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 cytometer.

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 asymptptomatically 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

UDC 579.261
DOI: 10.20535/1810-0546.2017.3.95070

L.M. Lazarenko†, L.P. Babenko1, V.V. Mokrozub1, M.A. Voronkevych1, D.V. Loseva1, L.M. Sichel1,2, M.Ya. Spivak1,3

1D.K. Zabolotny Institute of Microbiology and Virology of NASU, Kyiv, Ukraine
2Pure Research Products, LLC, Colorado, USA
3LCL “DIAPROF”, Kyiv, Ukraine

* corresponding author: LazarenkoLM@yandex.ru
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 non-pathogenic 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 5×10⁷ 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 1×10⁹ cells per animal, once a day for 7 days. When two, three or four
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. acidophilus 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. acidophilus 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          |
We used different probiotic compositions together, we used different probiotic compositions

phylococcus-infected mice by several probiotic strains have the same time-dependent immunomodulatory

as shown by our study, the most effective even in intact mice.

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
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+/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 mechanisms 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* Shirot 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 after staphylococcus infections was more effective in the spleen 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 — *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 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.
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.

Acknowledgements

The research work was partially carried out under the project “Development of probiotics with antibacterial and immunomodulating properties on the basis of lacto- and bifidobacteria” (No 5979) that was financed by Ukraine and EU countries through the STCU. These studies are the part of L.P. Babenko’s PhD thesis.

List of literature

1. Lodoen M.B. Natural killer cells as an initial defense against pathogens / M.B. Lodoen, L.L. Lanier // Curr. Opin. Immunol. – 2006. – Vol. 18, № 4. – P. 391—398.
2. Role of natural killer cells in antibacterial immunity / S. Schmidt, E. Ullrich, K. Bochenne [et al.] // Expert. Rev. Hematol. – 2016. – Vol. 9, № 12. – P. 1119—1127.
3. NK cell and DC interactions / M.A. Cooper, T.A. Fehniger, A. Fuchs [et al.] // Trends Immunol. – 2004. – Vol. 25, № 1. – P. 47—52.
4. Invasive surgery impairs the regulatory function of human CD56 bright natural killer cells in response to Staphylococcus aureus. Suppression of Interferon-γ synthesis / R. Reinhardt, S. Pohlmann, H. Kleinertz [et al.] // PLoS One. – 2015. – Vol. 10, № 6. – P. e0130155.
5. Sha W.-H. The correlation between NK cell and liver function in patients with primary hepatocellular carcinoma / W.-H. Sha, X.-H. Zeng, L. Min // Gut Liver. – 2014. – Vol. 8, № 3. – P. 298—305.
6. Decreased natural killer cell activity is associated with atherosclerosis in elderly humans / H. Bruunsgaard, A.N. Pedersen, M. Schroll [et al.] // Exp. Gerontol. – 2001. – Vol. 37, № 1. – P. 127—136.
7. Ojo-Amaize E.A. Decreased natural killer cell activity is associated with severity of chronic fatigue immune dysfunction syndrome / E.A. Ojo-Amaize, E.J. Conley, J.B. Peter // Clin. Infect. Dis. – 1994. – Vol. 18, № 1. – P. S157—S159.
8. Decreased risk of colorectal cancer with the high natural killer cell activity NKG2D genotype in Japanese / H. Furue, K. Matsuo, H. Kumimoto [et al.] // Carcinogenesis. – 2008. – Vol. 29, № 2. – P. 316—320.
9. NKC play a critical protective role in host defense against acute extracellular Staphylococcus aureus bacterial infection in the lung / C.L. Small, S. McCormick, N. Gill [et al.] // J. Immunol. – 2008 – Vol. 180, № 8. – P. 5558—5568.
10. Shirriff M.E. Acute septic arthritis / M.E. Shirriff, J.T. Mader // Clin. Microbiol. Rev. – 2002. – Vol. 15, № 4. – P. 527—544.
11. The emergence and evolution of methicillin-resistant Staphylococcus aureus // K. Hiramatsu, L. Cui, M. Kuroda [et al.] // Trends Microbiol. – 2001. – Vol. 9, № 10. – P. 486—493.
12. Exposure to particulate matter increases susceptibility to respiratory Staphylococcus aureus infection in rats via reducing pulmonary natural killer cells / H. Zhao, W. Li, Y. Gao [et al.] // Toxicology. – 2014. – Vol. 325. – P. 180—188.
13. Protective role of NK1.1+ cells in experimental Staphylococcus aureus arthritis / N. Nilsson, T. Bremell, A. Tarkowski [et al.] // Clin. Exp. Immunol. – 1999. – Vol. 117, № 1. – P. 63—69.
14. Amdekar S. Probiotic therapy: immunomodulating approach toward urinary tract infection / S. Amdekar, V. Singh, D.D. Singh // Curr. Microbiol. – 2011. – Vol. 63, № 5. – P. 484—490.
15. Frei R. Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence / R. Frei, M. Akdis, L. O’Mahony // Curr. Opin. Gastroenterol. – 2015. – Vol. 31, № 2. – P. 153—158.
16. Distinct gut-derived lactic acid bacteria elicit divergent dendritic cell-mediated NK cell responses / L.N. Fink, L.H. Zeuthen, H.R. Christensen [et al.] // Int. Immunol. – 2007. – Vol. 19, № 12. – P. 1319—1327.
References

