

INFLUENCE OF BACTERIAL LECTIN ON KEY REGULATORY LINKS OF FUNCTIONAL ACTIVITY OF MACROPHAGES IN MICE WITH EHRlich CARCINOMA

A.V. Chumak*, N.I. Fedosova, V.M. Shcherbina, N.L. Cheremshenko, O.M. Karaman, V.F. Chekhun
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine, Kyiv
03022, Ukraine

Background: Recent studies have shown the potential of using different approaches for immunotherapy in cancer treatment. Macrophages (Mph) are one of the promising targets for immunotherapy. **Aim:** To investigate changes in the functional activity of Mph in mice with Ehrlich carcinoma by nitric oxide (NO)/arginase (Arg), IRF4/IRF5 and STAT1/STAT6 ratios caused by administration of lectin from *B. subtilis* IMV-7724. **Materials and Methods:** From the 2nd day after Ehrlich carcinoma inoculation into female Balb/c mice, lectin from *B. subtilis* IMV B-7724 (0.02 mg/mouse) was administered for 10 days. The peritoneal Mph were isolated on days 14, 21, and 28 after tumor transplantation and their functional state (NO production, Arg activity and cytotoxic activity) was examined. The levels of mRNA expression of transcription factors STAT-1, STAT-6, IRF5, IRF4 were evaluated. **Results:** In lectin-treated animals with Ehrlich carcinoma, the functional state of Mph (NO/Arg ratio, index of cytotoxic activity) was maintained at the level of intact mice exceeding the values in untreated animals with Ehrlich carcinoma at late terms of tumor growth (21, 28 days). Analysis of mRNA expression levels of transcription factors in these animals showed a significant increase ($p < 0.05$) in the ratio of STAT1/STAT6 on the day 21 and IRF5/IRF4 on day 28 of tumor growth compared to that in untreated mice. **Conclusions:** Administration of lectin from *B. subtilis* IMV B-7724 to mice with Ehrlich carcinoma led to the prevalence of Mph exhibiting the functional properties of M1 type at late-term tumor growth. The transcription factors of the STAT and IRF signaling pathways are involved in the process of Mph polarization induced by lectin from *B. subtilis* IMV B-7724. **Key Words:** peritoneal macrophages, M1 and M2 polarization, lectin from *B. subtilis* IMV B-7724, functional state, transcription factors, Ehrlich carcinoma.

DOI: 10.32471/exp-oncology.2312-8852.vol-43-no-3.16537

Macrophages (Mph) play an important role in the innate immune response against infectious agents, maintaining homeostasis, tissue repair and differentiation. Mph are a heterogeneous cell population that can be divided into two subgroups: M1 and M2. M1-type Mph (classically activated) play an important role in the innate immune response to pathogen invasion. M2-type Mph (alternatively activated) normally play an important role in tissue repair. The nature of the functioning of Mph is regulated to a large extent by the extracellular signals, so, not surprisingly, the state of microenvironment is closely related to the state of their polarization. In particular, if a malignant neoplasm arises, tumor-associated macrophages (TAM) can perform opposite functions, depending on the signals they receive from the cells of the tumor microenvironment: M1 Mph exert antitumor properties, while M2 promote tumor progression. Such a well-known property of Mph as plasticity provides their rapid response to external stimuli and

allows them to polarize into M1 or M2 type in response to various signals of the microenvironment (cytokines, metabolic products, microbial signals, etc.). In this case, Mph are able to change their functional properties and acquire the phenotype of cells of another type. It is the ability of TAMs with protumoral functions to reprogram (from M2 to M1) that makes them interesting targets for antitumor therapy [1, 2]. The plasticity of Mph and their ability to change their properties under the influence of external signals have long attracted the attention of researchers. Significant results have been achieved in determining possible ways to reprogram the properties of these cells: signaling molecules, transcription factors, epigenetic changes, miRNA spectrum, which underlie the polarization of Mph.

Various mechanisms of TAMs reprogramming have been studied using monoclonal antibodies (mAbs), cytokines, miRNAs, and Toll-like receptor agonists (TLRs). In particular, the TLR7 ligand (imiquimod) acts mainly by increasing the number of infiltrating CD43⁺ lymphocytes. In addition, imiquimod induces nuclear translocation of NF- κ B in J774A Mph, leading to the synthesis of pro-inflammatory proteins tumor necrosis factor α (TNF α), interleukin (IL)-6, IL-12 and CCL2. Synthetic unmethylated cytosine guanine oligodeoxynucleotides (CpG-ODN), which are bind by TLR9, showed high immunostimulatory activity. These molecules have been shown to act by enhancing the production of proinflammatory cytokines (TNF α , IL-6, and IL-12) by Mph by activating the transcription factor nuclear factor kappa B (NF- κ B) in these cells. The combination of CpG-ODN with other

Submitted: July 02, 2021.

*Correspondence: E-mail: Alinkaivanchenko999@ukr.net

Abbreviations used: Arg – arginase; CpG-ODN – cytosine guanine oligodeoxynucleotides; CTA – cytotoxic activity; CTG – control of tumor growth; GM-CSF – granulocyte-macrophage colony-stimulating factor; IC – intact control; IL – interleukin; IFN – interferon; LPS – lipopolysaccharide; mAb – monoclonal antibody; Mph – macrophages; NO – nitric oxide; NO/Arg – ratio of NO production to arginase activity; NF- κ B – nuclear factor kappa B; PI3K γ – phosphoinositide-3-kinase γ ; TAM – tumor associated macrophage; TLRs – Toll-like receptors; TNF α – tumor necrosis factor α .

therapeutic agents, such as anti-CD40 mAbs, has shown promising results in TAM repolarization in pre-clinical models of experimental malignant glioma [3]. The possibility of local activation of innate immune cells and prevention of TAM polarization to the M2 phenotype using synthetic CpG-ODN oligonucleotides has been shown in experimental studies on the B16 melanoma model [4].

