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THE COMPLEX EFFECT OF *BIFIDOBACTERIUM* AND *LACTOBACILLUS* SPECIES AND METFORMIN ON MACROPHAGES POLARIZATION IN *IN VIVO* STUDIES

Aim: *to study the complex effect of the metformin and B. animalis subsp. lactis BB-12 or L. rhamnosus GG application on the polarization of peritoneal macrophages.* ***Object and Methods:*** *the studies were conducted on female Balb/c mice. Ehrlich adenocarcinoma (EAC) was used as an experimental tumor. Tumor-bearing animals were administered metformin or metformin in combination with B. animalis subsp. lactis BB-12 or L. rhamnosus GG for 20 days. The parameters of macrophages' (Mph) functional activity were determined by the levels of NO and reactive oxygen species (ROS) production, arginase (Arg) activity, phagocytic and cytotoxic activity, and the production of TNF-α and IL-10 cytokines.* ***Results:*** *Metformin administered as single treatment caused a binary effect on the polarization state of Mph: it positively influenced Mph cytotoxic activity, a high NO/Arg ratio, and ROS production (characteristics of M1 Mph), while simultaneously increasing phagocytic activity (a sign of M2 Mph) and having a weak effect on cytokine production. At the same time, when combined with bacterial preparations, metformin synergized with the influence of bacteria on Mph polarization. Due to the decreased NO/Arg ratio and cytotoxic activity concomitantly with the increased phagocytic activity, and the spectrum of produced cytokines metformin in combination with B. animalis subsp. lactis BB-12 supported Mph polarization toward one of the M2 subtypes. In contrast, the combined administration of metformin and L. rhamnosus GG promoted M1-type Mph polarization, as indicated by the prolonged preservation of Mph cytotoxic activity, their ability to produce high levels of NO, ROS, and TNF-α, while decreasing phagocytic activity and IL-10 production. Combined administration of metformin with bacterial preparations (but not metformin alone) contributed to tumor growth inhibition by 56.2% (B. animalis + metformin) and 62.0% (L. rhamnosus + metformin). Tumor volume inversely correlated with NO/Arg ratio or Mph cytotoxic activity (r equals to −0.785 and −0.742, respectively, $p < 0.05$ in both cases).* ***Conclusion:*** *combining metformin with bacterial preparations did not significantly alter the effect of the latter on macrophage (Mph) polarization, while demonstrating a pronounced antitumor effect. These findings highlight the potential of this combination for targeted immunocorrection, overcoming tumor-mediated immunosuppression, and enhancing the efficacy of cancer treatment.*

Keywords: *Ehrlich carcinoma, B. animalis subsp. lactis BB-12, L. rhamnosus GG, metformin, macrophages, polarization state, antitumor efficacy.*

Modern strategies of cancer patients' treatment include the development of approaches aimed at modifying antitumor immune responses and the metabolic profile of the tumor microenvironment. From this perspective, there is a growing interest in studying the efficacy of the complex action of human natural microbiota (strains of *Bifidobacterium* spp., *Lactobacillus* spp.) and the well-known hypoglycemic

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agent metformin. Although each of these components individually has proven biological activity and is widely used in medical practice, their combined effect on key reactions of the antitumor immune response remains insufficiently studied.

The widely recognized efficacy of *Bifidobacterium* spp. and *Lactobacillus* spp. strains in treating patients with various inflammatory diseases is largely due to the immunomodulatory properties of these microorganisms. Important role of microbiota in the formation of innate and adaptive immune responses has been demonstrated both under physiological conditions and in pathological states, including oncological diseases. The microbiota interacts with immune cells, influencing processes such as immune cell differentiation, cytokine production, and the maintenance of immunological tolerance [1]. However, the probiotics are often combined with other medications, the mutual influence of which may potentiate or inhibit each other's activity. A complex relationship has been demonstrated between the gut microbiome and anticancer therapy agents, resulting not only in changes in the qualitative and quantitative composition of the microbiota but also in microorganisms' metabolite-driven changes in the metabolism of anticancer drugs and immune reactions. As a result of such complex interaction, adverse reactions may occur, and a decrease in treatment efficacy may be observed [2, 3]. Mutual multidirectional effects of drugs have also been demonstrated for the combined use of probiotics and immunotherapy, which is becoming a common approach in cancer patients' treatment. It has been shown that, in addition to direct action on immune cells, immunomodulatory drugs affect the gut microbiota by changing its qualitative composition—specifically, by reducing the number of beneficial saprophytic bacteria (e.g., *Bifidobacterium*, *Lactobacillus*) and increasing the number of potentially pathogenic species. In turn, imbalance in the gut microbiota composition caused by medications can lead to secondary changes in the immune response and influence the treatment effectiveness [4, 5].

