycoerythrin-conjugated antibodies against NKC antigens (MACS, Miltenyi Biotec, Germany). Calculations of NKC as well as analysis of the results were performed using flow cytometry method on a FACStar Plus cytofluorometer.
Results. It is shown that the number of NKC in the spleen of intact mice did not change under the influence of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *B. animalis* VKL or *B. animalis* VKB (individually). But, using *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL and *B. animalis* VKB (individually) or their different compositions for colonization of the vagina in the case of intravaginal staphylococcosis associated with increasing of the number of NKC in spleen in different periods of observation. The number of NKC in the spleen of staphylococcus-infected mice completely normalized after treatment with some probiotic compositions. The probiotic bacteria (individually) only partially normalized the number of NKC in the spleen of staphylococcus-infected mice.
Conclusions. Thus, *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKL (individually) or their various compositions are promising to create highly effective immunobiotics, that are able to increase the innate immunity in cases of infections.

Keywords: lactic acid bacteria; bifidobacteria; natural killer cells; spleen; intravaginal staphylococcosis; mice.

Introduction

Infectious diseases caused by pathogenic or opportunistic bacteria are a vast group of human diseases that are frequently associated with immunosuppression. Recently obtained evidence that in addition to neutrophils and macrophages, natural killer cells (NKC) play an important role in host defense against extracellular bacterial infections [1–3]. Numerous experimental and clinical studies of antibacterial effects of activated NKC have demonstrated that they directly kill bacteria using soluble factors and have an indirect effect through interaction with other immune cells such as dendritic cells (DC), macrophages and neutrophils, through the production of cytokines (interleukin (IL)-12, IL-15, IL-18 and interferon (IFN)) [1, 4]. At several diseases, such as viral infections [5], atherosclerosis [6], chronic fatigue, immune dysfunction syndrome [7], cancer [8] the decrease of NKC cytotoxicity or a reduction in their number were observed that also confirms the importance of regular function of these cells in host defense.

Staphylococcus aureus that remains a common cause of nosocomial bacterial infections and asymptomatically colonize the nasal tract, rectum, mouth, genitals and skin, is often resistant to antibiotics and can cause various diseases, including pneumonia, sepsis, septic arthritis, etc. [9–11]. The cellular and molecular mechanisms of anti-staphylococcal host defense are closely associated with innate immune response especially with activity of neutrophils, macrophages and NKC [4, 11–13]. Experimental studies have shown that NKC involve in host defense against bacterial lung infection in mice [11] or in rats [13] as well as from arthritis in mice [12], which

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was induced by *S. aureus*. Thus, the enhancement of innate immunity, in the first place activation of the neutrophils, macrophages, and NKC is a promising direction of development of new therapeutic approaches for the treatment of patients with staphylococcal infection, especially in the case of infection caused by antibiotic-resistant strains.

The use of probiotics based on commensal nonpathogenic probiotic bacteria such as lactic acid bacteria (LAB) and bifidobacteria with antibacterial and immunomodulatory effects probably is an important part of treatment of patients with infectious diseases, including those induced by extracellular bacteria such as *Staphylococcus* spp etc. [14, 15]. It is known that immunomodulatory effects of probiotics are strain-specific and associated with activation of DC, macrophages, epithelial cells, T regulatory cells, effector lymphocytes, B-lymphocytes and NKC [15]. There is the evidence that commensal bacteria, including LAB, affect the regulation of NKC activity and their ability to product IFN-γ that may depends on the LAB-induced dendritic cells [16, 17], this helps them to develop a full range of special functions in the periphery and secondary lymphoid organs. On the one hand, NKC, activated by DC, kill infected or transformed cells in the periphery, and on the other hand, play a key role in Th1 polarization response upon interaction with DC [17]. Bifidobacteria also activated NKC in normal conditions and in case of pathologies [18, 19].