In addition to TLRs agonists, various chemical compounds can be used to reprogram TAMs. In particular, it was found that the flavonoid-like compound vadimezan, which has been the subject of numerous preclinical studies and clinical trials, repolarizes Mph into the M1 phenotype [5].

Experimental studies on the mechanisms of macrophage activation have shown that the enzyme phosphoinositide-3-kinase γ (PI3K γ), which normally controls processes such as apoptosis, metabolism, cell growth and proliferation, is also involved in activating the immune response. The consequence of selective deletion of the *PI3K γ* gene is the activation of NF- κ B with simultaneous inhibition of CCAAT/enhancer-binding protein C/EBP β in Mph. In combination with anti-PD-L1, a decrease in the level of PI3K γ expression contributed to tumor regression and increased survival of mice with carcinomas of the head and neck, lungs, breast [6]. A similar result was obtained in the study of the role of serine/threonine protein kinase 1 in the processes of TAM activation in pancreatic adenocarcinoma [7].

Another way to reprogram Mph is to use mAbs against CD40. Experimental and clinical studies have shown an increase in the antitumor activity of Mph when using antibodies against CD40. Binding of mAb against CD40 with corresponding receptors on the plasma membrane of Mph led to an increase in their antitumor activity due to increased secretion of NO and TNF α . It has been experimentally proven that antibodies against CD40 activate TAMs and block the growth of pancreatic carcinoma, glioma and melanoma [8].

A number of studies have been conducted on the role of miRNA in TAM reprogramming. miRNA-33 directly controls the polarization of Mph by acting on the energy sensor and a key integrator of cellular energy homeostasis — AMP-activated protein kinase, reducing the oxidation of fatty acids and polarizing Mph to the M1 phenotype. *In vivo*, the use of anti-miRNA-33 in mice with knockout of low-density lipoprotein receptors on a high-fat diet resulted in the accumulation of FOXP3 $^{+}$ Treg lymphocytes and M2 Mph. Overexpression of miRNA-125a-5p promoted the expression of M2 markers by acting on the transcription factor KLF13, which is involved in the activation of T lymphocytes. Increased miR-26a expression in a mouse hepatocellular carcinoma model inhibited tumor growth, which is inversely correlated with M-CSF expression and macrophage infiltration into tumor tissues of patients with hepatocellular carcinoma [9].

The influence of thymus hormones on Mph polarization is also shown. Thymosin alpha 1 has been shown to activate the immune system by several mechanisms, including stimulation of T cell differentiation, activation

of NK cells, dendritic cells, and Mph. There are studies that show that thymosin alpha 1 is able to activate TAM M2 and reprogram them to proinflammatory Mph, which produce significant amounts of IL-1, TNF- α , ROS and NO. As a result, inhibition of tumor growth and increased survival in mice with Dalton's lymphoma were observed [10].

The effect of products of bacterial origin on Mph has been studied to a much lesser extent. An *in vitro* study demonstrated the ability of β -glucan to reprogram immunosuppressive TAMs into M1 phenotype cells with significant immunomodulatory activity. This process was accompanied by reprogramming of Mph metabolism with increased glycolysis, Krebs cycle and glutamine utilization. Repolarization of M2 TAMs in M1 phenotype under the action of β -glucan is mediated by canonical Syk-Card9-Erk pathway dependent on the C-type lectin receptor dectin-1. Further studies were performed *in vivo* in a model of Lewis lung carcinoma. It has been shown that oral administration of β -glucan to mice was accompanied by activation of the T-cell response, the presence of cells with the M1 phenotype among TAMs and, accordingly, a significant inhibition of tumor growth [11].

Therefore, the plasticity of Mph, the ability to change their properties under the influence of external stimuli makes these cells an interesting target for attempts to reprogram TAMs in the direction of proinflammatory antitumor phenotype and determines one of the strategies of modern immunotherapy.

The aim of the work was to investigate changes in the functional activity of Mph of mice with Ehrlich carcinoma by ratio of NO production to arginase activity (NO/Arg), IRF4/IRF5 and STAT1/STAT6 ratios caused by administration of lectin from *B. subtilis* IMV-7724.

MATERIALS AND METHODS

Bacterial lectin. The lectin was produced by a spore-forming gram-positive saprophytic bacteria *B. subtilis* IMV B-7724 (deposited in the collection of the DK Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv) [12]. The lectin was obtained from the culture fluid of the microorganism as described in [13]. After purification and lyophilization, the substance was stored in a powder form at -20°C .

Animals. Male Balb/c mice 2–2.5 month old weighing 19–20 g, bred at the vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology were used in the study. The use and care of experimental animals have been performed in accordance with standard international rules on biologic ethics and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [14] and was approved by Institutional Animal Care and Use Committee. In total 36 animals were used, of which 12 as intact control (IC) and 24 were transplanted with solid variant of Ehrlich carcinoma cells. Tumor cells were injected intramuscularly into the hind limb ($5 \cdot 10^5$ cells/mouse).

