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N. FEDOSOVA *, A. CHUMAK, N. CHEREMSHENKO, O. KARAMAN, T. SYMCHYCH, I. VOYEYKOVA

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine, Kyiv, Ukraine

* Correspondence: Email: dn2003@ukr.net

IN VIVO STUDY OF POTENTIAL MECHANISMS OF MACROPHAGE REPOLARIZATION ON THE BACKGROUND OF TUMOR GROWTH

Aim. To study the activity of antitumor immunity effectors and to analyze possible mechanisms of peritoneal Mph M1/M2 repolarization of Balb/c mice under the influence of lectin from *B. subtilis* IMV B-7724 in the dynamics of the model tumor growth. **Materials and Methods**. Studies were performed on Balb/c mice; Ehrlich adenocarcinoma (ACE) was used as an experimental tumor. Lectin from *B. subtilis* IMV B-7724 was administered to ACE-bearing mice at a dose of 1 mg/kg of body weight, 10 times. Immunological testing was performed on days 21 and 28 after tumor grafting. The functional activity of peritoneal macrophages (Mph), natural killer (NK) cells, cytotoxic lymphocytes (CTL), and cytokine levels (IFN-γ, IL-4) were studied by the standard methods. mRNA expression levels of transcription factors STAT-1, STAT-6, IRF5, and IRF4 in Mph were evaluated. **Results**. The administration of lectin from *B. subtilis* IMV B-7724 to mice with solid ACE led to the preservation of the initial functional state of peritoneal Mph M1 during the experiment. The bacterial lectin ensured the preservation of the cytotoxic activity of $CD8^+$ T-lymphocytes and a significant ($p < 0.05$) increase in the NK activity (by 2.7 times compared to the intact animals and by 12.9 times compared to the untreated mice). A strong positive correlation was noted between the levels of the functional activity of Mph and $CD8^+$ T-lymphocytes of animals with tumors and the indices of the antitumor effectiveness of bacterial lectin. The indirect polarization of Mph was evidenced by a strong positive correlation between the level of the NO/Arg ratio (which characterizes the direction of Mph polarization) and the cytotoxic activity of $CD8^+$ T-lymphocytes, NK cells, and the expression of STAT1/STAT6 (the 21st day) and IRF5/IRF4 (the 28th day). **Conclusion**. In ACE-bearing mice, repolarization of the peritoneal Mph toward M1 can occur not only due to the direct action of bacterial lectin on the cellular receptors but also with the involvement of other effectors of antitumor immunity (NK cells, T-lymphocytes). The transcription factors of the STAT and IRF signaling pathways are involved in the polarization process.

Keywords: peritoneal macrophages, M1/M2 polarization, natural killer cells, cytotoxic lymphocytes, transcription factors, Ehrlich adenocarcinoma.

Macrophages (Mph) are among the key effector cells of antitumor immunity. In the modern classification, the classically activated pro-inflammatory Mph M1 and the alternatively activated Mph M2 with anti-inflammatory and reparative properties are most often distinguished. The division

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of Mph into M1 and M2 is based on the expression of specific surface markers, the spectrum of produced factors, and the functions performed by these cells.

The classical pathway of Mph activation is initiated by microbial products (in particular, lipopolysaccharide (LPS)), interferon-γ (IFN-γ), and tumor necrosis factor (TNF-α). Mph M1 are characterized by increased production of pro-inflammatory cytokines (TNF-α; interleukins IL-1, IL-6, IL-12) and nitric oxide synthase 2 (NOS 2). Such a spectrum of produced cytokines promotes the activation of effector cells of both natural (natural killer cells (NK cells)) and adaptive ($CD4^+$, $CD8^+$ T-lymphocytes) immunity and their involvement in the inflammatory process. A high level of the inducible nitric oxide synthase (iNOS) ensures the production of nitric oxide (NO) and reactive oxygen species (ROS) by Mph M1 which play a significant role in the direct cytotoxic effect [1, 2].

An alternative pathway of Mph activation is due to the influence of IL-4, IL-13 and IL-10. Mph M2 produce high levels of anti-inflammatory cytokines (IL-10, IL-4, IL-13), transforming growth factor beta (TGF-β), and epidermal growth factor (EGF). These factors contribute to immunosuppression necessary to stop inflammation. During cancer development, Mph with M2 phenotype and protumoral properties prevail [3, 4].

Mph polarization is a regulated process mediated by several key molecular signaling pathways. Primary stimulating signals from TLRs, receptors for particular cytokines (IFN-γR, IL-10R, and IL-4Rα) are important for the activation of the STAT/IRF signaling pathway. Their mediators are signal molecules — interferon regulatory factors (IRF), signal transducers, and transcriptional activators (STAT) [5].