As we have shown in animal models [20, 21] the probiotic strains *Lactobacillus acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *Bifidobacterium animalis* VKL and *B. animalis* VKB from our collection of the probiotic bacteria have a high level of immunomodulatory properties in normal conditions and in cases of infectious and inflammatory diseases. These probiotic bacteria and their various compositions with different efficacy inhibited the persistence of *S. aureus* strain 8325-4 in the vagina of staphylococcus-infected BALB/c mice. In the case of the experimental intravaginal staphylococcosis *L. casei* IMV B-7280, as well as most compositions on the basis of these strains of LAB and bifidobacteria caused a normalization of cellular immunity indicators [21].

Problem statement

This study aimed to investigate if the *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL and *B. animalis* VKB (individually) or their different compositions can alter the number of NKC in the spleen of mice at normal conditions and in the case of the experimental intravaginal staphylococcosis.

Materials and methods

Experimental studies were performed on six-week-old female BALB/c mice, synchronized in their estral cycle. All studies were performed taking into account the rules of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986) and in accordance with "General ethical principles of experiments on animals". Mice were kept in standard vivarium conditions at a temperature of 22 ± 1 °C, they were provided with the full mixed feed and had free access to automatic water bowls.

The bacterial strains used in the study were: *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (deposited in the Depositary of microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine), *B. animalis* VKL and *B. animalis* VKB isolated from content of gut of healthy people in the course of laboratory study of fermented biological materials. The lyophilized in Cuddon Freeze Dryer FD1500 (New Zealand) probiotic bacteria were used in our study. The viability of the LAB and bifidobacteria strains was tested before each experiment by monitoring their growth on the Man-Rogosa-Sharpe (MRS) agar medium or Bifidum-agar medium (respectively) at 37 °C for 24–48 h.

S. aureus strain 8325-4 (kindly provided to us by Professor V.S. Zuyeva, N.F. Gamaleya Institute of Epidemiology and Microbiology, Russian Federation) that has a plasmid of resistance to gentamicin was chosen for modelling the intravaginal staphylococcosis in mice. *S. aureus* strain 8325-4 was grown on selective agar medium for staphylococci (BAIRD-PARKER-Agar, Merck, Germany), which contained gentamicin at a concentration of 15 mg/ml, at 37 °C for 24 h. After that bacterial cells were washed twice with sterile phosphate-buffered saline (PBS).

Suspension of the *S. aureus* 8325-4 in PBS was administered once into vagina of BALB/c mice, in the dose of 5×10^7 cells per animal. The following clinical manifestations of the infection process were observed in the infected mice: significant increase in whitish mucous secretions of the vagina, elevation of body temperature, inactivity and loss of appetite. Suspension of the probiotic bacteria or their different compositions in PBS was administrated into the vagina of intact and staphylococcus-infected mice 1 day after infection at the dose of 1×10^6 cells per animal, once a day for 7 days. When two, three or four

strains of probiotic bacteria were used as a composition, they were used at the concentration to achieve the total number of bacterial cells = 1×10^6. The probiotic compositions used in the study were: *L. casei* IMV B-7280 – *B. animalis* VKB, *L. casei* IMV B-7280 – *B. animalis* VKL, *L. acidophilus* IMV B-7279 – *B. animalis* VKB, *L. acidophilus* IMV B-7279 – *B. animalis* VKL, *L. acidophilus* IMV B-7279 – *L. casei* IMV B-7280, *B. animalis* VKL – *B. animalis* VKB, *L. casei* IMV B-7280 – *B. animalis* VKL – *B. animalis* VKB, *L. casei* IMV B-7280 – *B. animalis* VKL – *L. acidophilus* IMV B-7279, *L. acidophilus* IMV B-7279 – *B. animalis* VKL – *B. animalis* VKB, *L. casei* IMV B-7280 – *B. animalis* VKB – *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280 – *B. animalis* VKB – *B. animalis* VKL – *L. acidophilus* IMV B-7279.

