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B. animalis AND THE LECTIN OF *B. subtilis* DIFFERENTLY REGULATE CYTOKINES PRODUCTION BY MACROPHAGES IN TUMOUR-BEARERS

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By producing a variety of soluble factors, including cytokines, macrophages (Mph) modulate the activity of other immune cells and have direct effects on cancer cells. This makes Mph a promising target for cancer immunotherapy.

Aim. This work purposed to study the effect of the simultaneous use of *B. animalis* and the lectin of *B. subtilis* on the production of some cytokines by Mph in tumour -bearing mice.

Materials and Methods. Female Balb/c mice bearing solid Ehrlich adenocarcinoma (SEA) were treated with the lectin (s/c, 1 mg/kg body weight) or *B. animalis* (per os, 7×10^5 CFU/mouse) or with their combination. The lectin was isolated from the cultural fluid of *B. subtilis* IMV B-7724. Lyophilized cells of *B. animalis* subsp. *lactis* BB-12 (Lek Pharmaceuticals, Slovenia) was used as a probiotic. On day 28 of tumour growth, Mph from the peritoneal cavity (pMph) and tumour nodule (tMph) were isolated and analyzed for TNF-α and IL-10 production.

Results. The pMph and tMph of untreated mice produced low levels of TNF-α (739.8 and 800.4 pg/mL respectively) and high levels of IL-10 (1169.8 and 1090.5 pg/mL respectively), the TNF-α/IL-10 ratio was 0.68 and 0.95 respectively. The lectin improved the TNF-α/IL-10 ratio to 1.13 (in the intact mice, it was 1.11). In the combined group, the effect was similar but less profound. The results of the *B. animalis* group did not differ from the untreated SEA group.

Conclusions. The changes in the production levels of cytokines indicate the lasting preservation of Mph functional activity in the tumour -bearers due to the influence of probiotics or their metabolites.

Key words: macrophages, cytokines, *B. animalis*, bacterial lectin, cancer.

Macrophages (Mph) attract attention from researchers due to their ability to rapidly change their functional properties depending on the stimuli of the local microenvironment or therapeutic agents. Depending on the microenvironmental signal, they polarise into different phenotypes of proinflammatory M1 or suppressive M2. Tumour -associated macrophages (TAMs) are predominantly of the M2 phenotype. Clinical studies have shown that the accumulation of M2 TAMs correlates with poor clinical outcomes [1]. Mph penetrates the tumour -affected tissues or infiltrates the microenvironment of various solid tumours. There, they can force tumour progression by inducing proliferation, angiogenesis, and degradation of the extracellular matrix, which promotes metastasis [2].

On the other hand, M1 Mph can efficiently eliminate cancer cells, present cancer antigens, and direct immune response toward the Th1 type [3]. Due to the

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production of a broad spectrum of cytokines, Mph can regulate the immune response by enhancing or suppressing the activity of immune cells. Mph reprogramming is one of the interventional strategies that is under intensive study today [4].

The microbiota plays a significant role in shaping both immunity and tumour — microenvironment. Data from experimental and clinical studies suggest a link between the gut microbiome and the tumour -environment, especially in the regulation of tumour -infiltrating Mph. The possible effect of probiotic bacteria on the immune system is increasingly attracting interest for potential therapeutic and prophylactic applications in various diseases. There is data that probiotic bacteria have a favourable effect on the host immunity due to modulation of Mph polarisation with different strains polarizing Mph towards different types [5, 6].

However, the precise mechanism by which microbiota stimulate the anticancer immune response is poorly understood. The possibility of enhancing the immunomodulatory effect by simultaneous application of different bacteria (or their metabolites) is also under-studied. This work aimed to study the impact of the concurrent use of *B. animalis* and the lectin of *B. subtilis* on the production of some cytokines by Mph in tumour-bearing mice.