The activation of Mph M1 occurs due to the effect of LPS and IFN-γ on the corresponding surface receptors TLR4 and IFN-γR. The binding of LPS or other microbial products to TLR4 results in the activation of the receptor-associated adapter proteins MyD88 followed by the transcription of the NF-kB-p65/p50-dependent genes. NF-κB regulates gene expression of pro-inflammatory cytokines (including TNF-α, IL-1β, IL-6, IL-12, and cyclooxygenase 2) and chemokines (CXCL10, CXCL11). The effect on TLR4 also leads to the activation and translocation of IRF3 and IRF5 in the nucleus and the initiation of signaling cascades of M1 polarization. The IFN-γ/JAK/STAT1 signaling pathway is believed to be a key component in the modulation of the Mph M1 phenotype. The binding of IFN-γ to IFN-γR induces an increase in the expression level of the transcription factor STAT1 and activates the transcription of the STAT1-dependent genes that regulate the production of pro-inflammatory cytokines, chemokines, and antigen-presenting molecules (MHC II). Some studies of the features of the expression of transcription factors of the IRF family in cells of the immune system have shown that IRF5 is expressed at a higher level in Mph with the M1 phenotype. This regulatory factor is activated under the influence of all the stimuli listed above (LPS, IFN) and plays a central role in the initiation of the inflammatory process. IRF5 mediates the high-level synthesis of the pro-inflammatory cytokines IL-6, IL-12, IL-23, and TNF-α [6—8].

The induction of the alternative pathway of Mph activation occurs under the influence of IL-4/IL-13 or IL-10 cytokines on the surface receptors. As a result of the activation of the corresponding signaling pathways involving JAK1 and JAK3, the transcription factors STAT6, IRF4 (via IL-4Rα), and STAT3 (via IL-10R) translocate into the nucleus and activate the expression of the corresponding genes. In particular, STAT6 and IRF4 control the synthesis of arginase 1 (Arg1), Ym1, Fizz1, CCL17, and CD206, which are the markers of Mph M2 [9, 10]. That is the signs of Mph M1 polarization are the activation of transcription factors STAT1, STAT5, IRF3, and IRF5. The polarization of Mph M2 is accompanied by activation of STAT3, STAT6, and IRF4.

As far as Mph are highly plastic cells sensitive to the signals of the surrounding microenvironment, their repolarization could be used for the therapeutic purposes. By applying certain stimuli, it is possible to achieve reprogramming of Mph M2, the number of which increases during tumor growth, and to ensure the predominance of Mph M1, which is necessary for an antitumor response [11—13]. The understanding of the specificity of the Mph activation mechanisms can become the basis for the development of M1-directed repolarization of these cells for the purposes of antitumor immunotherapy.

The ability of the bacterial lectin from *B. subtilis* IMV B-7724 to change the polarization state of Mph of the Balb/c mice has been demonstrated in our previous studies [14, 15]. In order to determine a possible mechanism of Mph repolarization under the influence of bacterial lectin *ex vivo*, their functional state was assessed by determining the levels of NO production, Arg activity, and mRNA expression levels of transcription factors STAT1 and STAT6. It was demonstrated that the effect of *B. subtilis* IMV B-7724 lectin on Mph is accompanied by a significant increase in the levels of NO/Arg ratios and *STAT1*/*STAT6* mRNA expression and is comparable to the effect of the combined action of LPS and IFN-γ [14]. Similar results were obtained *in vivo* when studying the effect of bacterial lectin on the changes in the polarization of peritoneal Mph of mice in the growth dynamics of Ehrlich adenocarcinoma [15]. The obtained *ex vivo* results may indicate Mph polarization toward pro-inflammatory M1 due to the direct effect of lectin on their surface receptors (in particular, TLR4). However, when evaluating the results of *in vivo* studies, one should take into account that the immune response is a complex multicomponent process and the effect of lectin on Mph can be not only direct but also be mediated through the soluble factors (cytokines, chemokines, etc.) produced by other cells of the immune system. Therefore, the aim of the study was to analyze possible mechanisms of the peritoneal Mph M1/M2 repolarization in Balb/c mice under the influence of lectin from *B. subtilis* IMV B-7724 in the dynamics of the model tumor growth.

Materials and Methods

The study was carried out on female Balb/c mice 2—2.5 month old weighting 20—22 g, bred at the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR). The use and care of the experimental animals have been performed in accordance with the standard international rules on biologic ethics and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [16] and was approved by the Institutional Animal Care and Use Committee.