On the 1st, 3rd, 6th and 9th days after the probiotic bacteria first administration, spleens were obtained from decapitated mice of all groups who had previously received anesthesia. Leukocytes were extracted from the spleen cell suspension by fractionating cells in ficoll-verohrafin density gradient (ρ = 1.077 g/cm^3) by centrifuging at 1500 rev/min for 15 min. The cells were then washed twice in the RPMI-1640 culture medium by centrifuging at 1500 rev/min for 10 min. Monoclonal phycoerythrin-conjugated antibodies against NKC antigens (MACS, Miltenyi Biotec, Germany) were used for phenotyping of the isolated cells. Calculations of NKC, as well as analysis of the results, were performed using flow cytometry method on a FACStar Plus cytofluorometer (Becton-Dickinson, USA).

All digital data received were processed with the help of the Epi Info software (version 6.0) through analysis of variance. The null hypothesis for the control and experimental comparative groups was checked using Wilcoxon-Mann-Whitney (U) criteria. The differences between the groups were considered statistically meaningful at $P < 0.05$.

Results and discussion

We have established that the effect of probiotic strains of LAB and bifidobacteria on the number of NKC in the spleen of intact and staphylococcus-infected mice was different. The number of NKC in the spleen of intact mice, whose vagina was colonized with *L. casei* IMV B-7280, *L. acidophillus* IMV B-7279, *B. animalis* VKL or *B. animalis* VKB (individually) remain unchanged throughout the observation period compared with intact mice that did not receive probiotic bacteria (Table 1).

In the spleen of staphylococcus-infected mice the number of NKC changed compared with intact mice, depending on the periods of observation. As shown in Table 2, statistically significant decrease in the number of NKC in the spleen of staphylococcus-infected mice was on the 1st and 6th days. The downward trend in the number of NKC in the spleen of staphylococcus-infected mice we observed on the 3rd day. But, the number of NKC in the spleen of these mice was normalized on the 9th day.

The data presented here demonstrate a time-dependent immunomodulatory effect of probiotic bacteria that we used for the purposes of vagina colonization in the case of intravaginal staphylococcosis in mice. So, treatment of staphylococcus-infected mice with *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKL (individually) resulted in increasing the number of NKC in the spleen in different periods of observation compared with staphylococcus-infected mice that did not receive probiotic bacteria (control group) (see Table 2). We observed a slight decrease in the number of NKC in the spleen of staphylococcus-infected mice that received *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (on the 6th day) or *L. casei* IMV B-7280 (on the 3rd day) compared with intact mice. But these changes were incomprehensible. The number of NKC in the spleens of staphylococcus-infected mice treated with *B. animalis* VKB on the 1st, 3rd and 6th days was the same as in the control group.

***Table* 1.** The number of NKC in the spleen of intact mice who receiving probiotic strains of LAB or bifidobacteria (individually)

Group of mice	NKC (%)/day of study			
	1st day	3rd day	6th day	9th day
Intact mice	9.4 ± 1.8	10.2 ± 1.9	9.9 ± 1.2	9.8 ± 1.3
Received *L. acidophillus* IMV B-7279	10.1 ± 1.9	12.3 ± 1.8	10.5 ± 1.8	9.8 ± 1.6
Received *L. casei* IMV B-7280	9.3 ± 1.6	15.9 ± 1.8	11.2 ± 1.6	9.5 ± 1.5
Received *B. animalis* VKL	9.0 ± 1.1	10.9 ± 1.7	7.5 ± 1.7	7.2 ± 1.8
Received *B. animalis* VKB	8.4 ± 1.6	8.7 ± 1.6	8.1 ± 1.4	7.8 ± 1.5

***Table* 2.** The number of NKC in the spleens of staphylococcus-infected mice who receiving probiotic bacteria (individually)