Other medications including metformin can be expected to impose similar effects on the microbiota. Metformin is one of the most common hypoglycemic drugs used in the treatment of patients with type 2 diabetes mellitus especially in overweight patients. However, its effectiveness in treating several other diseases is drawing scientists' attention. It has been shown that in addition to its hypoglycemic effect metformin exhibits multiple biological activities including immunomodulatory, anti-inflammatory, antioxidant, cardioprotective, hepatoprotective, and regenerative properties [6–9]. The immunomodulatory effect of metformin is due, in part, to its ability to influence the functional state and polarization of macrophages (Mph) [7]. The metformin-induced suppression of M1 Mph polarization and maintaining of M2 Mph polarization is one of the mechanisms of its anti-inflammatory properties which underlie its protective effects in chronic inflammatory diseases, atherosclerosis, and obesity [10–12]. At the same time, literature data suggest that in case of cancer, metformin promotes the reprogramming of tumor associated Mph from the anti-inflammatory M2 phenotype to the pro-inflammatory M1 phenotype significantly enhancing antitumor resistance. The metformin-induced repolarization of Mph leads to changes in cytokine production: an increase in M1 associated IL-12 and TNF-α cytokines and a decrease in the levels of immunosuppressive IL-8, IL-10, and TGF-β which are associated with M2 Mph [7, 13, 14]. Given that the interaction of bacterial metabolites with Mph can also radically alter their polarization state, combining of human *Bifidobacterium* and *Lactobacillus* microbiomic bacteria with metformin may prove to be a promising approach for modulating both local and systemic immune responses in the context of malignant growth.

Therefore, the aim of the research was to study the complex effect of the metformin and *B. animalis* subsp. *lactis* BB-12 or *L. rhamnosus* GG application on the polarization of peritoneal macrophages.

OBJECTS AND METHODS

Experimental animals. The study was performed on female Balb/c mice (age 2.0–2.5 months, weight 19.0–22.0 g) obtained from the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the National Academy of Sciences of Ukraine. The animals were kept under standard vivarium conditions with a natural light cycle and a balanced diet. Animal maintenance and experimental procedures were carried out in accordance with standard international rules on bioethics and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [15]. The research protocol has been approved by the Bioethics Committee of the IEPOR (Protocol No. 5 dated May 6, 2025). All animals underwent a 10-day quarantine before being included in the study. After the adaptation period, the animals were weighed, divided into groups, and marked with serial numbers.

Tumor model. Ehrlich solid adenocarcinoma (ECA) was used as a model of tumor growth [16]. Tumor cells were obtained from the IEPOR's Bank of Human and Animal Tissue Cell Lines. For tumor transplantation, EAC cells were injected intramuscularly into the thigh (5×10^5 cells/mouse).

Preparations. Lyophilized cells of *B. animalis* subsp. *lactis* BB-12 (Lek Pharmaceuticals, Ljubljana, Slovenia) and *L. rhamnosus* GG (Probiotical S.p.A., Switzerland) were used as probiotic preparations. Probiotics were administered at a dose of 7×10^5 CFU/mouse per administration, and metformin at a dose of 4 mg/mouse per administration in 0.2 ml of 0.9% NaCl. All preparations were administered *per os* daily via a gastric tube for 20 days.

Experimental Design. Experimental animals ($n = 50$) were divided into 5 groups: "Intact Control, IC" — mice without tumors that were administered 0.9% NaCl ($n = 10$); "EAC" — tumor-bearing mice that were administered 0.9% NaCl ($n = 10$); "Metformin" — tumor-bearing mice that were administered metformin starting from the 2nd day after EAC transplantation ($n = 10$); "*B. animalis* + metformin" — mice bearin EAC that were administered metformin and *B. animalis* ($n = 10$); "*L. rhamnosus* + metformin" — mice bearing EAC that were administered metformin and *L. rhamnosus* ($n = 10$).

On days 14 and 21 of tumor growth, the functional activity of peritoneal Mph was assessed: phagocytic activity, levels of NO production and arginase (Arg) activity, production of reactive oxygen species (ROS), and amount of TNF-α and IL-10 in Mph supernatants. Throughout the study, standard tumor growth parameters were assessed (grafting frequency, latent period of tumor appearance, volume of the tumor nodule). The methods are described in detail in [17].