A solid variant of Ehrlich adenocarcinoma (ACE) was used as a model of tumor growth. Cancer cells were provided by the Bank of Cell Lines from Human and Animal Tissues, IEPOR NASU. Tumor cells were injected intramuscularly into the hind limb (5×10^5 cells/mouse).

As an agent with immunomodulatory properties, we used bacterial lectin from *B. subtilis* IMV B-7724, spore forming, aerobic, and gram-positive bacteria. The lectin was isolated from the cultural fluid as described in [17]. Then it was freeze-dried and stored as a powder at –20 °C.

The scheme of the experiment. The animals were distributed in 4 groups: IC — intact control (n = 10); ACE — control of tumor growth, tumor-bearing mice that were injected with 0.9% NaCl solution (n = 15); ACE + lectin — tumor-bearing mice that were injected with lectin (n = 15). Lectin was used in a dose of 1 mg/kg of body weight per one administration. The complete course consisted of 10 administrations of lectin; the cumulative dose reached 10 mg/kg of body weight. Immunological testing was performed on days 21 and 28 after tumor grafting. The cytotoxic activity (Mph, NK, and cytotoxic T-lymphocytes — CTL), production of NO and arginase (Arg) activity (Mph), and cytokine levels (IFN-γ, IL-4) in serum were investigated. Besides, on the 21th and 28th days after the tumor transplantation, the mRNA expression levels of STAT-1, STAT-6, IRF5, and IRF4 in Mph were evaluated.

The antitumor effect was evaluated by the calculated indexes of tumor growth inhibition (TGI, %) and increased survival time (ST, %) of animals as described in [18].

Cytotoxic activity (CTA) was determined by a MTT-assay [19]. ACE cells were used as a target for the peritoneal Mph and CTL; K562 cells were used as a target for NK. In brief, target cells (2×10^4 cells/well) in the RPMI medium supplemented with 10% fetal bovine serum (Sigma, USA) and antibiotics, were placed in flat-bottom 96-well plates, where Mph (4×10^5 cells/well) or lymphocytes (1×10^6 cells/well) were adhered beforehand, and incubated for 18 h in a 100% humidity atmosphere with 5% CO_2 at 37 °C. The control wells contained target cells or effector cells only. Then 0.01 mL of MTT solution/well (5 mg/mL, Sigma, USA) was added, and incubation continued for 2 h. The plates were centrifuged (550 g for 15 min) and washed twice with a 0.9% NaCl solution. After that, 0.12 mL of 2 M KOH and 0.14 mL of di-

methyl sulfoxide (50% solution) were added into each well. The optical density was measured at λ = 545 nm *vs*. λ = 630 nm using a microplate ELISA reader (StatFax-2100, USA). Each sample was done in triplicate. The cytotoxic activity index (CTAI, %) was calculated by the formula:

$$CTAI = [1 - (OD_{effector+tc} - OD_{effector})/$$
$$(OD_{tc} - OD_{blank})]\cdot 100\%,$$

where $OD_{effector}$ is the optical density of the wells in which only effector cells were incubated, OD_{tc} is the optical density of the wells in which only tumor cells were incubated, $OD_{effector+tc}$ is the optical density of the wells in which tumor cells and effector cells were incubated, and OD_{blank} is the optical density of the wells with the culture medium only.

NO production was measured by the standard Griess reaction [20]. In brief, cell suspensions (2×10^6 cells/well) were placed in a volume of 200 μL in 96-well flat-bottom tissue culture plates and cultured for 24 h. Each cell culture was investigated in duplicate. At the end of the incubation period, supernatants were collected and NO production was assessed by the accumulation of nitrite (as stable metabolite of NO) by the Griess reaction. The aliquot of culture supernatant (100 μL) was mixed with an equal volume of Griess reagent (Acros Organics, Belgium) and incubated for 1 h at room temperature in the dark. The reaction products were quantified by colorimetry at λ = 550 nm. The standard curve plotted by the results of measurements of the solutions containing known concentration of $NaNO_2$ was used for converting the absorbance to micromolar concentrations of NO expressed in μM NO_2^- per 10^6 cells.

Arg activity was determined based on urea measurement [21]. Mph were lysed by double freezing and thawing. Then 50 μL of 50 mM Tris-HCl (pH 7.4) and 10 μL of 50 mM $MnCl_2$ were added to each sample. Samples were heated at 56 °C for 10 min, and upon addition of 100 μL of 0.5 M L-arginine (pH 9.7) heated for further 30 min (37 °C). The reaction was stopped with 800 μL of acidic mixture (1:3:7, 96% H_2SO_4 : 85% H_3PO_4 : H_2O). Then 40 μL of α-isonitrosopropriophenone (Sigma — Aldrich) was added to the solution, which was heated for 30 min (95 °C) and incubated for 30 min at 4 °C. Urea concentration was measured by spectrophotometry at λ = 550 nm. The values of optical density were converted to mass of urea based on the calibration curve of standard urea solution. One unit of Arg activity means the amount of the enzyme hydrolyzing 1 μM of arginine per min. The results are expressed as units/10^6 cells.