Materials and Methods

The study was performed on female Balb/c mice 2.0–2.5-month-old, weighting 19.0–21.0 g, breed at the vivarium of IEPOR of NAS of Ukraine. The use and care of experimental animals were performed in accordance with the standard international rules on biological ethics and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Solid Ehrlich adenocarcinoma (EAC) was used as a tumour model. Cancer cells were kindly granted by the Bank of Cell Lines from Human and Animal Tissues, IEPOR of NASU. Tumour's cells were injected intramuscularly into the hind limb (5×10^5 cells/mouse).

The *bacterial lectin* was isolated from the cultural fluid of *B. subtilis IMV* B-7724, as described earlier [7].

Lyophilized cells of *B. animalis* subsp. lactis BB-12 (Lek Pharmaceuticals, Slovenia) were used in the experiment as a probiotic.

Animals were divided into five groups (5 mice per group): IC — intact control; EAC — tumour — bearing mice that were injected with 0.9% NaCl solution; Lectin — tumour — bearing mice that were injected with B. *subtilis* IMB B-7724 lectin (subcutaneously, 1 mg/kg body weight); *B. animalis* — tumour-bearing mice that were feed with *B. animalis* subsp. lactic BB-12 (*per os*, 7×10^5 CFU/mouse); Lectin + *B. animalis* — tumour -bearing mice that were administered with both the lectin and *B. animalis*.

On day 28 of tumour growth, peritoneal (pMph), and tumour-infiltraiting (tMph) Mphs were isolated and analyzed for TNF-α and IL-10 production as was described in [8]. Cytokines concentration was detected by BD OptEIA Set Mouse TFN-α and BD OptEIA Set Mouse IL-10 (BD Biosciences, USA) kits according to the manufacturer's recommendations. Statistical analisys was performed by nonparametric Mann-Whitney U test using Prism software Version 8.0. The difference was considered as significant at $P < 0.05$.

Results and Discussion

The levels of proinflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines production by pMph and tMph of experimental mice are shown in Figs. 1–2 respectively. Regardless of the anatomical niche, Mph of control EAC group mice produced low levels of TNF-α: pMph — 739.8±7.3 pg/mL; tMph — 800.4±23.0 pg/mL. The amount of IL-10 was high in the supernatants of both pMph (1169.8±25.2 pg/mL) and tMph (1090.5±19.7 pg/mL). The TNF-α/IL-10 ratio was 0.68 and 0.95 units respectively.

The application of probiotics and/or lectins led to changes in TNF-α and IL-10 production depending on the agents used. The lectin injections led to an increase in TNF-α production (pMph 1.2-fold and tMph 1.3-fold, $P < 0.05$) and to a simultaneous decrease in IL-10 production (pMph and tMph 1.1-fold, $P < 0.05$). The TNF-α/IL-10 ratio was 1.14 and 1.13 units respectively, which did not differ from the intact control. Changes in the cytokine production in the group of combined application were similar but less pronounced: TNF-α/ IL-10 was 0.82 and 1.08 units, respectively. Such results are more typical for proinflammatory M1 Mph.

The results of *B. animalis* application did not differ significantly from the EAC group: TNF-α/IL-10 was 0.68 and 0.95 units for pMph and tMph, respectively, which is typical for Mph with the M2 phenotype. The

influence on TNF-α and IL-10 production correlate with the effect on tumour growth. It was demonstrated that the administration of the lectin alone or in combination with *B. animalis* led to a suppression of tumour growth (by 55.0 and 50.5%, respectively); the use of *B. animalis* alone did not effect on tumour volume.

Our results are consistent with data on the immunomodulatory effects of microbial products obtained in ex vivo and *in vivo* studies. It is known that different species and strains of probiotic bacteria can modulate Mph function in other ways (activate M1 or M2 cells), thus potentiating or suppressing the immune response [9]. For instance, *Bifidobacterium lactis* BB-12 can induce polarisation of M2 Mph, which results in a decrease in IL-6 and IL-12 while increasing the IL-10 production [10]. On the contrary, probiotic strains of Lactobacillus are prone to stimulate Mph and dendritic cells to secrete IL-12 and TNF (M1 cytokines) and thus direct the immune response towards the Th1 type [11]. It gives a key to influence immune response through the application of probiotics and bacterial metabolites.