Group of mice	NKC (%)/day of study			
	1st day	3rd day	6th day	9th day
Intact mice	10.4 ± 1.0	10.4 ± 1.0	10.4 ± 1.0	10.4 ± 1.0
Infected mice (control group)	7.5 ± 1.1*	9.1 ± 0.7	7.2 ± 1.0*	11.6 ± 0.9
Received *L. acidophilus* IMV B-7279	10.7 ± 0.7•	10.4 ± 1.2	6.2 ± 0.8*	13.0 ± 1.4
Received *L. casei* IMV B-7280	9.7 ± 1.0	7.9 ± 0.6*	12.7 ± 0.8•	11.0 ± 1.2
Received *L. delbrueckii* subsp.*bulgaricus* IMV B-7281	10.9 ± 0.4•	11.9 ± 1.3	7.0 ± 0.5*	12.3 ± 0.6
Received *B. animalis* VKL	9.8 ± 1.3	10.1 ± 0.7	12.3 ± 0.6•	9.6 ± 1.1
Received *B. animalis* VKB	7.4 ± 0.5*	6.2 ± 0.9*	7.6 ± 1.4	6.5 ± 1.1*•

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

***Table* 3.** The number of NKC in the spleens of staphylococcus-infected mice who receiving probiotic compositions

Group of mice	NKC (%)/day of study			
	1st day	3rd day	6th day	9th day
Intact mice	10.4 ± 1.0	10.4 ± 1.0	10.4 ± 1.0	10.4 ± 1.0
Infected mice (control group)	7.5 ± 1.1*	9.1 ± 0.7	7.2 ± 1.0*	11.6 ± 0.9
Received *L. casei* IMV B-7280 – *B. animalis* VKB	6.5 ± 0.3*	6.3 ± 1.2*	14.5 ± 1.1*•	8.0 ± 2.9
Received *L. casei* IMV B-7280 – *B. animalis* VKL	13.3 ± 1.3•	7.5 ± 2.5	11.1 ± 1.4•	10.0 ± 1.6
Received *L. acidophilus* IMV B-7279 – *B. animalis* VKB	12.3 ± 1.2•	9.4 ± 0.7	11.3 ± 1.7•	9.8 ± 0.9
Received *L. acidophilus* IMV B-7279 – *B. animalis* VKL	8.6 ± 0.5	7.0 ± 0.3	6.8 ± 0.2*	6.2 ± 3.1*
Received *L. acidophilus* IMV B-7279 – *L. casei* IMV B-7280	12.0 ± 1.4•	9.8 ± 0.9	9.8 ± 0.8	10.4 ± 1.1
Received *B. animalis* VKL – *B. animalis* VKB	11.5 ± 1.1•	18.4 ± 0.9*•	17.4 ± 1.0*•	12.3 ± 1.6
Received *L. casei* IMV B-7280 – *B. animalis* VKL – *B. animalis* VKB	7.3 ± 1.1	15.0 ± 0.75*•	14.0 ± 2.4*	13.2 ± 1.1
Received *L. casei* IMV B-7280 – *B. animalis* VKL – *L. acidophilus* IMV B-7279	18.3 ± 0.6*•	11.5 ± 1.9	15.2 ± 1.1*•	9.0 ± 1.3
Received *L. acidophilus* IMV B-7279 – *B. animalis* VKL – *B. animalis* VKB	9.8 ± 0.6	8.0 ± 0.8	11.0 ± 0.2•	16.1 ± 0.8*•
Received *L. casei* IMV B-7280 – *B. animalis* VKB – *L. acidophilus* IMV B-7279	7.4 ± 0.3	16.0 ± 0.1*•	17.7 ± 0.7*•	16.1 ± 0.5*•
Received *L. casei* IMV B-7280 – *B. animalis* VKB – *B. animalis* VKL – *L. acidophilus* IMV B-7279	12.0 ± 0.2•	9.8 ± 0.3	16.9 ± 0.4*•	10.4 ± 0.2

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

On the 9th day the number of NKC in the spleens of these mice was less than in the control mice and even in intact mice.