The design of the experiment. Animals were divided into groups: IC ($n = 12$); CTG — control of tumor

growth, tumor-bearing mice that were not injected with lectin ($n = 12$); Lectin — tumor-bearing mice that were injected with lectin ($n = 12$). From the 2nd day after tumor grafting mice from Lectin group received subcutaneous injection of lectin from *B. subtilis* IMV B-7724 (0.02 mg per day) for 10 days. The mice (4 animals injected with lectin, 4 tumor-bearing and 4 intact per one observation point) were sacrificed on days 14, 21 and 28 after the tumor transplantation. Mph from peritoneal cavity were isolated and subjected to functional activity analysis. The nitric oxide (NO) production, arginase (Arg) activity, cytotoxic activities (CTA) of the Mph were studied. Data on the tumor-bearing mice were compared with the intact mice of the same strain, sex and age (referred as the IC). Besides, on 21th and 28th days after the tumor transplantation mRNA expression levels of TFs STAT-1, STAT-6, IRF5, IRF4 in Mph were evaluated.

Isolation and cultivation of Mph. Mice were euthanized, and 3 ml of ice-cold PBS supplemented with heparin (5 U/ml) was injected into the abdomen. The fluid was withdrawn and the abdomen was washed twice with the same volume of heparin-containing PBS. The resulting cells suspension was centrifuged (550 g for 10 min), and the cell pellet was resuspended in 1 ml of 0.9% NaCl solution supplemented with 2.0% penicillin-streptomycin. The peritoneal exudate cells were counted and cultured in 96-well flat-bottomed plates for 2 h (37 °C, 5% CO₂, 100% humidity). After that, non-adherent cells were removed, and the adherent cells were washed two times with 0.9% NaCl and taken for further investigation. In all subsequent studies, Mph were cultured at 37 °C in a humidified atmosphere with 5% CO₂ in complete culture medium: RPMI-1640 (Sigma, USA), 10% fetal calf serum, (Sigma, USA), and 40 µg/ml gentamicin.

CTA assay. CTA was determined by MTT-assay [15]. Autologous Ehrlich carcinoma cells were used as target. The target cells ($2 \cdot 10^4$ cells/well) in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics, were placed in a flat-bottom 96-well plates where Mph ($4 \cdot 10^5$ cells/well) were adhered beforehand, and incubated for 18 h in a 100% humidity atmosphere with 5% CO₂ at 37 °C. Control wells contained target cells or adhered Mph. 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 0.9% NaCl solution. After that, 0.12 ml of 2 M KOH and 0.14 ml of dimethyl sulfoxide (50% solution) were added into each well. Optical density was measured at $\lambda = 545$ nm vs $\lambda = 630$ nm using a microplate ELISA reader (StatFax-2100, USA). Each sample was done in triplicate. Cytotoxic activity index (CTAI, %) was calculated by the formula: $CTAI = [1 - (OD_{mph+tc} - OD_{mph}) / (OD_{tc} - OD_{blank})] \cdot 100\%$, where OD_{mph} — optical density of wells in which only adhered Mph were incubated; OD_{tc} — optical density of wells in which only tumor cells were incubated; OD_{mph+tc} — optical density of wells in which tumor cells and Mph were incubated; OD_{blank} — optical density of wells with the culture medium only.

NO production. Cell suspensions ($2 \cdot 10^5$ cells/well) were placed in a volume of 200 µl in 96-well flat-bottom tissue culture plates and cultured for 24 h. Each experiment was done 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 Griess reaction [16]. An 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 colorimetrically quantified at $\lambda = 550$ nm. The standard curve plotted by the results of measurements of the solutions containing known concentration of NaNO₂ was used for converting the absorbance to micromolar concentrations of NO expressed in µM NO₂ per 10^6 cells.

Arg activity. Arg activity was determined based on urea measurement [16]. Mph were lysed by double freezing and melting. Then 50 µl of 50 mM Tris-HCl (pH 7.4) and 10 µl of 50 mM MnCl₂ 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% H₂SO₄:85% H₃PO₄:H₂O). Then 40 µl of α -isonitrosopropiophenone (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 spectrophotometrically at $\lambda = 550$ nm. Values of optical density were converted to mass of urea based on calibration curve of standard urea solution. Arg activity was calculated as described in [17]. One unit of Arg activity means the amount of the enzyme hydrolyzing 1 µM of arginine per 1 min. Results are expressed as units/ 10^6 cells.

qRT-PCR. Total RNA was isolated from $2 \cdot 10^5$ Mph using NucleoZOL (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to 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 following forward (For) and reverse (Rev) list of primers: IRF4: For 5'-GGATTGTTCCAGAGGAGCC-3', Rev 5'-GGGCATA-ATCCCTCCAGCTC-3', IRF5: For 5'-CCTCAGCCG-TACAAGATCTACGA-3', Rev 5'-GTAGCATTCTCTG-GAGCTCTTCCT-3', STAT1 For 5'-TCCTTCTGGCCTTG-GATTGA-3', Rev 5'-ACCGTCCACCCATGTGAA-3', STAT6 For 5'-AGATGAGGCTTTCGGAGTCA-3', Rev 5'-CCCATATCTGAGCTGAGTTGCA-3', TBP For 5'-CCAATGACTCCTATGACCCC-3', Rev 5'-GTTGTC-CGTGGCTCTCTTATTC-3'. The target genes Ct values were normalized to Ct value of internal control gene (TBP) using ddCt method.

Statistical analysis. Statistical significance was evaluated by nonparametric Mann-Whitney U test and correlation analysis was determined according to Spearman's correlation using Prism software Version

8.0. Statistical significance between examined groups was assessed as $p < 0.05$.

RESULTS AND DISCUSSION

Polarization of peritoneal Mph of Balb/c mice was evaluated according to their functional status (NO production levels and Arg activity) and mRNA expression of transcription factors (STAT-1, STAT-6, IRF5, IRF4) on days 14, 21 and 28 after transplantation of solid Ehrlich adenocarcinoma.