Cytokine levels (IFN-γ and IL-4) in serum were determined by the immunoassay using commercial test systems BD OptEIA Set Mouse IFN-γ and BD OptEIA Set Mouse IL-4 (BD Biosciences, USA).

qRT-PCR. The total RNA was isolated from 2×10^5 Mph using NucleoZOL (MACHEREY-NAGEL GmbH & Co. KG Germany) according to the manufacturer's protocol. cDNA synthesis was performed using RevertAid Reverse Transcriptase, RiboLock Inhibitor, dNTP mix and Oligo(dT) primer (Thermo Scientific, USA). qRT-PCR was held on sequence detection system 7500 (Applied Biosystems, CA USA) using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, USA) and the following primers:

STAT1 — forward 5`-TCCTTCTGGCCTTGGATTGA-3`,
reverse 5`-ACCGTTCCACCCATGTGAA-3`,
STAT6 — forward 5`-AGATGAGGCTTTCCGGAGTCA-3`,
reverse 5`-CCCATATCTGAGCTGAGTTGCA-3`,
TBP — forward 5`-CCAATGACTCCTATGACCCC-3`,
reverse 5`-GTTGTCCGTGGCTCTCTTATTC-3`.

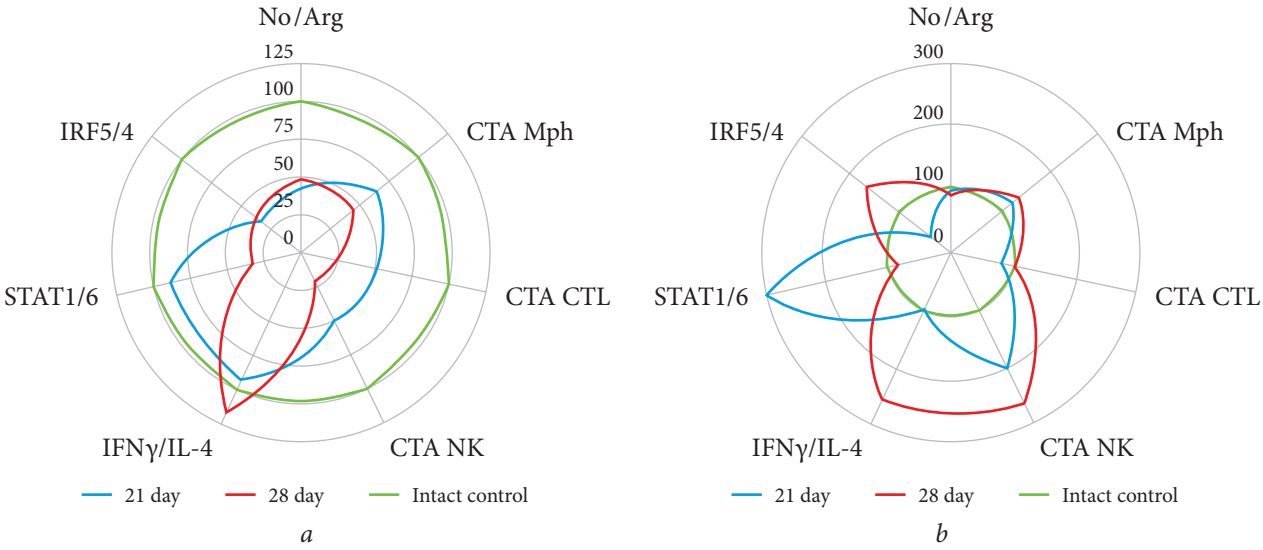
The target genes` Ct values were normalized to the Ct value of the internal control gene (*TBP*) using a ddCt method.

Statistical analysis was carried out by the nonparametric Mann — Whitney U test, and correlation analysis was conducted according to Spearman`s correlation using Prism software Version 8.0. The difference between examined groups was considered significant if $p < 0.05$.

Results and Discussion

The analysis of tumor growth indices has shown that the lectin treatment of mice with transplanted ACE resulted in the significant inhibition of the primary tumor growth by 53.2% compared to the untreated animals with a 1.8-fold increase in their average ST ($p < 0.05$).