To answer the question of whether we will have the same time-dependent immunomodulatory effect, if we are going to colonize the vagina of staphylococcus-infected mice by several probiotic strains together, we used different probiotic compositions (Table 3). As shown by our study, the most effective probiotic compositions of two probiotic strains, which significantly increased the number of NKC in the spleen of staphylococcus-infected mice, were *L. acidophilus* IMV B-7279 – *B. animalis* VKB, *B. animalis* VKL – *B. animalis* VKB and *L. casei* IMV B-7280 – *B. animalis* VKL. We have observed an increase in the number of NKC in the spleen of staphylococcus-infected mice treated with *B. animalis* VKL – *B. animalis* VKB composition on the

1st, 3rd and 6th days or *L. acidophilus* IMV B-7279 – *B. animalis* VKB or *L. casei* IMV B-7280 – *B. animalis* VKL compositions on the 1st and 6th days compared with control group. The number of NKC in the spleens of staphylococcus-infected mice that received *L. acidophilus* IMV B-7279 – *L. casei* IMV B-7280 composition was increased only on the 1st day. However, other probiotic compositions of two strains were not effective.

Among the compositions of the three probiotic strains most effective was *L. casei* IMV B-7280 – *B. animalis* VKL – *L. acidophilus* IMV B-7279. After administration of this probiotic composition into staphylococcus-infected mice the increase in the number of NKC in the spleen was observed on the 1st and 6th days.

The number of NKC increased in the spleen of staphylococcus-infected mice that received *L. casei* IMV B-7280 – *B. animalis* VKB – *B. animalis* VKL composition on the 3rd and 6th days. Treatment of staphylococcus-infected mice with *L. casei* IMV B-7280 – *B. animalis* VKB – *L. acidophilus* IMV B-7279 composition resulted in increasing of the NKC number in the spleen of staphylococcus-infected mice on the 3rd, 6th and 9th days. *L. acidophilus* IMV B-7279 – *B. animalis* VKL – *B. animalis* VKB composition was less effective. The number of NKC in the spleens of these mice was increased only on the 6th and 9th days. There was a significant increase in the number of NKC in the spleen of staphylococcus-infected mice that received *L. casei* IMV B-7280 – *B. animalis* VKB – *B. animalis* VKL – *L. acidophilus* IMV B-7279 composition on the 3rd and 6th days (see Table 3).

Thus, we established that the number of NKC in the spleen of intact mice did not change under the influence of any probiotic strains that we investigated. But, using *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKL (individually) or their different compositions for the purposes of vagina colonization in the case of intravaginal staphylococcosis was associated with increasing in the number of NKC in the spleen compared with indicators of staphylococcus-infected mice that did not receive probiotic bacteria or even with intact mice in different periods of observation.

The previous study in our laboratory showed that after *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *B. animalis* VKL or *B. animalis* VKB (individually) and their different compositions administration into staphylococcus-infected BALB/c mice the growth of *S. aureus* in the vagina was inhibited and the number of $CD3^+$ and $CD4^+$ T-cells in the spleen, and $CD4^+/CD8^+$ index, which decreased after staphylococcus infection, were increased [21]. *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 induced the IL-12 and IFN-γ production by murine macrophages *in vitro* [22]. Thus, the use of these probiotic bacteria and their different compositions for the purposes of vagina colonization of staphylococcus-infected mice led to activation of the innate and adoptive immunity.

It should be noted that the effect of probiotic bacteria on the NKC is one of the key mechanism for strengthening of the innate immunity, which plays an important role in host defense against infections. Therefore most researchers have directed their efforts to study the effect of probiotic bacteria on the NKC activity and/or change in their number in normal conditions and in cases of pathologies. It has been found that such probiotic strains of LAB as *L. pentosus* S-PT84 [23], *L. brevis* KB290 [24], *L. paracasei* [25] after oral administration into intact mice significantly increased the activity of NKC. The cytotoxic activity of NKC and production of cytokines in the spleen and blood were increased in immunosuppressed mice which were treated with *L. sakei* K101 and *L. plantarum* K55-5 [26]. The number of NKC and their cytotoxicity were significantly increased in tumor-bearing C3H/HeN mice after *L. casei* Shirota use for treatment [27]. Oral administration of *L. plantarum* A into tumor-bearing BALB/c mice led to increase of the NKC infiltration into tumor tissue and activation of the effector functions of CD8+ T-cells [28]. *L. casei* ssp *casei* significantly increased the cytotoxicity of NKC and up-regulated the production of IFN-γ and IL-12 in the spleen cells culture in invasive ductal carcinoma bearing BALB/c mice [29].