[1] Lodoen MB, Lanier LL. Natural killer cells as an initial defense against pathogens. Curr Opin Immunol. 2006 Aug;18(4):391-8. DOI 10.1016/j.coi.2006.05.002

[2] Schmidt S, Ullrich E, Bochennek K, Zimmermann SY, Lehnhöcher T. Role of natural killer cells in antibacterial immunity. Expert Rev Hematol. 2016 Dec;9(8):1119-27. DOI 10.1080/17474086.2016.1254546

[3] Cooper MA, Fehniger TA, Fuchs A, Caligiuri MA. NK cell and DC interactions. Trends Immunol. 2004 Jan;25(1):47-52. DOI 10.1016/j.it.2003.10.012

[4] Reinhardt R, Pohlmann S, Kleinertz H, Hepner-Scheefczyk M, Paul A, Flohé SB. Invasive surgery impairs the regulatory function of human CD56 bright natural killer cells in response to Staphylococcus aureus. Suppression of Interferon-γ synthesis. PLoS One. 2015 Jun 19;10(6):e0130155. DOI 10.1371/journal.pone.0130155

[5] Sha WH, Zeng XH, Min L. The correlation between NK cell and liver function in patients with primary hepatocellular carcinoma. Gut Liver. 2014 May;8(3):298-305. DOI 10.5009/gnl.2014.8.3.298

[6] Bruunsgaard H, Pedersen AN, Schroll M, Skinhøj P, Pedersen BK. Decreased natural killer cell activity is associated with atherosclerosis in elderly humans. Exp Gerontol. 2001 Dec;36(1):127-36. DOI 10.1016/S0531-5565(01)00162-0

[7] Ojo-Amaize EA, Conley EJ, Peter JB. Decreased natural killer cell activity is associated with severity of chronic fatigue immune dysfunction syndrome. Clin Infect Dis. 1994 Jan;18 Suppl 1:S157-9.