The evaluation of a number of parameters of the peritoneal Mph activity (NO production, Arg activity, and cytotoxic activity) showed that the use



Changes in the immunological parameters of mice with transplanted ACE treated (*b*, the ACE + lectin group) or not treated (*a*, the ACE group) with bacterial lectin. The parameters are given as percentages of the parameters of the intact control, the levels of which are taken as 100%

of the lectin contributed to the preservation of the initial functional state of Mph of mice with the transplanted solid ACE throughout the entire observation period. All the indices were determined at the level of the intact control and exceeded those of the animals of the ACE group (Figure). That is at the terminal stages of tumor growth (the 21st and 28th days after tumor transplantation), in the population of peritoneal Mph of mice of the ACE group, cells with the functional properties of M2 prevailed (Fig. *a*), while in mice of the ACE + lectin group, Mph with the M1 properties were prevailing (Fig. *b*).

The polarization state of Mph in these animals was also determined by the level of the mRNA expression of the STAT and IRF transcription factors. The direction of Mph polarization was evaluated based on the ratio of the STAT1/STAT6 and IRF5/IRF4 mRNA expression levels, taking into account that the M1 phenotype is characterized by the expression of STAT1 and IRF5, while M2 — by STAT6 and IRF4. On the 21st day of tumor growth, a significant increase in the STAT1/STAT6 ratio was observed in mice of the ACE + lectin group compared to that in the ACE group ($p < 0.05$). On the 28th day, this indicator decreased to the level of the intact control and there was observed a significant increase in the level of IRF5/IRF4 ($p < 0.05$) (Fig. *b*).

Analyzing the obtained data, it can be assumed that after the administration of lectin from *B. subtilis* IMV B-7724 to mice with solid ACE, the significant antitumor effect was probably caused by the preservation of the initial functional state of the peritoneal Mph M1 throughout the entire period of tumor growth. At the same time, the polarization of Mph M1 on the 21st day of tumor growth occurred due to the activation of transcription factors of the STAT signaling pathway, and subsequently, an increase in the expression of the IRF signaling pathway factors was observed.

Polarized Mph M1 produce a significant number of pro-inflammatory factors (IL-1, IL-6, IL-12, IL-23, and TNF-α), which affect other effector cells of the immune system. In particular, IL-12 is known as an immunoregulatory cytokine that plays a key role in ensuring a cellular immune response. Under the influence of IL-12 the differentiation of $CD4^+$ T cells to T helper type 1 (Th1) occurs, and cytotoxic $CD8^+$ T-lymphocytes and NK cells are activated. The activation of Th1 and NK cells is accompanied by an increase in the production of IFN-γ by these cells, which in turn leads to the polarization of Mph M1 through the IFN-γ/JAK/STAT1 signaling pathway [22—24]. Taking into account the given data, we investigated the effect of bacterial lectin on the cytotoxic activity of $CD8^+$ T-lymphocytes and NK cells and the content of cytokines IFN-γ and IL-4 in the peripheral blood serum.

In the animals that were not treated, the indicators of the cytotoxic activity of the NK cells and

$CD8^+$ T-lymphocytes in the late periods of tumor growth were significantly lower than those in the intact mice by 1.9 and 1.8 times on the 21st day and 4.8 and 4.7 times on the 28th day, respectively (Figure, *a*). The administration of bacterial lectin resulted in the preservation of the cytotoxic activity of $CD8^+$ T-lymphocytes at the level of the intact control and led to a significant increase in the NK activity on the 28th day of tumor growth. The cytotoxic activity of NK cells at this time point exceeded the indicators of the intact animals by 2.7 times and mice of the ACE group by 12.9 times ($p < 0.05$). The IFN-γ content in the blood serum of the experimental animals also changed in a similar way (Figure, *b*). The data obtained suggest that the activation of cells of $CD8^+$ T-lymphocytes and NK cells can be mediated by the influence of pro-inflammatory cytokines produced by Mph M1.

Then we determined correlations between the modulation of indicators characterizing the specific and non-specific links of antitumor immunity and the indicators characterizing the antitumor activity of bacterial lectin. A strong positive correlation was noted between the levels of functional activity of Mph and $CD8^+$ T-lymphocytes and TGI and the increased ST of animals with tumors (Table 1). That is the antitumor effectiveness of lectin largely depends on the functional state of these cells.

The assessment of the relationships between the indicators of the functional activity of Mph, NK and $CD8^+$ T-lymphocytes and the mRNA expression levels of STAT/IRF transcription factors demonstrated the likelihood of an indirect way of polarization of pro-inflammatory Mph M1 under the influence of bacterial lectin (Table 2). In particular, a strong positive correlation was observed between the NO/Arg ratio (which characterizes the direction of Mph polarization) and the cytotoxic activity of Mph, $CD8^+$ T-lymphocytes, NK cells, the ratios of STAT1/STAT6 (the 21st day) and IRF5/IRF4 (the 28th day).