Statistical analysis of the results was performed using conventional methods of variational statistics with GraphPad Prism 8.0.1 (GraphPad Software Inc., USA) software. Pearson's correlation coefficient was calculated using the GraphPad Prism 8.0.1 software (GraphPad Software Inc., USA). The significance of the difference between the control and experimental groups was assessed using Student's t-test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

One of the most important characteristics of macrophages is their ability to exhibit cytotoxic activity against various target cells, including malignant cells. However, tumor cells reprogram macrophages both locally within the tumor microenvironment and systemically throughout the organism [18, 19]. Consequently, macrophages lose their cytotoxic properties and actively adopt pro-tumor and immunosuppressive roles. Therefore, researchers are actively investigating methods to reprogram or modulate macrophages. Modulating the microbiome or altering energy metabolism has been shown to influence macrophage polarization and functional state.

According to the obtained results, the administration of metformin preserved Mph cytotoxic activity throughout the entire observation period (*Fig. 1*). On day 21 of tumor growth, despite a decrease compared to the "IC" group (by 1.3 times, $p < 0.05$), the cytotoxic activity of Mph in animals receiving metformin was twice as high ($p < 0.05$) compared to the control tumor-bearing animals. Combined use of metformin with bifidobacteria and lactobacilli had multidirectional effects on the cytotoxic activity of Mph: in animals of the "*B. animalis* + metformin" group, the administration of bifidobacteria cancelled the protective effect of metformin on Mph cytotoxic activity. It decreased to the level of untreated EAC-bearing mice and was significantly lesser compared to the "IC" group (by 1.8 times, $p < 0.05$). In contrast, the combined administration of metformin and L. rhamnosus maintained the specific cytotoxic activity of Mph at the IC level throughout the entire experiment (see *Fig. 1*). The cytotoxic activity in animals of this group was significantly higher compared to both the control tumor-bearers and the animals of the "*B. animalis* + metformin" group.

As mentioned above, the loss of cytotoxic activity is a consequence and a sign of tumor-induced Mph reprogramming, which is also reflected in alterations in the balance of L-arginine metabolizing enzymes. Tumor-suppressive M1 Mph actively produce NO, while in pro-tumor M2 Mph, arginine is metabolized by arginase more intensively. Therefore, a progressive decrease in the NO/Arg ratio was observed in control tumor-bearing animals compared to intact mice (*Fig. 2*). The NO/Arg ratio in Mph samples from mice administered metformin was significantly lower than

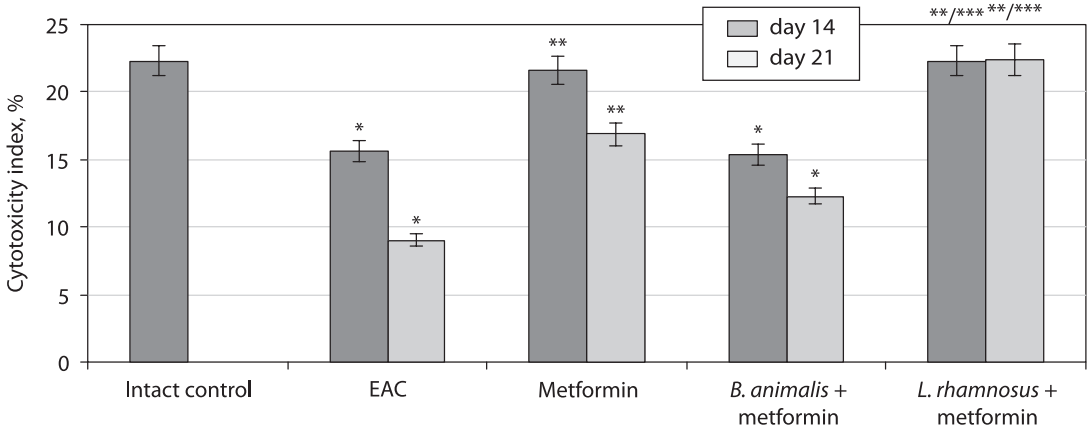

Fig. 1. The level of cytotoxic activity of peritoneal Mph in mice administered metformin and *B. animalis* or *L. rhamnosus*
* — $p < 0.05$ compared to the "IC" group; ** — $p < 0.05$ compared to the "EAC" group; *** — $p < 0.05$ compared to the "*B. animalis* + metformin" group.