Our results show that in the case of bacterial infections the majority of probiotic compositions used in the present study more effectively affect the number of NKC in the spleen than probiotic bacteria individually. Thus, in the spleen of staphylococcus-infected mice the number of NKC that decreased on the 1st and 6th days after mice were infected with *S. aureus* 8325-4, completely normalized after use for treatment of such probiotic compositions as *L. casei* IMV B-7280 – *B. animalis* VKL, *L. acidophilus* IMV B-7279 – *B. animalis* VKB, *B. animalis* VKL – *B. animalis* VKB, *L. casei* IMV B-7280 – *B. animalis* VKL – *L. acidophilus* IMV B-7279 and *L. casei* IMV B-7280 – *B. animalis* VKB – *B. animalis* VKL – *L. acidophilus* IMV B-7279. The probiotic bacteria individually only partially normalized the number of NKC in the spleen of staphylococcus-infected mice. Perhaps, this is due to the fact that the

probiotic bacteria used in the compositions can enhance the growth of each other after the administration into animals that may increase their ability to colonize vagina and immunomodulatory effects.

Conclusions

So, our results indicate that *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKB and *B. animalis* VKL (individually) or their different probiotic compositions are promising to create highly effective immunobiotics, that are able to increase the innate immunity in cases of bacterial infections and, possibly, other pathologies. But in the case of intravaginal staphylococcosis, probiotic bacteria individually were less effective than probiotic compositions. It should be noted that for creation of highly effective immunobiotics consisting of several probiotic bacteria it is important to determine their optimal combination and study their activity in different experimental conditions. However, additional studies should be conducted to ensure that these probiotic strains or their different compositions could be used in treatment or prevention of bacterial infections.

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ВПЛИВ ЛАКТОБАЦИЛ І БІФІДОБАКТЕРІЙ НА КІЛЬКІСТЬ ПРИРОДНИХ КІЛЕРНИХ КЛІТИН У НОРМІ ТА ЗА ІНТРАВАГІНАЛЬНОЇ СТАФІЛОКОКОВОЇ ІНФЕКЦІЇ У МИШЕЙ

Проблематика. Розробка нових імунобіотиків на основі непатогенних коменсальних пробіотичних бактерій, таких як лактобацили і біфідобактерії з антибактеріальною та імуномодулювальною дією, є важливим напрямом сучасної біотехнології.

Мета дослідження. Метою роботи є визначення впливу *Lactobacillus acidophilus* IMB B-7279, *L. casei* IMB B-7280, *L. delbrueckii* subsp. *bulgaricus* IMB B-7281, *Bifidobacterium animalis* VKL та *B. animalis* VKB (окремо) або їх різних композицій на кіль-

кість природних кілерних клітин (ПКК) у селезінці мишей лінії BALB/c у нормі та за експериментальної інтравагінальної стафілококової інфекції.

Методика реалізації. Кількість ПКК у селезінці визначали з використанням моноклональних фікоеритрин-кон'югованих антитіл проти антигенів ПКК (MACS, Miltenyi Biotec, Німеччина). Підрахунок ПКК, а також аналіз результатів проводили на цитофлюориметрі FACStar Plus.