The role of NK cells in the process of Mph polarization is suggested by a strong positive correlation between the indicators of the cytotoxic activity of these cells, the level of IFN-γ synthesized by them and the level of the expression of the transcription factor STAT1 in Mph. The obtained data are in line with the role of above-described IFN-γ/JAK/STAT1 pathway of mutual activation of Mph and NK through the production of cytokines (IL-12, IFN-γ) [22—24]. The revealed strong positive correlations between the cytotoxic activity of $CD8^+$ T-cells and such indicators of Mph M1 polarization as the levels of NO/Arg and STAT1/STAT6 ratios may also indicate the mutual influence of these cells in the antitumor immune response.

Table 1. **Correlation (*r*) between the changes in some immunological parameters of mice with ACE treated with bacterial lectin and the indicators of its antitumor effectiveness**

Immunological parameters	TGI index		Index of ST increase
	Day 21	Day 28	
CTA Mph	**0.80**	**0.89**	**0.85**
CTA NK	0.35	0.34	0.57
CTA $CD8^+$ T-cells	**0.99**	**0.94**	**0.95**
IFN-γ/IL-4	0.11	0.15	0.33
NO/Arg	**0.88**	**0.94**	**0.98**

Note: Statistically significant correlations at $p < 0.05$ are indicated in bold.

Table 2. **Correlation (r) between the changes in some immunological parameters of mice with ACE treated with bacterial lectin**

Index	CTA Mph		CTA NK		CTA $CD8^+$ T-cells		STAT1/STAT6		IRF5/IRF4	
	21	28	21	28	21	28	21	28	21	28
CTA Mph	1	1	**0.96**	0.73	0.67	**0.99**	**0.85**	**0.99**	0.12	**0.85**
CTA NK	**0.96**	0.73	1	1	0.44	0.64	**0.965**	0.61	0.15	**0.98**
CTA $CD8^+$ T-cells	0.67	**0.99**	0.44	0.64	1	1	0.18	**0.99**	**0.81**	**0.78**
STAT1/STAT6	**0.85**	**0.99**	**0.96**	0.61	0.18	**0.99**	1	1	0.41	0.75
IRF5/IRF4	0.12	**0.85**	0.15	**0.98**	**0.82**	0.78	0.41	0.75	1	1
IFN-γ/IL-4	**0.86**	0.50	**0.97**	**0.96**	0.20	0.39	**0.99**	0.36	0.39	**0.88**
NO/Arg	**0.90**	**0.99**	0.75	0.85	**0.92**	**0.99**	**0.99**	0.68	0.54	**0.78**

Note: Statistically significant correlations at $p < 0.05$ are indicated in bold.

During cancer progression, a gradual change in the polarization of Mph from pro-inflammatory M1 to protumoral M2 occurs due to the influence of the factors of the tumor microenvironment. Mph M2 produce a number of suppressor factors (Arg-1, IL-10) and suppress the activity of cytotoxic lymphocytes, activating other types of cells with immunosuppressive properties (T-regulatory lymphocytes, bone marrow suppressor cells, and MDSC). Such processes lead to a decrease in antitumor immune surveillance resulting in tumor progression [25, 26]. However, it should be taken into account that the polarization of Mph M1/M2 is a reversible process and depends on the signals of the microenvironment. Mph M2 can be repolarized into Mph M1 in response to the stimulation by certain cytokines (for example, IFN-γ or TNF-α). It has been shown that cytokines such as IL-4 and IFN-γ can cross-regulate the process of macrophage polarization to the M1 or M2 phenotype. Therefore, $CD4^+$ T-lymphocytes (Th1) and NK cells, which are able to produce IFN-γ, can also be involved in the process of activation of M1 macrophages in a STAT1-dependent way [23, 24, 27].

To sum up, the obtained results allow us to conclude that in *in vivo* conditions, Mpf repolarization also occurs at the systemic level, under the influence of factors of other effector cells of antitumor immunity ($CD4^+$ T-lymphocytes and NK cells). The ability of lectin to support the induction of a significant number of cells with proinflammatory properties for a long time can be used to develop anticancer immunotherapy approaches, expanding the arsenal of new natural antitumor immunotherapeutic agents.