On day 14 of tumor growth, in peritoneal Mph of untreated mice, a decreased NO production and simultaneously increased Arg activity were observed, as evidenced by a 1.6-fold decrease in the NO/Arg ratio compared to intact animals. In the group of animals injected with lectin, the level of this index was significantly higher ($p < 0.05$) compared with CTG group (10.83 a.u. vs 8.4 a.u.), although it did not reach the level of IC (Fig. 1).

Subsequently, under the introduction of lectin, the level of the NO/Arg ratio significantly exceeded ($p < 0.05$) the indices of animals of the CTG group (13.85 a.u. vs 5.98 a.u. and 12.45 a.u. vs 6.38 a.u. at 21 and 28 days of observation, respectively) and was at the level of IC group (Fig. 1).

The data obtained indicate that the use of lectin from *B. subtilis* IMV B-7724 contributed to the preservation of the initial functional state of peritoneal Mph of mice with grafted tumors throughout the observation period. That is, in the late stages of tumor growth in mice treated with lectin, cells with the M1 phenotype prevailed in the population of peritoneal Mph.

Similar dynamics was observed in the study of the specific CTA of peritoneal Mph of mice with Ehrlich adenocarcinoma at different terms of tumor growth (Table).

Table. Index of specific CTA (%) of peritoneal Mph of Balb/c mice with Ehrlich carcinoma

Group	Terms of tumor growth, days		
	14	21	28
IC		25.6 ± 1.9	
CTG	25.0 ± 4.1	16.9 ± 1.4 ¹	16.8 ± 0.5 ¹
Lectin	20.4 ± 2.5	32.1 ± 2.2 ^{1,2}	38.5 ± 4.5 ^{1,2}

Notes: ¹ $p < 0.05$ compared with IC; ² $p < 0.05$ compared with CTG

In the early stages of tumor growth, no significant differences were observed between the specific CTA of peritoneal Mph of mice that received or did not receive lectin *B. subtilis* IMV B-7724. From the 21 day of tumor growth in the group of untreated animals, this index de-

creased significantly ($p < 0.05$ compared with intact mice). Under the use of bacterial lectin on days 21 and 28 of tumor growth (complete course of treatment), the index of CTA of peritoneal Mph was significantly ($p < 0.05$) higher than the corresponding values of untreated and intact mice. The latter may also indicate long-term preservation of cells with the M1 phenotype among peritoneal Mph of mice injected with the lectin.

The dynamics and direction of the detected changes were also assessed at the mRNA expression level of STAT and IRF transcription factor on days 21 and 28 of tumor growth, as they are known as regulators of Mph polarization to M1 (STAT1 and IRF5) or M2 phenotype (STAT6 and IRF4) [18–20].

Similar to the NO/Arg ratio, the assessment of the M1/M2 polarization direction was assessed by the STAT1/STAT6 and IRF5/IRF4 mRNA ratio. On day 21 of tumor growth in mice of the Lectin group (1 day after the last administration of lectin) there was a significant increase in STAT1/STAT6 ratio compared with that in the CTG group (7.14 a.u. vs 2.65 a.u., $p < 0.05$) (Fig. 2, a). That is, changes in the STAT1/STAT6 ratio in Mph samples exert the same dynamics as their functional parameters (NO production, CTA). Instead, due to the action of lectin, the IRF5/IRF4 ratio on day 21 of tumor growth did not differ from that in the CTG group (Fig. 2, b).

Analysis of the STAT1/STAT6 and IRF5/IRF4 ratio on day 28 of tumor growth (7 days after the last lectin administration) revealed the opposite trend. In particular, there was no significant difference in STAT1/STAT6 ratio in the group of mice treated with lectin, compared with the CTG group (Fig. 3, a). In contrast, the IRF5/IRF4 ratio in Mph of lectin-treated animals was significantly higher than that in control animals (5.2 a.u. vs 1.07 a.u., respectively, $p < 0.05$) (Fig. 3).

Thus, the results of the evaluation of the functional activity of peritoneal Mph of mice treated with bacterial lectin, indicate their gradual polarization in the M1 direction. Instead, the results of analysis of STAT1/6 and IRF5/4 transcription factor mRNA expression levels are ambiguous. We can assume that on day 21 of tumor growth, Mph are polarized in the M1 direction due to the activation of STAT1 transcription factors. Given that NO synthase belongs to the list of targets of STAT1, we obtained high values of the NO/Arg ratio on the 21th

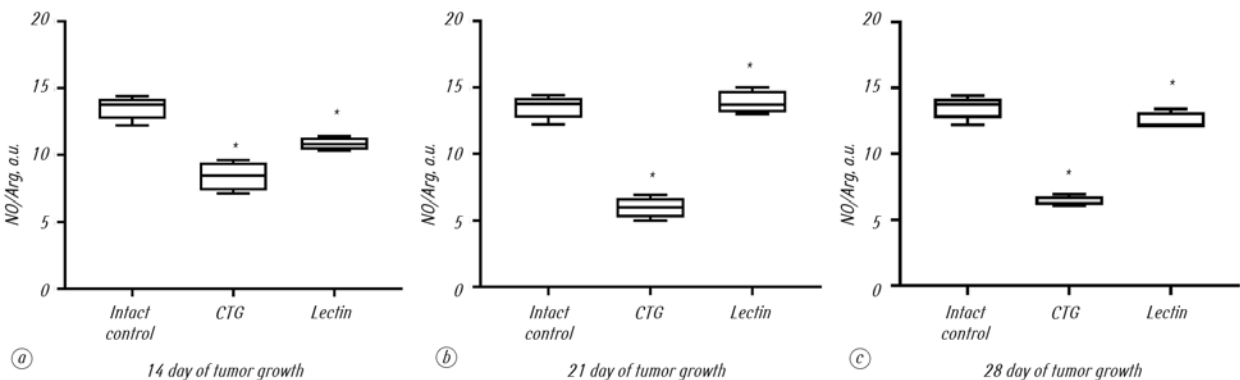


Fig. 1. Changes in NO/Arg ratio in peritoneal Mph isolated from Balb/c mice with Ehrlich adenocarcinoma: a — 14 day of tumor growth; b — 21 day of tumor growth; c — 21 day of tumor growth; * $p < 0.05$ compared with IC