Результати дослідження. Показано, що під впливом *L. acidophilus* IMB B-7279, *L. casei* IMB B-7280, *B. animalis* VKL та *B. animalis* VKB (окремо) кількість ПКК у селезінці інтактних мишей не змінювалась. Проте використання для колонізації піхви *L. acidophilus* IMB B-7279, *L. casei* IMB B-7280, *L. delbrueckii* subsp. *bulgaricus* IMB B-7281, *B. animalis* VKL та *B. animalis* VKB (окремо) або їх різних композицій за інтравагінальної стафілококової інфекції було пов'язане зі збільшенням кількості ПКК у селезінці мишей у різні періоди спостереження. В селезінці інфікованих стафілококом мишей кількість ПКК повністю нормалізувалась після використання для лікування певних пробіотичних композицій. Пробіотичні бактерії (окремо) нормалізували кількість ПКК у селезінці інфікованих стафілококом мишей лише частково.

Висновки. *L. acidophilus* IMB B-7279, *L. casei* IMB B-7280, *L. delbrueckii* subsp. *bulgaricus* IMB B-7281 та *B. animalis* VKL (окремо) або їх різні композиції є перспективними для створення високоефективних імунобіотиків, які здатні посилити вроджений імунітет при інфекційних хворобах.

Ключові слова: лактобацили; біфідобактерії; природні кілерні клітини; селезінка; інтравагінальна стафілококова інфекція; миші.

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ВЛИЯНИЕ ЛАКТОБАЦИЛЛ И БИФИДОБАКТЕРИЙ НА КОЛИЧЕСТВО ЕСТЕСТВЕННЫХ КИЛЛЕРНЫХ КЛЕТОК В НОРМЕ И ПРИ ИНТРАВАГИНАЛЬНОЙ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ У МЫШЕЙ

Проблематика. Разработка новых иммунобиотиков на основе непатогенных коменсальных пробиотических бактерий, таких как лактобациллы и бифидобактерии с антибактериальным и иммуномодулирующим действием, является важным направлением современной биотехнологии.

Цель исследования. Целью работы является определение влияния *Lactobacillus acidophilus* ИМВ B-7279, *L. casei* ИМВ B-7280, *L. delbrueckii* subsp. *bulgaricus* ИМВ B-7281, *Bifidobacterium animalis* VKL и *B. animalis* VKB (индивидуально) или их различных композиций на количество естественных киллерных клеток (ЕКК) в селезенке мышей линии BALB/c в норме и при экспериментальной интравагинальной стафилококковой инфекции.

Методика реализации. Количество ЕКК в селезенке определяли с использованием моноклональных фикоэритрин-конъюгированных антител против антигенов ЕКК (MACS, Miltenyi Biotec, Германия). Подсчет ЕКК, а также анализ результатов проводили на цитофлюориметре FACStar Plus.

Результаты исследования. Показано, что под влиянием *L. acidophilus* ИМВ B-7279, *L. casei* ИМВ B-7280, *B. animalis* VKL и *B. animalis* VKB (индивидуально) количество ЕКК в селезенке интактных мышей не изменялось. Однако использование для колонизации влагалища *L. acidophilus* ИМВ B-7279, *L. casei* ИМВ B-7280, *L. delbrueckii* subsp. *bulgaricus* ИМВ B-7281, *B. animalis* VKL и *B. animalis* VKB (индивидуально) или их различных композиций в случае интравагинальной стафилококковой инфекции было связано с увеличением количества ЕКК в селезенке мышей в различные периоды наблюдения. В селезенке инфицированных стафилококком мышей количество ЕКК полностью нормализовалось после использования для лечения пробиотических композиций. Пробиотические бактерии (индивидуально) нормализовали количество ЕКК в селезенке инфицированных стафилококком мышей лишь частично.

Выводы. *L. acidophilus* ИМВ B-7279, *L. casei* ИМВ B-7280, *L. delbrueckii* subsp. *bulgaricus* ИМВ B-7281 и *B. animalis* VKL (индивидуально) и их различные композиции являются перспективными для создания высокоэффективных иммунобиотиков, способных усилить врожденный иммунитет при инфекционных болезнях.

Ключевые слова: лактобациллы; бифидобактерии; естественные киллерные клетки; селезенка; интравагинальная стафилококковая инфекция; мыши.

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