[8] Furue H, Matsuo K, Kumimoto H, Hiraki A, Suzuki T, Yatabe Y, et al. Decreased risk of colorectal cancer with the high natural killer cell activity NKG2D genotype in Japanese. Carcinogenesis. 2008 Feb;29(2):316-20. DOI 10.1093/carcin/bgm260

[9] Small CL, McCormick S, Gill N, Kugathasan K, Santoussuo M, Donaldson N, et al. NK cell critical role in host defense against acute extracellular Staphylococcus aureus bacterial infection in the lung. J Immunol. 2008 Apr 15;180(8):5558-68. DOI 10.1049/jimmunol.180.8.5558

[10] Shirreff ME, Mader JT. Acute septic arthritis. Clin Microbiol Rev. 2002 Oct;15(4):527-44. DOI 10.1128/CMR.15.4.527-544.2002
[11] Hiramatia K, Cui L, Kuroda M, Ito T. The emergence and evolution of meticillin-resistant *Staphylococcus aureus*. Trends Microbiol. 2001 Oct;9(10):486-93.

[12] Zhao H, Li W, Gao Y, Li J, Wang H. Exposure to particular matter increases susceptibility to respiratory *Staphylococcus aureus* infection in rats via reducing pulmonary natural killer cells. Toxicology. 2014 Nov 5;325:180-8. DOI 10.1016/j.tox.2014.09.006

[13] Nilsson N, Bremell T, Tarkowski A, Carlsten H. Protective role of NK1.1+ cells in experimental *Staphylococcus aureus* arthritis. Clin Exp Immunol. 1999 Jul;117(1):63-9. DOI 10.1046/j.1365-2249.1999.00922.x

[14] Amdekar S, Singh V, Singh DD. Probiotic therapy: immunomodulating approach toward urinary tract infection. Curr Microbiol. 2011 Nov;63(5):484-90. DOI 10.1007/s00284-011-0006-2

[15] Frei R, Akdis M, O’Malony L. Prebiotics, probiotics, symbiotics, and the immune system: experimental data and clinical evidence. Curr Opin Gastroenterol. 2015 Mar;31(2):153-8. DOI 10.1097/MOG.0000000000000151

[16] Fink LN, Zeuthen LH, Christensen HR, Morandi B, Frokiaer H, Ferlazzo G. Distinct gut-derived lactic acid bacteria elicit divergent dendritic cell-mediated NK cell responses. Int Immunol. 2010 Dec;19(12):1319-27. DOI 10.1093/intimm/dxm103

[17] Rizzello V, Bonaccorsi I, Dongarra ML, Fink LN, Ferlazzo G. Role of natural killer and dendritic cell crosstalk in immunomodulation by commensal bacteria probiotics. J Biomed Biotechnol. 2011;2011:473097. DOI 10.1155/2011/473097

[18] Kawahara T, Takahashi T, Oishi K, Tanaka H, Masuda M, Takahashi S, et al. Essential role of Toll-like receptors for dendritic cell mediated NK cell responses in murine model. Microbiol Immunol. 2015 Jan;59(1):1-12. DOI 10.1111/1348-0421.12210

[19] Fan J, Hou Y, Zhou S, Cai X. Effect of *Bifidobacterium* on the immunity in BALB/c mice. Wei Sheng Wu Xue Bao. 2015 Apr 4;55(4):484-91.

[20] Lazarenko L, Babenko L, Sichel LS, Pidgorsky V, Mokrozub V, Voronkova O, et al. Antagonistic action of *Lactobacilli* and *Bifidobacteria* in relation to *Staphylococcus aureus* and their influence on the immune response in cases of intravaginal staphylococcosis in mice. Probiotics Antimicrob Proteins. 2012 Jun;4(2):78-89. DOI 10.1007/s12602-012-9093-z

[21] Spivak MYa, Pidgorsky VS, Lazarenko LM, Shynkarenko LM, Rachkova LT, Olevinska ZM. Antagonistic action of Lactobacillus and Bifidobacteria on the immunity of innate immune response of the organism showed on experimental model. Microbiol Biotechnol. 2009;1(5):39-46.