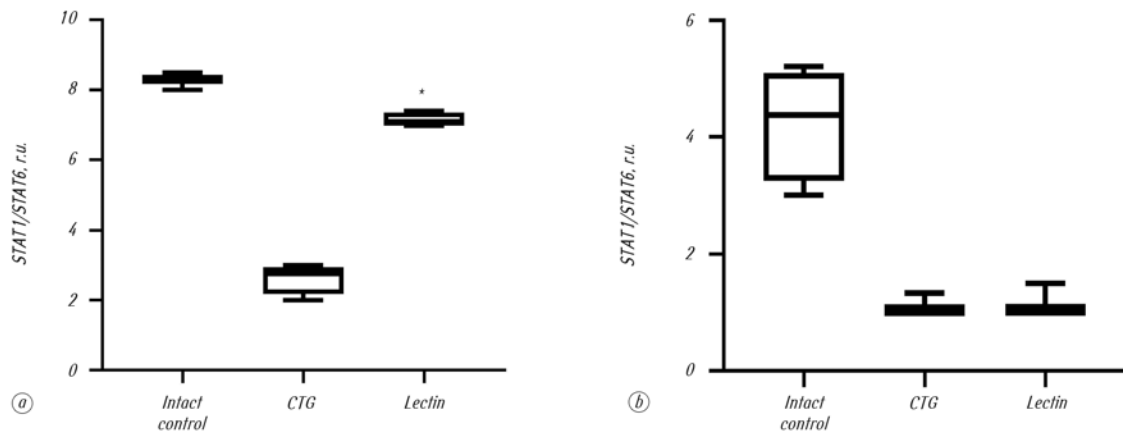


Fig. 2. STAT1/STAT6 (a) and IRF5/IRF4 (b) mRNA expression levels ratio in peritoneal Mph isolated in Balb/c mice on the 21st day of tumor growth; * $p < 0.05$ compared with CTG

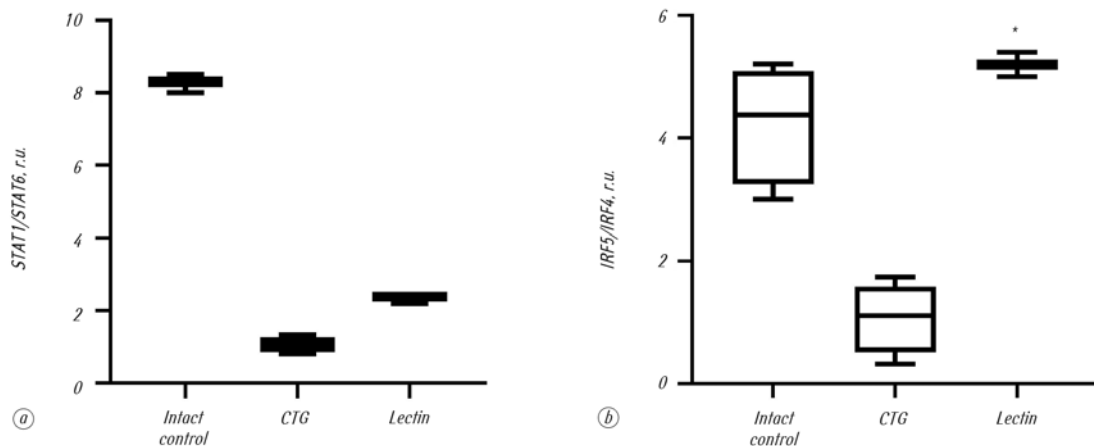


Fig. 3. STAT1/STAT6 (a) and IRF5/IRF4 (b) mRNA expression levels ratio in peritoneal Mph isolated in Balb/c mice on the 28th day of tumor growth; * $p < 0.05$ compared with CTG

day (due to increased NO levels) in Mph of mice from the Lectin group possibly due to the effect of lectin on the level of STAT1 expression. Regarding the terminal stage of tumor growth (28 days), high levels of NO/Arg ratio and preservation of CTA of Mph under the treatment with the lectin also indicate the prevalence of type M1 Mph. Polarization of cells at this stage may be due to activation of transcription factors of the IRF signaling pathway.

The functions (dysfunctions) of the IRF5 signaling pathway in autoimmune diseases and cancer are closely related to the level of its expression. In the case of autoimmune diseases, polymorphism in the *IRF5* gene leads to increased expression of IRF5. IRF5 expression is often suppressed (or absent) in malignant cells [21]. In a study of the expression of transcription factors of the IRF5 signaling pathway in human immune cells (Mph, dendritic and natural killer cells), it was found that IRF5 is expressed at a higher level in Mph possessing M1 phenotype compared to that in M2. Similar results were obtained in the study of changes in the polarization of Mph isolated from the bone marrow of C57Bl/6 mice under the *in vitro* action of granulocyte-macrophage colony-stimulating factor (GM-CSF), which is responsible for the induction of M1 type cells. Incubation of Mph with GM-CSF led to a significant increase in the expression of IRF5 mRNA and IRF5 protein, synthesis of proinflammatory cytokines and expression of surface markers of M1 Mph. The results

confirmed the importance of IRF5 as a marker of proinflammatory Mph [22]. A number of studies suggest the possibility of activation of the IRF5 signaling pathway in Mph under the influence of inflammatory factors, such as GM-CSF and interferon (IFN)- α/β [23].