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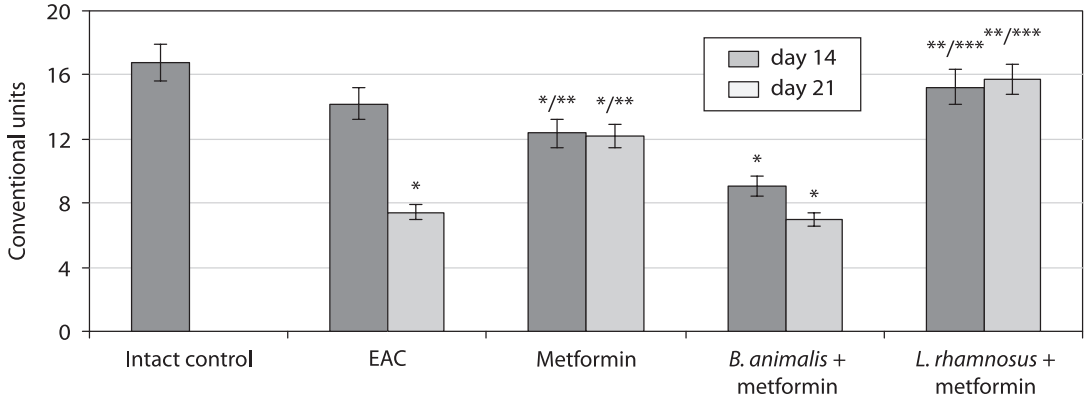

Fig. 2. The NO/Arg ratio in peritoneal Mph of mice administered metformin and *B. animalis* or *L. rhamnosus*
* — $p < 0.05$ compared to the "IC" group; ** — $p < 0.05$ compared to the "EAC" group; *** — $p < 0.05$ compared to the "*B. animalis* + metformin" group.

in the "IC" group, yet it exceeded the values of untreated EAC-bearing mice. Similarly to the influence on cytotoxic activity, the combined administration of metformin with bacterial preparations had a multidirectional effect. Specifically, in the "*L. rhamnosus* + metformin" group, the NO/Arg ratio remained at the level of intact mice throughout the observation period and significantly exceeded the values of both control tumor-bearing animals and mice from the "*B. animalis* + metformin" group. Conversely, in the "*B. animalis* + metformin" group, the NO/Arg ratio significantly decreased compared to the intact mice on day 14 of tumor growth and remained so until the end of the experiment.

Investigated preparations influenced ROS production to a lesser extent (*Fig. 3*). The administration of metformin caused a significant increase in the level of ROS production on day14 of tumor growth ($p < 0.05$ compared to both the intact control and untreated tumor-bearing animals). In the combined treatment groups, ROS production remained at the level of the intact control throughout the entire observation period and was higher compared to the "EAC" group on day 21 of tumor growth. No significant difference was observed between the combined treatment groups.

Pronounced differences between the studied groups were observed in phagocytic activity, a high level of which is a hallmark of M2 macrophage polarization. As shown in *Fig. 4*, on day 14 of tumor growth, there was a significant increase (by 1.6 times, $p < 0.05$) in the phagocytic activity of Mph in mice administered metformin alone or in combination with *B. animalis*. On day 21, this parameter remained elevated only in mice of the "*B. animalis* + metformin" group. In mice of the "*L. rhamnosus* + metformin" group, Mph phagocytic activity remained at the level of intact mice throughout the entire experiment.

Thus, according to the studied functional characteristics, the administration of metformin alone had a dualistic effect on Mph polarization, which is consistent with literature data regarding the context-dependent activity of metformin in its influence on the immune system in general and Mph polarization in particular [7, 20]. The administration of metformin in combination with B. animalis promoted M2 polarization of peritoneal Mph, while its combination with *L. rham-*

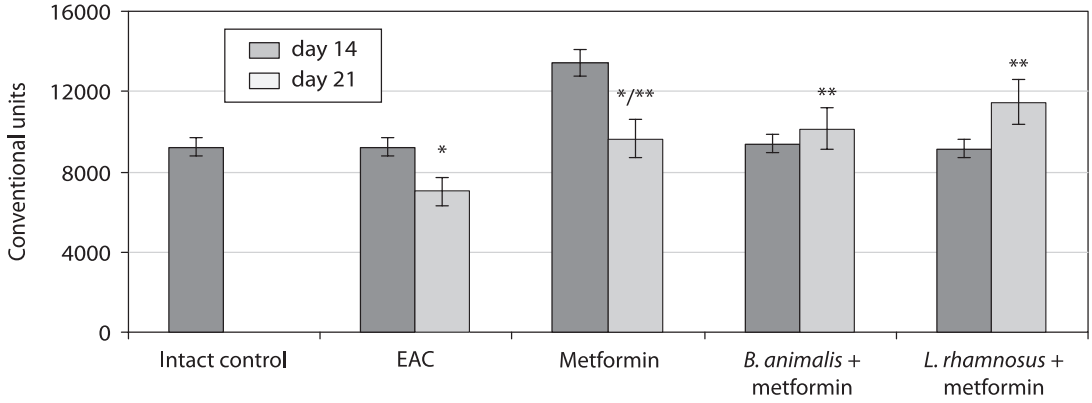

Fig. 3. The level of ROS production by Mph of mice administered metformin and *B. animalis* or *L. rhamnosus*
* – $p < 0.05$ compared to the "IC" group; ** – $p < 0.05$ compared to the "EAC" group.