[22] Mokrozub VV, Lazarenko LM, Sichel LM, Babenko LP, Lytvyn PM, Demchenko OM, et al. The role of beneficial bacteria wall elasticity in regulating innate immune response. EPMA J. 2015 Jun 19;6(1):13. DOI 10.1186/s12602-015-0035-1

[23] Koizumi S, Wakita D, Sato T, Mitamura R, Izumo T, Shibata H, et al. Essential role of Toll-like receptors for dendritic cell crosstalk and NK1.1(+) cell-dependent activation of type 1 immunity by *Lactobacillus pentosus* strain S-PT84. Immunol Lett. 2008 Oct 30;120(1-2):14-9. DOI 10.1016/j.imlet.2008.06.003

[24] Fukui Y, Sasaki F, Eke N, Nakai Y, Ishiihama T, Abe K, et al. Effect of *Lactobacillus brevis* KB290 on the cell-mediated cytotoxicity of mouse splenocytes: a DNA microarray analysis. Br J Nutr. 2013 Nov 14;110(9):1617-29. DOI 10.1017/S0007114513000767

[25] Kou X, Chen Q, Ju X, Liu H, Chen W, Xue Z. A tolerant lactic acid bacteria, *Lactobacillus paracasei*, and its immunoregulatory function. Can J Microbiol. 2014 Nov;60(10):729-36. DOI 10.1139/cjm-2014-0383

[26] Lee YD, Hong YF, Jeon B, Jung BJ, Chung DK, Kim H. Differential cytokine regulatory effect of three *Lactobacillus* strains isolated from fermented foods. J Microbiol Biotechnol. 2016 Sep 28;26(9):1517-26. DOI 10.4014/jmb.1601.01044

[27] Takagi A, Matsuaki T, Sato M, Nomoto K, Morotomi M, Yokokura T. Enhancement of natural killer cytotoxicity delayed murine carcinogenesis by a probiotic microorganism. Carcinogenesis. 2001 Apr;22(4):599-605. DOI 10.1093/carcin/22.4.599

[28] Lee JW, Shin JG, Kim EH, Kang HE, Yim IB, Kim JY, et al. Immunomodulatory and antitumor effects in vivo by the cosupplementation of *Lactobacillus casei* and *Bifidobacterium animalis*. J Vet Sci. 2004 Mar;5(1):41-8.

[29] Soltan Dallal MM, Yazdi MH, Holakuyee M, Hassan ZM, Abolhassani M, Mahdavi M. *Lactobacillus casei* spp. *casei* induced TH1 cytokine profile and natural killer cells activity in invasive ductal carcinoma bearing mice. Iran J Allergy Asthma Immunol. 2012 Jun;11(2):183-9. DOI 01.112/jiaai.183189

Л.М. Лазаренко, Л.П. Бабенко, В.В. Мокроузб, М.А. Воронкевич, Д.В. Лосева, Л.М. Сіщел, М.Я. Співак

ВПЛИВ ЛАКТОБАЦІЙ І БІФІДОБАЦІЙ НА КІЛЬКІСТЬ ПРИРОДНИХ КІЛЕРНИХ КЛІТИН У НОРМІ ТА ЗА ІНТРАВАГІНАЛЬНОЮ СТАФІЛОКОКОВОЮ ІНФЕКЦІЄЮ У МІШІ

Проблематика. Розробка нових імунобіотиків на основі непатогенних коменсальних бактерій, таких як лактобацилли і біфідобацилли з антибактеріальним та імуномодулюючим 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 кількісному рості івану імунобіотиків на основі непатогенних коменсальних бактерій, таких як лактобацилли і біфідобацилли з антибактеріальним та імуномодулюючим дією, є важливим напрямом сучасної біотехнології.
кість природних кілерних клітин (ПКК) у селезінці миші лінії BALB/c у нормі та за експериментальної інтравагінальної стафіло-кооккової інфекції.

Методика реалізації. Кількість ПКК у селезінці визначали з використанням моноокліональних фіксованих конюгованих антитіл проти антигенів ПКК (MACS, Miltenyi Biotec, Німеччина). Підрахунок ПКК, а також аналіз результатів проводили на цитофлуориметрі FACStar Plus.