Among other factors influencing the polarization of Mph, bacterial lipopolysaccharide (LPS) should be mentioned. It has been shown that binding of LPS to TLR4 on Mph activates IRF5, which in turn causes an increase in IL-12 expression and inhibition of IL-10 [24, 25]. The pathway of Mph activation in response to LPS via the TLR4 receptor and the autocrine production of IFN- β , which initiates the pathway of activation of STAT1 and STAT2 transcription factors via phosphorylation, have been demonstrated. The consequence of this process is the polarization of Mph into M1 type [26].

Given the above, we can assume that the predominance of cells with M1 phenotype in the peritoneal Mph population in mice with Ehrlich carcinoma treated with lectin may occur due to direct binding of bacterial lectin to TLR4 on the cell surface, and due to the influence of factors of the tumor microenvironment. These include tumor cells, stromal cells (fibroblasts, endothelial and immune cells) and some components of the extracellular matrix, such as collagen, fibronectin and others. Cytokines, in particular GM-CSF and IFN, can be the key factors influencing the effect of microenvironment on the Mph polarization

process. It is known that GM-CSF is produced in response to certain stimuli by a wide range of non-immune cell types, including fibroblasts, keratinocytes and endothelial cells. In addition, due to the activation of innate immune processes, activated T- and B-lymphocytes, Mph, mast cells, vascular endothelial cells and fibroblasts in response to cytokine stimulation are able to secrete GM-CSF [27, 28]. In mice, GM-CSF is produced mainly by lung epithelial cells and congenital lymphoid cells present in the intestine. Thus, GM-CSF plays a key role in the differentiation of Mph of lung and intestinal tissue and controls the process of their polarization [29].

On the other hand, most cells of innate immunity in the process of forming a response to infectious agents or transformed cells of the body are capable of producing IFN. The ability of IFN type I (IFN- α/β) to regulate the effector functions of natural and adaptive immunity, influencing the activation, differentiation, migration of different types of immune cells (dendritic, NK, Mph, T- and B-lymphocytes) is considered important for the formation of the antitumor response [30]. IFN- β is produced by fibroblasts, and leukocyte IFN- α — by leukocytes, especially Mph and dendritic cells. The latter were previously even called “natural cells that produce IFN” [31]. But further studies have disproved the relation of IFN- α production only to dendritic cells. Indeed, the populations of cells responsible for the production of type I IFN are different depending on the type of pathogen, its tissue tropism and the route of infection [32].

Due to the progression of the tumor process via accumulation of suppressor factors, the immune response is suppressed, the number of cells with anti-inflammatory and protumor properties increases, including M2 Mph. This is exactly the effect we observed in Balb/c mice in the late-term growth of Ehrlich carcinoma. On day 28 of tumor growth, regardless of the niche of functioning (isolated from the peritoneal cavity, spleen, tumor), all Mph by their functional properties belonged to the M2 type [33]. The treatment of mice with the lectin allowed achieving the prevalence of the M1 type Mph at late terms of tumor growth. Given the information on the interferonogenic properties of lectins produced by related strains of *B. subtilis* [34], we can assume that the lectin from *B. subtilis* IMV B-7724 may support Mph activation indirectly by inducing the production of IFN- γ in lymphocytes, NK cells. In this case, possibly, the transcription factors of the STAT signaling pathway may be involved. It is known that STAT1, which is responsible for the polarization of Mph in the M1 direction, is activated in response to IFN α , IFN γ release and TLR signaling [35].

In addition, our previous studies have shown the ability of lectin from *B. subtilis* IMV B-7724 at low concentrations (0.02 and 0.05 mg/ml) to stimulate *in vitro* peritoneal Mph of intact Balb/c mice. Under the influence of lectin there was a significant increase in NO production, a decrease in Arg activity, which is characteristic of Mph with the M1 phenotype. The changes in the expression of transcription factors of the STAT signaling pathway were similar to those in the combined effect of LPS and IFN- γ [36].

In conclusion, the results showed the ability of lectin from *B. subtilis* IMV B-7724 to change the polarization of Mph in the direction of M1 and to maintain for a long time the induction of a significant number of cells with proinflammatory properties not only *in vitro* but also *in vivo*. In the process of Mph polarization under the influence of the studied lectin, the transcription factors of the STAT and IRF signaling pathways are involved. The mechanisms of activating effect of the lectin on effector cells of antitumor immunity require further study.