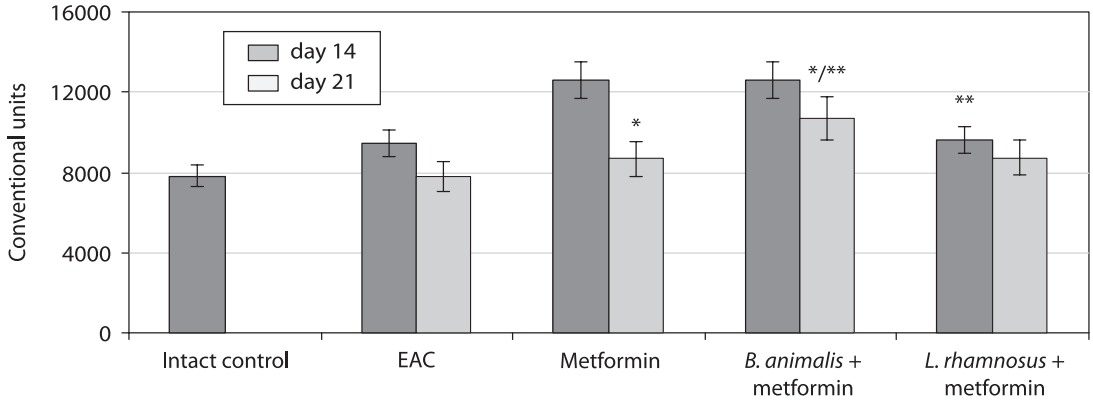

Fig. 4. Phagocytic activity of Mph in mice administered metformin and *B. animalis* or *L. rhamnosus*

* — $p < 0.05$ compared to the "IC" group; ** — $p < 0.05$ compared to the "EAC" group.

nosus polarized Mph toward the M1 type. These data align with previously described results of determining Mph functional activity after the administration of the studied bacterial preparations alone (Gogol et al., 2025). Specifically, an increase in cytotoxic activity, the NO/Arg ratio, and ROS production—indicating the prevalence of M1 Mph with pro-inflammatory properties — was noted in the group of mice administered *L. rhamnosus*. In contrast, the use of *B. animalis* was accompanied by the increase in phagocytic and arginase activity accompanied with the decreased NO and ROS production levels, which are characteristic features of M2 Mph polarization with anti-inflammatory properties. That is, the administration of metformin in combination with bacterial preparations did not interfere with the direction of Mph polarization determined by these preparations.

As a result of tumor-induced Mph reprogramming toward the M2 type, the spectrum of cytokines produced by Mph changes: the amount of pro-inflammatory cytokines (specifically TNF-α) decreases, while the level of the immunosuppressive IL-10 simultaneously increases. Corresponding changes, particularly pronounced on day 21 of tumor growth, were observed in the group of control tumor-bearing animals (*Fig. 5*). By this observation point, compared to intact mice, a statistically significant decrease in TNF-α production by 39.8% occurred alongside an increase by 37.3% in IL-10 production. This is characteristic of M2 Mph and indicates the development of an immunosuppressive state in the mice of this group.

A decrease in TNF-α production accompanied with an increase in IL-10 level was also observed in the experimental animals, but with differences depending on the preparations administered. Thus, in animals administered metformin only, changes in the production of both cytokines did not differ significantly from the data of both control groups. Combined administration of metformin with bacterial preparations positively influenced the cytokine balance of the experimental animals: TNF-α production was at the level of intact mice and significantly exceeded this parameter in control tumor-bearing animals. Similarly, the level of IL-10 production in animals receiving combined treatment did not differ from the intact mice level and was significantly lower compared to the "EAC" group. The most pronounced effect on cytokine production was observed in mice of the "*L. rhamnosus* + met-