Результати дослідження. Показано, що під впливом L. acidophilus IMB-7279, L. casei IMB-7280, B. animalis VKL та B. animalis VKB (окремо) кількість ПКК у селезінці інтенсивні не змінювалась. Проте використання для колонізації лінії L. acidophilus IMB-7279, L. casei IMB-7280, L. delbrueckii subsp. bulgaricus IMB-7281, B. animalis VKL та B. animalis VKB (окремо) або їх різні комбінації за інтравагінальної стафілоокооккової інфекції було пов’язане зі збільшенням кількості ПКК у селезінці миші у різні періоди спостереження. В селезінці інфікованих стафілоокооккового миші кількість ПКК повісно нормалізувалась після використання для лікування певних пробіотичних композицій. Пробіотичні бактерії (окремо) нормалізували кількість ПКК у селезінці інфікованих стафілоокоокковий миші лише частково.

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

Ключові слова: лактобацилли; бифідобактерії; природні кілерні клітини; селезенка; інтравагінальна стафілоокооккова інфекція; миші.

Л.Н. Лазаренко, Л.П. Бабенко, В.В. Мокрозуб, М.А. Воронкевич, Д.В. Лосева, Л.Н. Сишен, Н.Я. Спивак

ВИЯЗАННЯ ЛАКТОБАЦИЛЛИ I БИФИДОБАКТЕРІЙ НА КОЛИЧЕСТВО ЕСТЕСТВЕННИХ КИЛЕРНЫХ КЛЕТОК В НОРМЕ И ПРИ ИНТРАВАГИНАЛЬНОЙ СТАФИЛОКОКОКОВОЙ ИНФЕКЦИИ У МЫШЕЙ

ПРОБЕЛЕМТИКА. Разработка новых иммунобиотиков на основе непатогенных комменсальных пробиотических бактерий, таких как лактобациллы и бифидобактерии с антибактериальным и иммуномодулирующим действием, является важным направле- нием современной биотехнологии.

Цель исследования. Целью работы является определение влияния Lactobacillus acidophilus IMB-7279, L. casei IMB-7280, L. delbrueckii subsp. bulgaricus IMB-7281, Bifidobacterium animalis VKL и B. animalis VKB (индивидуально) или их различ- них 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 моноокліональных фіксованих конюгованих антитіл против антигенов ЕКК (MACS, Miltenyi Biotec, Германия). Подсчет ЕКК, а также анализ результатов прово- дили на цитофлюориметре FACStar Plus.

Результаты исследования. Показано, что под влиянием L. acidophilus IMB-7279, L. casei IMB-7280, B. animalis VKL и B. animalis VKB (индивідуально) количество ЕКК в селезенке интактных мышей не изменялось. Однако использование для колонизации влагалища L. acidophilus IMB-7279, L. casei IMB-7280, L. delbrueckii subsp. bulgaricus IMB-7281, B. animalis VKL и B. animalis VKB (индивідуально) 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 стафилококковом мышей количество ЕКК полностью нормализовалось после использования для лечения пробиотических композиций. Пробиотические бактерии (индивідуально) нормализовали количество ЕКК в селезенке инфицированных стафилоко- кковом мышей лишь частично.

Выводы. L. acidophilus IMB-7279, L. casei IMB-7280, L. delbrueckii subsp. bulgaricus IMB-7281 и B. animalis VKL (индивідуально) и их различные композиции являются перспективными для создания высокоэффективных иммунобиотиков, способ- ных усиливать врожденный иммунитет при инфекционных болезнях.

Ключевые слова: лактобациллы; бифидобактерии; естественные киллерные клетки; селезенка; интравагинальная ста- филококковая инфекция; мыши.

Reprinted from: Naukovi Visti NTUU KPI, 2017;3:48-56

Received 6 November 2017
Accepted 4 December 2017