REFERENCES

1. Kovaleva OV, Mikhailenko DS, Alekseev BYa, Grachev AN. The role of tumor-associated macrophages in the pathogenesis of renal cell carcinoma 2017; **1**: 20–6 (in Russian). doi: 10.17 650 / 1726-9776-2017-13-1-20-26.
2. Lyamina S.V. Polarization of macrophages in the modern concept of the formation of the immune response. Fundamental'nyye Issledovaniya 2014; **10**: 930–5 (in Russian). ISSN 1812-7339.
3. Jordan M, Waxman DJ. CpG-1826 immunotherapy potentiates chemotherapeutic and anti-tumor immune responses to metronomic cyclophosphamide in a preclinical glioma model. Cancer Lett 2016; **373**: 88–96. doi: 10.1016/j.canlet.2015.11.029.
4. Le Noci V, Tortoreto M, Gulino A, et al. Poly(I:C) and CpG-ODN combined aerosolization to treat lung metastases and counter the immunosuppressive microenvironment. Oncoimmunology 2015; **4**: e1040214. doi: 10.1080/2162402X.2015.1040214.
5. Daei Farshchi Adli A, Jahanban-Esfahlan R, Seidi K, et al. An overview on Vadimezan (DMXAA): The vascular disrupting agent. Chem Biol Drug Des 2018; **91**: 996–1006. doi: 10.1111/cbdd.13166.
6. Kaneda MM, Messer KS, Ralainirina N, et al. PI3K-gamma is a molecular switch that controls immune suppression. Nature 2016; **539**: 437–42. doi: 10.1038/nature19834.
7. Pan Y, Yu Y, Wang X, et al. Tumor-associated macrophages in tumor immunity. Front Immunol 2020; **11**: 583084. doi: 10.3389/fimmu.2020.583084.
8. Fraternali A BS, Magnani M. Polarization and repolarization of macrophages. Clin Cell Immunol 2015; **6**: 1–6. doi: 10.4172/2155-9899.1000319.
9. Chai ZT, Zhu XD, Ao JY, et al. microRNA-26a suppresses recruitment of macrophages by down-regulating macrophage colony-stimulating factor expression through the PI3K/Akt pathway in hepatocellular carcinoma. J Hematol Oncol 2015; **8**: 56. doi: 10.1186/s13045-015-0150-4.
10. Garaci E, Pica F, Sinibaldi-Vallebona P, et al. Thymosin alpha(1) in combination with cytokines and chemotherapy for the treatment of cancer. Int Immunopharmacol 2003; **3**: 1145–50. doi: 10.1016/S1567-5769(03)00053-5.
11. Liu M, Luo F, Ding C, et al. Correction: dectin-1 activation by a natural product beta-glucan converts immunosuppressive macrophages into an m1-like phenotype. J Immunol 2016; **196**: 3968. doi: 10.4049/jimmunol.1600345.
12. Chekhun VF, Didenko GV, Cheremshenko NL, et al. [Strain of bacteria Bacillus subtilis IMB B-7724 — producer of cytotoxic substances with antitumor activity] (Patent UA 131824). Publ. 25.01.2019 (in Ukrainian).
13. Podgorsky VS. The method for the obtaining bacterial lectin, specific to sialic acids (Patent UA 1791). Publ. 23.01.1991. (in Ukrainian).
14. Kozhemyakin UM, Filonenko MA, Saifetdinova GA. Scientific and Practical Recommendations for Keeping Laboratory Animals and Working with Them. K: Avicenna 2002; 156 p (in Ukrainian).

15. van de Loosdrecht AA Beelen RH, Ossenkuppe GJ. 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 Met* 1994; **174**: 311–20. doi: 10.1016/0022-1759(94)90034-5.
16. Reiner NE. Methods in molecular biology. Macrophages and dendritic cells. Methods and protocols. Preface. *Methods Mol Biol* 2009; **531**. doi: 10.1007/978-1-59745-396-7.
17. Dovgii RS, Shitikov, DV, Pishel, IN, *et al.* Functional state and metabolic polarization of splenic macrophages of old immunized mice. *Problemy Stareniya Dolgoletiya* 2015; **24**: 111–9. ISSN 08691703 (in Ukrainian).
18. Horhold F, Eisel D, Oswald M, *et al.* Reprogramming of macrophages employing gene regulatory and metabolic network models. *PLoS Comput Biol* 2020; **16**: e1007657. doi: 10.1371/journal.pcbi.1007657.
19. Zhou J, Li Z, Wu T, *et al.* LncGBP9/miR-34a axis drives macrophages toward a phenotype conducive for spinal cord injury repair via STAT1/STAT6 and SOCS3. *J Neuroinflammation* 2020; **17**: 134. doi: 10.1186/s12974-020-01805-5.
20. van Dalen FJ, van Stevendaal M, Fennemann FL, *et al.* Molecular repolarisation of tumour-associated macrophages. *Molecules* 2018; **24**: 9. doi: 10.3390/molecules24010009.
21. Li D, De S, Li D, *et al.* Specific detection of interferon regulatory factor 5 (IRF5): A case of antibody inequality. *Sci Rep* 2016; **6**: 31002. doi: 10.1038/srep31002.
22. Weiss M, Blazek K, Byrne AJ, *et al.* IRF5 is a specific marker of inflammatory macrophages in vivo. *Mediators Inflamm* 2013; **2013**: 245804. doi: 10.1155/2013/245804.
23. Almuttaqi H, Udalova IA. Advances and challenges in targeting IRF5, a key regulator of inflammation. *FEBS J* 2019; **286**: 1624–37. doi: 10.1111/febs.14654.
24. Arora S, Dev K, Agarwal B, *et al.* Macrophages: Their role, activation and polarization in pulmonary diseases. *Immunobiology* 2018; **223**: 383–96. doi: 10.1016/j.imbio.2017.11.001.
25. Liu YC, Zou XB, Chai YF, *et al.* Macrophage polarization in inflammatory diseases. *Int J Biol Sci* 2014; **10**: 520–9. doi: 10.7150/ijbs.8879.
26. Tugal D, Liao X, Jain MK. Transcriptional control of macrophage polarization. *Arterioscler Thromb Vasc Biol* 2013; **33**: 1135–44. doi: 10.1161/ATVBAHA.113.301453.
27. Baghban R, Roshangar L, Jahanban-Esfahlan R, *et al.* Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Commun Signal* 2020; **18**: 59. doi: 10.1186/s12964-020-0530-4.
28. Hong IS. Stimulatory versus suppressive effects of GM-CSF on tumor progression in multiple cancer types. *Exp Mol Med* 2016; **48**: e242. doi: 10.1038/emmm.2016.64.
29. Jeannin P, Paolini L, Adam C, *et al.* The roles of CSFs on the functional polarization of tumor-associated macrophages. *FEBS J* 2018; **285**: 680–99. doi: 10.1111/febs.14343.
30. Abdolvahab MH, Darvishi B, Zarei M, *et al.* Interferons: role in cancer therapy. *Immunotherapy* 2020; **12**: 833–55. doi: 10.2217/imt-2019-0217.
31. Budhwani M, Mazzeri R, Dolcetti R. Plasticity of type I interferon-mediated responses in cancer therapy: from anti-tumor immunity to resistance. *Front Oncol* 2018; **8**: 322. doi: 10.3389/fonc.2018.00322.
32. Ali S, Mann-Nuttel R, Schulze A, *et al.* Sources of type I interferons in infectious immunity: plasmacytoid dendritic cells not always in the driver's seat. *Front Immunol* 2019; **10**: 778. doi: 10.3389/fimmu.2019.00778.
33. Symchych TV, Fedosova NI, Chumak AV, *et al.* Functions of tumor-associated macrophages and mac-