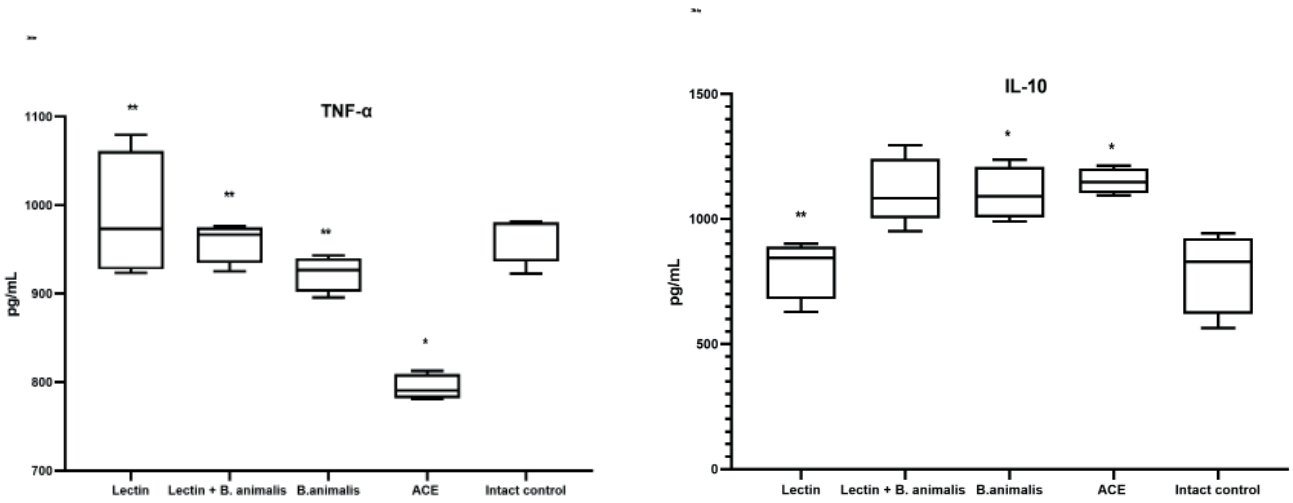

Fig. 1. **The level of TNF-α and IL-10 production by pMph of treated and control EAC-bearing mice. * — *P* <0.05 compared with IC, ** — *P* <0.05 compared with EAC**