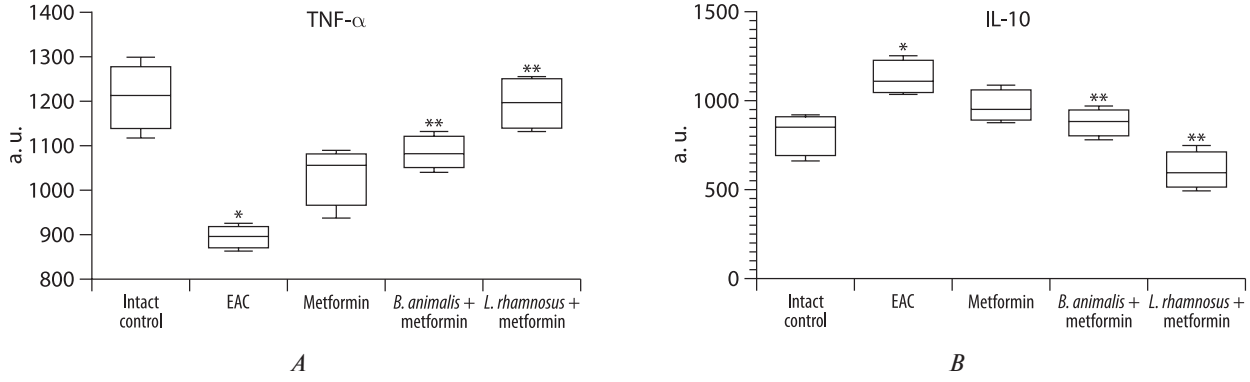

Fig. 5. Levels of TNF-α (*A*) and IL-10 (*B*) production by Mph on day 21 of tumor growth in mice administered metformin and *B. animalis* BB-12 or *L. rhamnosus* GG

* — $p < 0.05$ compared to the "IC" group; ** — $p < 0.05$ compared to the "EAC" group.

formin" group: TNF-α production was 1.4 times higher ($p < 0.05$) compared to the "EAC" group, and IL-10 production was the lowest among all groups: 1.9 times ($p < 0.05$) lower than the "EAC" group values, and 1.5 times lower compared to the "IC" and "*B. animalis* + metformin" groups ($p < 0.05$ in both cases).

The TNF-α/IL-10 ratio can also be used to infer the direction of Mph polarization: its increase is associated with the prevalence of M1 Mph, while its decrease is linked to polarization toward M2 Mph. It is generally accepted that non-activated Mph (for example, in intact animals and healthy donors) are non-polarized, i.e., M0 [21,22]. The TNF-α/IL-10 ratio in intact mice was 1.41 ± 0.01. On the other hand, the polarization state of Mph in tumor-bearing mice is typically characterized as M2 (some researchers distinguish it as a separate subtype M2d, characterized by high IL-10 and low TNF-α production [21, 23]). In the experiment, the TNF-α/IL-10 ratio in the "EAC" group was 0.84 ± 0.02. Based on these data, it can be concluded that in the "*L. rhamnosus* + metformin" group, Mph were polarized toward type 1, as the TNF-α/IL-10 ratio of 2.09 ± 0.01 significantly exceeded the values of both control groups. In the "Metformin" and "*B. animalis* + metformin" groups, the TNF-α/IL-10 ratio was 1.18 ± 0.03 and 1.62 ± 0.03, respectively, which is close to the values of intact animals. However, considering the functional test results that matched M2 Mph characteristics, it can be suggested that Mph in these groups exhibit an intermediate/transitional polarization type or a combination of several polarized Mph types. In previous studies on the effect of bacterial preparations on the polarization state of tumor-associated Mph, we showed that the administration of lactobacilli promotes M1-type Mph polarization, while the administration of bifidobacteria polarizes Mph toward one of the M2 types, likely M2b [24]. This polarization subtype is characterized, in particular, by the production of both pro-inflammatory (IL-1β, IL-6, TNF-α, IL-12) and anti-inflammatory (IL-10) cytokines, which classifies them as immunoregulatory Mph [23]. Therefore, it can be assumed that in the "*B. animalis* + metformin" group, peritoneal Mph were also polarized toward the M2b subtype.

Thus, by day 21 of tumor growth, the Mph of the control tumor-bearing mice acquired the properties of M2 Mph, which are associated with the formation of an immunosuppressive state and tumor progression. Adding metformin to the bacterial administration regimen did not alter the character of the bacteria's influence on the Mph polarization. Combined administration of *L. rhamnosus* GG and metformin promoted Mph polarization toward the pro-inflammatory M1 type with antitumor properties. In the "*B. animalis* + metformin" group, Mph acquired M2(b) polarization with immunoregulatory properties.