REFERENCES

1. Strizova Z, Benesova I, Bartolini R, et al. M1/M2 macrophages and their overlaps – myth or reality? *Clin Sci (Lond)*. 2023;137(15):1067-1093. https://doi.org/10.1042/CS20220531
2. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep*. 2014;6:13. https://doi.org/10.12703/P6-13
3. Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. *Nat Rev Drug Discov*. 2022;21(11):799-820. https://doi.org/10.1038/s41573-022-00520-5
4. Lu Y, Han G, Zhang Y, et al. M2 macrophage-secreted exosomes promote metastasis and increase vascular permeability in hepatocellular carcinoma. *Cell Commun Signal*. 2023;21(1):299. https://doi.org/10.1186/s12964-022-00872-w
5. Tzeng HT, Chyuan IT, Lai JH. Targeting the JAK-STAT pathway in autoimmune diseases and cancers: A focus on molecular mechanisms and therapeutic potential. *Biochem Pharmacol*. 2021;193:114760. https://doi.org/10.1016/j.bcp.2021.114760
6. Almuttaqi H, Udalova IA. Advances and challenges in targeting IRF5, a key regulator of inflammation. *FEBS J*. 2019;286(9):1624-1637. https://doi.org/10.1111/febs.14654
7. Chen X, Tang J, Shuai W, et al. Macrophage polarization and its role in the pathogenesis of acute lung injury/acute respiratory distress syndrome. *Inflamm Res*. 2020;69(9):883-895. https://doi.org/10.1007/s00011-020-01378-2
8. Chen S, Saeed AFUH, Liu Q, et al. Macrophages in immunoregulation and therapeutics. *Signal Transduct Target Ther*. 2023;8(1):207. https://doi.org/10.1038/s41392-023-01452-1
9. Abdelaziz MH, Abdelwahab SF, Wan J, et al. Alternatively activated macrophages; a double-edged sword in allergic asthma. *J Transl Med*. 2020;18(1):58. https://doi.org/10.1186/s12967-020-02251-w
10. Filipek A, Mikołajczyk TP, Guzik TJ, Naruszewicz M. Oleacein and foam cell formation in human monocyte-derived macrophages: a potential strategy against early and advanced atherosclerotic lesions. *Pharmaceuticals (Basel)*. 2020;13(4):64. https://doi.org/10.3390/ph13040064
11. Wang Y, Wang D, Yang L, Zhang Y. Metabolic reprogramming in the immunosuppression of tumor-associated macrophages. *Chin Med J (Engl)*. 2022;135(20):2405-2416. https://doi.org/10.1097/CM9.0000000000002426
12. Crezee T, Rabold K, de Jong L, et al. Metabolic programming of tumor associated macrophages in the context of cancer treatment. *Ann Transl Med*. 2020;8(16):1028. https://doi.org/10.21037/atm-20-1114
13. Hörhold F, Eisel D, Oswald M, et al. Reprogramming of macrophages employing gene regulatory and metabolic network models. *PLoS Comput Biol*. 2020;16(2):e1007657. https://doi.org/10.1371/journal.pcbi.1007657
14. Chumak A, Shcherbina V, Fedosova N, et al. Polarization of macrophages of mice under the influence of lectin from Bacillus Subtilis IMB B-7724 *EUREKA: Life Sci*. 2021;(3):3-10. https://doi.org/10.21303/2504-5695.2021.001878
15. Chumak AV, Fedosova NI, Shcherbina VM, et al. Influence of bacterial lectin on key regulatory links of functional activity of macrophages in mice with Ehrlich carcinoma. *Exp Oncol*. 2021;43(3):197-203. https://doi.org/10.32471/exp-oncology.2312-8852.vol-43-no-3.16537