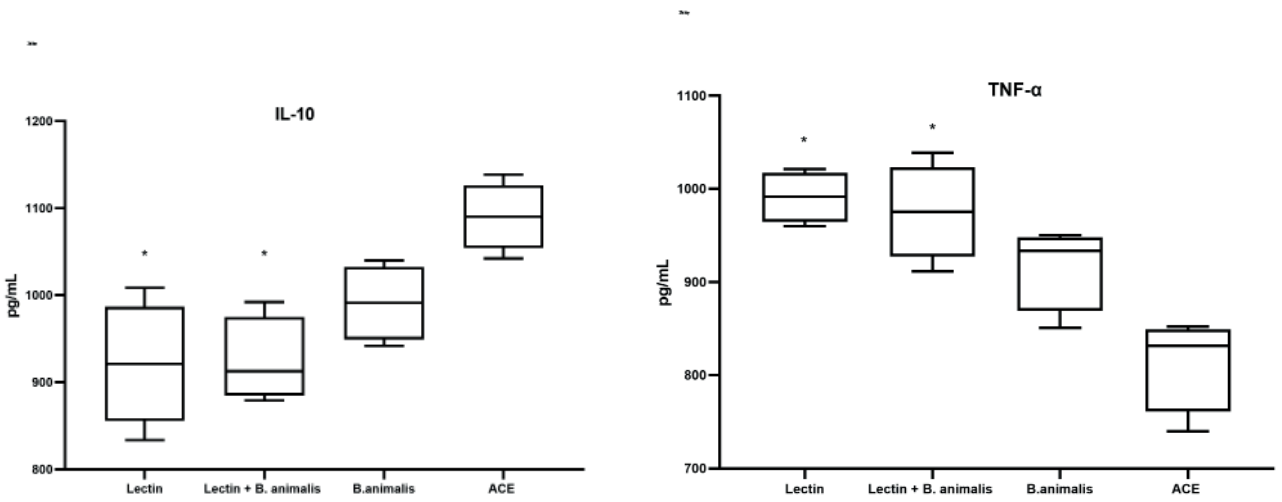

Fig. 2. **The level of TNF-α and IL-10 production by tMph of treated and control EAC-bearing mice.**
* — *P* <0.05 compared with IC

Conclusions

The changes in the production levels of cytokines indicate the lasting preservation of Mph functional activity in the tumour -bearers due to the influence of probiotics or their metabolites.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

N.I. Fedosova — performing enzyme immunoassay, writing an article; N.L. Cheremshenko, S.V. Gogol — experimental work, primary analysis of results; T.V. Symchych — statistical processing of results and figure preparation; I.M. Voyeykova — manuscript editing; O.O. Lykhova, V.F. Chekhun — idea of the study, guided the conception of the research.

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ВІДМІННОСТІ ВПЛИВУ *B. animalis* ТА ЛЕКТИНУ *B. subtilis* НА ПРОДУКЦІЮ ЦИТОКІНІВ МАКРОФАГАМИ ТВАРИН З ПУХЛИНАМИ

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Макрофаги (Мф) здатні модулювати активність інших ефекторів протипухлинного імунітету та впливати на взаємодію пухлинних клітин з мікрооточенням завдяки продукції низки розчинних факторів, зокрема цитокінів. Такі властивості роблять Мф привабливою мішенню для імунотерапії раку.

Метою роботи було дослідити вплив *B. animalis* **та** метаболіту *B. subtilis* на продукціію низки цитокінів Мф мишей з модельною пухлиною.

Матеріали і методи. Мишам лінії Balb/c з солідною формою аденокарциноми Ерліха (АКЕ), вводили бактеріальний лектин (п/шк, 1 мг/гк ваги), *B. animalis* (*per os*, 7×10^5 КУО/мишу) або їхню комбінацію. Бактеріальний лектин отримували з культуральної рідини *B. subtilis IM*V B-7724. Ліофілізовані клітини *B. animalis* subsp. *lactis* BB-12 (Lek Pharmaceuticals, Slovenia) використані як пробіотик. На 28-му добу росту пухлини визначали рівні продукції TNF-α та IL-10 Мф, виділеними з перитонеальної порожнини (пМф) та пухлинної тканини (пхМф).

Результати. Для пМф та пхМф нелікованих мишей з АКЕ характерні низькі рівні продукції TNF-α (відповідно 739,8 та 800,4 pg/mL) та підвищені рівні IL-10 (відповідно 1169,8 та 1090,5 pg/mL), співвідношення TNF-α/ IL-10 становило 0,68 та 0,95 ум.од., відповідно. Введення бактеріального лектину призводило до нормалізації показників: TNF-α/IL-10 = 1,13 (проти 1,11 в інтактному контролі). Зміни рівнів продукції цитокінів за комбінованого застосування бактеріального лектину та *B. animalis* були аналогічними, але менш вираженими. При застосуванні окремо *B. animalis* досліджені показники статистично достовірно не відрізнялись від групи АКЕ.

Висновки. Зміни рівня продукції цитокінів свідчать про тривале збереження функціональної активності Mph у носіїв пухлини внаслідок впливу пробіотиків або їхніх метаболітів.

Ключові слова: макрофаги, цитокіни, *B. animalis*, бактеріальний лектин, рак.