Analysis of the standard parameters of tumor growth showed that the administration of metformin or its combination with B. animalis and L. rhamnosus to the experimental animals did not have a significant effect on the engraftment rate or the latency period of EAC appearance. Tumors developed in all animals on days 6–9 after tumor cell transplantation, regardless of the preparations administered (*Table 1*).

However, the dynamics of EAC growth differed among the groups. As shown in *Table 1*, the largest tumor volume was recorded in mice from the "EAC" group: on day 14 and 21 after tumor cell transplantation, the tumor volume reached 2162.0 ± 232.7 and 4074.6 ± 767.0 mm^3, respectively. The administration of bacterial preparations and metformin to mice with adenocarcinoma differently effected the tumor growth. In the group of animals administered metformin alone, the tumor size on day 21 of growth was 1.7 times smaller than that of the "EAC" group, yet it did not reach a statistically significant difference ($p > 0.05$ due to significant heterogeneity of the results). However, the combined use of *B. animalis* or *L. rhamnosus* with metformin had a significant antitumor effect — throughout the entire observation period, the tumor volume in mice of these groups were statistically significantly smaller than the corresponding values of the control animals. To illustrate, in the group "*B. animalis* + metformin", the tumor volume was smaller ($p < 0.05$) than the corresponding values of the "EAC" group: by 1.7 times on day 14 day and by 2.3 times on day 21. Combined use of *L. rhamnosus* and metformin resulted in even more pronounced inhibition of tumor growth: the tumor volume was significantly smaller than the

Table 1

Parameters of tumor growth in control and experimental Balb/c mice

Group	Tumor inhibition index, %	Tumor volume, mm^3		
		Day 7	Day 14	Day 21
Metformin	40.6 ± 9.6	268.2 ± 24.7	1562.0 ± 235.4	2420.0 ± 886.3
B. animalis + metformin	56.2 ± 5.0	268.2 ± 24.7	1274.5 ± 215.0*	1783.4 ± 204.6*
L. rhamnosus + metformin	62.0 ± 2.7	268.2 ± 24.7	1068.8 ± 81.9*	1547.3 ± 110.2*
Control EAC bearing mice	—	429.1 ± 111.0	2162.2 ± 232.7	4075.0 ± 767.0

* — $p < 0.05$ compared to the "EAC" group.

Table 2

Pearson correlation coefficient between parameters of Mph functional activity and tumor volume in EAC-bearing Balb/c mice

	NO/Arg	Cytotoxic activity	ROS production	Phagocytic activity	TNF-α/IL-10 ratio
r	–0,7851	–0,7418	–0,08249	–0,03971	–0,5303
p	0,0009	0,0024	0,7792	0,8928	0,0511

corresponding values of the "EAC" group by 2.0 times (on day 14) and by 2.7 times (on 21 day).

The meaningful inhibition of tumor growth is reflected in high indexes of tumor growth inhibition, which reached 56.2% and 62.0% in the "*B. animalis* + metformin" and "*L. rhamnosus* + metformin" groups, respectively (see *Table 1*). Indexes exceeding the 50% threshold indicates the presence of an antitumor effect. Thus, according to the results, a pronounced antitumor effect was observed in groups of combined application of bacterial preparations and metformin.

Correlation analysis between tumor volume and parameters of Mph functional activity revealed strong inverse correlation with only two of all the studied indicators of Mph functional activity (*Table 2*). The correlation coefficients (*r*) between tumor size and the NO/Arg ratio or Mph cytotoxic activity were –0.785 and –0.742, respectively ($p < 0.05$ in both cases). These results emphasize the important role of M1 Mph in effective antitumor immune response and, as a result, inhibition of tumor growth, since an increase in NO production and cytotoxic activity with a simultaneous decrease in Arg activity is a hallmark of Mph polarized toward the M1 type. Accordingly, the most pronounced antitumor effect was observed specifically in the "*L. rhamnosus* + metformin" group, in which Mph demonstrated M1-type polarization according to all studied parameters.

Surprisingly, an antitumor effect was also recorded in the mice of the "*B. animalis* + metformin" group, although the Mph in these animals matched M2 characteristics (likely M2b). Probably, the observed effect could be due to increased TNF-α production, which is a characteristic feature of this Mph polarization subtype. This is partially reflected by a moderate inverse correlation between tumor volume and the TNF-α/IL-10 ratio ($r = -0.53$, $p = 0.0511$). However, this assumption does not rule out the possibility that the combination of *B. animalis* and metformin may exert other effects on the tumor microenvironment and/or the immune system of the experimental animals and requires further investigation.