16. European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes ETS 123. Protection of Vertebrate Animals, 18.III.1986. 48. https://norecopa.no/media/2iydns5h/ets-123-original.pdf
17. Fedosova NI, Cheremshenko NL, Hetman KI, et al. Physicochemical and cytotoxicity properties of *Bacillus subtilis* IMV B-7724 extracellular lectin. *Mikrobiol Z*. 2021;83:39-48. https://doi.org/10.15407/microbiolj83.01.039
18. Stefanov OV. *Doklinichni doslidzhennya likarskykh zasobiv* [Preclinical studies of drugs] Kyiv: Avitsena; 2001 (in Ukrainian).
19. Van de Loosdrecht AA, Beelen RH, Ossenkoppele GJ, et al. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *J Immunol Methods*. 1994;174:311-320.
20. Reiner NE. Methods in molecular biology. Macrophages and dendritic cells. Methods and protocols. Preface. *Methods Mol Biol*. 2009;531:v-vi. https://doi.org/10.1007/978-1-59745-396-7
21. Corraliza IM, Campo ML, Soler G, et al. Determination of arginase activity in macrophages: amicromethod. *J Immunol Methods*. 1994;174:231-235. https://doi.org/10.1016/0022-1759(94)90027-2
22. Ullrich KA, Schulze LL, Paap EM, et al. Immunology of IL-12: An update on functional activities and implications for disease. *EXCLI J*. 2020;19:1563-1589. https://doi.org/10.17179/excli2020-3104
23. Beizavi Z, Zohouri M, Asadipour M, Ghaderi A. IL-27, a pleiotropic cytokine for fine-tuning the immune response in cancer. *Int Rev Immunol*. 2021;40(5):319-329. https://doi.org/10.1080/08830185.2020.1840565
24. Powell MD, Read KA, Sreekumar BK, et al. IL-12 signaling drives the differentiation and function of a TH1-derived TFH1-like cell population. *Sci Rep*. 2019;9(1):13991. https://doi.org/10.1038/s41598-019-50614-1
25. Kowal J, Kornete M, Joyce JA. Re-education of macrophages as a therapeutic strategy in cancer. *Immunotherapy*. 2019;11(8):677-689. https://doi.org/10.2217/imt-2018-0156
26. Kadomoto S, Izumi K, Mizokami A. Macrophage polarity and disease control. *Int J Mol Sci*. 2021;23(1):144. https://doi.org/10.3390/ijms23010144
27. Li M, Li M, Yang Y, et al. Remodeling tumor immune microenvironment via targeted blockade of PI3K-γ and CSF-1/CSF-1R pathways in tumor associated macrophages for pancreatic cancer therapy. *J Control Release*. 2020;321:23-35. https://doi.org/10.1016/j.jconrel.2020.02.011

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Н.І. Федосова [*], *А.В. Чумак, Н.Л. Черемшенко,*
О.М. Караман, Т.В. Симчич, І.М. Воєйкова
Інститут експериментальної патології, онкології і радіобіології
ім. Р.Є. Кавецького НАН України, Київ 03022, Україна

ДОСЛІДЖЕННЯ *IN VIVO* ПОТЕНЦІЙНИХ МЕХАНІЗМІВ
РЕПОЛЯРИЗАЦІЇ МАКРОФАГІВ НА ТЛІ ПУХЛИННОГО РОСТУ

Мета. Дослідити активність ефекторів протипухлинного імунітету та проаналізувати можливі механізми зміни напрямку М1/М2 поляризації перитонеальних макрофагів (Мф) у мишей лінії Balb/c в динаміці модельного пухлинного процесу за умови впливу лектину *B. subtilis* IMB B-7724. **Матеріали та методи**. Дослідження проведено на мишах лінії Balb/c; в якості експериментальної модельної пухлини використана аденокарцинома Ерліха (АКЕ). Лектин вводили мишам з пухлинами в разовій дозі 1 мг/кг ваги, 10 введень. Імунологічні дослідження проводили на 21 та 28 доби росту пухлини. Визначали параметри функціональної активності перитонеальних Мф, природних клітин-кілерів (ПКК), цитотоксичних Т-лімфоцитів (ЦТЛ), а також вміст цитокінів (IFN-γ, IL-4) у сироватці крові. Оцінювали рівень експресії мРНК транскрипційних факторів STAT-1, STAT-6, IRF5, IRF4 в Мф. **Результати**. Введення лектину *B. subtilis* IMB B-7724 мишам із солідною АКЕ сприяло збереженню вихідного функціонального стану перитонеальних Мф М1 протягом усього періоду експерименту. Застосування бактеріального лектину забезпечувало також збереження цитотоксичної активності $CD8^+$ Т-лімфоцитів та суттєве ($p < 0,05$) збільшення активності ПКК (порівняно з інтактними тваринами у 2,7 раза, а мишами з АКЕ, яким не вводили лектин, — у 12,9 раза). Відмічали сильну позитивну кореляцію між показниками функціональної активності Мф та ЦТЛ мишей з пухлинами та показниками протипухлинної ефективності бактеріального лектину. Про можливість опосередкованого шляху активації Мф свідчить виявлена сильна позитивна кореляція між рівнем співвідношення NO/Arg (яке характеризує напрямок поляризації Мф) та цитотоксичною активністю ЦТЛ, ПКК, експресією STAT1/STAT6 (21 доба) та IRF5/IRF4 (28 доба). **Висновок**. У мишей з АКЕ реполяризація перитонеальних Мф у напрямку М1 може відбуватися не лише за рахунок прямої дії бактеріального лектину на клітинні рецептори, а також під впливом факторів інших клітин-ефекторів протипухлинного імунітету (ПКК, Т-лімфоцити). У процес поляризації Мф М1 залучені транскрипційні фактори сигнальних шляхів STAT та IRF.

Ключові слова: перитонеальні макрофаги, М1/М2 поляризація, природні кілерні клітини, цитотоксичні лімфоцити, транскрипційні фактори, аденокарцинома Ерліха.