Thus, the study results not only reveal certain aspects of the interaction between metformin and probiotic strains but also lay the foundation for developing personalized immunocorrection strategies. These results demonstrate that targeted modulation of the body's metabolic profile and the functional state of macrophages could be applied in enhancing the efficacy of antitumor therapy and overcoming immunosuppression in cancer patients.

CONCLUSIONS

1. Applied as a single preparation, metformin has a dual effect on the polarisation of peritoneal macrophages; however, when used in combination with bacterial preparations, it maintains the direction of macrophage polarisation induced by the bacterial preparation.

2. Combined application of metformin with *B. animalis* promotes polarization of macrophages towards the anti-inflammatory and immunoregulatory M2 phenotype.

3. Combined application of metformin and *L. rhamnosus* GG acts as a potent immune response modulator supporting the pro-inflammatory macrophage phenotype (M1) till terminal periods of tumor growth.

4. The combined application of metformin with bacterial preparations does not interfere with effects induced by bacteria and inhibit tumor growth by 56.2% and 62.0% when combined with *B. animalis* and *L. rhamnosus* respectively.

5. The strong inverse correlation between tumour volume and NO/Arg ratio and macrophage cytotoxic activity suggests that these parameters may be used as prognostic markers of immunotherapy efficacy.

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КОМПЛЕКСНИЙ ВПЛИВ БАКТЕРІЙ РОДУ *BIFIDOBACTERIUM* І *LACTOBACILLUS* ТА МЕТФОРМІНУ НА ПОЛЯРИЗАЦІЮ ПЕРИТОНЕАЛЬНИХ МАКРОФАГІВ У ДОСЛІДЖЕННЯХ IN VIVO

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Резюме. ***Мета:*** *вивчення комплексного впливу інгібітора енергетичного обміну метформіну та B. animalis subsp. lactis BB-12 або L. rhamnosus GG на поляризаційний стан перитонеальних макрофагів.* ***Об'єкт і методи:*** *дослідження проведені на мишах-самках лінії Balb/c. В якості експериментальної моделі використана аденокарцинома Ерліха (АКЕ). Тваринам з пухлинами протягом 20 діб вводили метформін або метформін в комбінації з B. animalis subsp. lactis BB-12 або L. rhamnosus GG. Параметри функціональної активності макрофагів (Мф) визначали за рівнем продукції NO та активних форм кисню, аргіназною (Arg), фагоцитарною та цитотоксичною активністю, продукцією цитокінів TNF-α та IL-10.* ***Результати:*** *введення метформіну окремо проявляло бінарний вплив на поляризаційний стан Мф: сприяло збереженню цитотоксичної активності Мф, високого співвідношення NO/Аргіназа та продукції АФК (що є ознаками М1 Мф), водночас збільшувало фагоцитарну активність (ознака М2 Мф), та мало невиразний вплив на продукцію цитокінів. Водночас, при комбінованому введенні з бактеріальними препаратами метформін підкреслював вплив бактерій на поляризацію Мф. За показниками співвідношенням рівня продукції NO та активності аргінази, зниженням цитотоксичної активності на тлі зростання фагоцитарної та за спектром продукованих цитокінів продемонстровано, що введення метформіну в комбінації з B. animalis subsp. lactis BB-12 сприяє поляризації Мф за одним з підтипів М2. Натомість, комбіноване введення метформіну та*

L. rhamnosus GG сприяє поляризації Мф за типом М1, на що вказують тривале збереження цитотоксичної активності Мф, їх здатності до продукції високих рівнів NO, АФК та TNFα, на тлі зниженої фагоцитарної активності та продукції ІЛ-10. Комбіноване введення метформіну з бактеріальними препаратами (але не метформіну окремо) сприяло гальмуванню росту пухлини на 56,2% (B. animalis + метформін) та 62,0% (L. rhamnosus + метформін). Об'єм пухлини обернено корелював із співвідношенням NO/Arg або цитотоксичною активністю Мф (коефіцієнт кореляції (r) становив, відповідно, –0,785 та –0,742, $p < 0,05$ в обох випадках). ***Висновок:*** *комбінація метформіну з бактеріальними препаратами суттєво не змінювала вплив бактеріальних препаратів на поляризаційний стан Мф і мала виражений протипухлинний ефект. Отриманий результат вказує на перспективність застосування комбінації метформіну з бактеріальними препаратами для цілеспрямованої імунокорекції, подолання пухлиноопосередкованої імуносупресії та збільшення ефективності лікування онкологічних захворювань.*

Ключові слова: карцинома Ерліха, *B. animalis* subsp. *lactis* BB-12, *L. rhamnosus* GG, метформін, макрофаги, поляризаційний стан, протипухлинна ефективність.

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