rophages residing in remote anatomical niches in Ehrlich carcinoma bearing mice. *Exp Oncol* 2020; **42**: 197–203. doi: 10.32471/exp-oncology.2312-8852.vol-42-no-3.14928.

34. Podgorskii VS, Kovalenko EA, Karpova IS, *et al.* [Extracellular lectins from saprophytic strains of bacteria of the genus *Bacillus* (review)]. *Prikl Biokhim Mikrobiol* 2014; **50**: 256–63 (in Russian). <https://doi.org/10.1134/S0003683814030120>.

35. Piaszyk-Borychowska A, Szeles L, Csermely A, *et al.* Signal integration of IFN-I and IFN-II with TLR4 involves sequential recruitment of STAT1-complexes and NFkappaB to enhance pro-inflammatory transcription. *Front Immunol* 2019; **10**: 1253. doi: 10.3389/fimmu.2019.01253.

36. Chumak A, Shcherbina V, Fedosova N, Chekhun V. Polarization of macrophages of mice under the influence of lectin from *Bacillus subtilis* IMV B-7724. *EUREKA: Life Sci* 2021; **43**: 15–20. doi: 10.21303/2504-5695.2021.001878.

ВПЛИВ ЛЕКТИНУ БАКТЕРІАЛЬНОГО ПОХОДЖЕННЯ НА КЛЮЧОВІ ЛАНКИ ФУНКЦІОНАЛЬНОЇ АКТИВНОСТІ МАКРОФАГІВ МИШЕЙ З КАРЦИНОМОЮ ЕРЛІХА

А.В. Чумак, Н.І. Федосова, В.М. Щербіна, Н.Л. Черемшенко, О.М. Караман, В.Ф. Чехун

Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького НАН України, Київ, Україна

Стан питання: Дослідження останніх років демонструють перспективність використання різних напрямків імунотерапії в лікуванні онкологічних хворих. Одними з перспективних мішеней для імунотерапії є макрофаги (Мф). Тривають пошуки можливих агентів для активації та підтримання прозапальних властивостей цих клітин на тлі пухлинного процесу. **Мета:** дослідити зміни функціональної активності макрофагів мишей з карциномою Ерліха за рівнем співвідношення оксиду азоту (NO)/аргіназної активності (Arg), IRF4/IRF5 та STAT1/STAT6 при дії лектину *B. subtilis* IMV B-7724. **Матеріали та методи:** У дослідженні використовували мишей-самців Balb/c з карциномою Ерліха. З 2-го дня після прищеплення пухлини частини тварин вводили лектин *B. subtilis* IMV B-7724 (0,02 мг/мишу) протягом 10 днів. На 14-, 21- та 28-й день після перещеплення пухлини виділяли перитонеальні Мф та досліджували їх функціональний стан (продукцію NO та аргіназну активність (Arg), цитотоксичну активність). На 21- та 28-й день після трансплантації пухлини в Мф оцінювали рівні експресії мРНК транскрипційних факторів STAT-1, STAT-6, IRF5, IRF4. **Результати:** За умов застосування бактеріального лектину на віддалені терміни росту пухлини (21-, 28- доби) показники, що характеризують функціональний стан Мф (рівень співвідношення NO/Arg, індекс цитотоксичної активності) зберігалися на рівні інтактних мишей та достовірно ($p < 0.05$) перевищували показники тварин, яким не вводили лектин. Аналіз рівнів експресії мРНК транскрипційних факторів у цих тварин показав значне збільшення ($p < 0.05$) показника співвідношення STAT1/STAT6 на 21-шу та IRF5/IRF4 — на 28-му доби росту пухлини порівняно з таким у мишей, яким не вводили лектин. Наведені дані свідчать про збереження поляризації перитонеальних Мф у мишей з пухлинами, які отримували лектин, в напрямку M1-типу. **Висновки:** Введення мишам з карциномою Ерліха лектину *B. subtilis* IMV B-7724 призводило до превалювання на віддалених термінах пухлинного росту Мф, які проявляють функціональні властивості M1-типу. У процесі поляризації Мф під впливом досліджуваного лектину задіяні транскрипційні фактори сигнальних шляхів STAT та IRF.

Ключові слова: перитонеальні макрофаги, M1 та M2 поляризація, лектин *B. subtilis* IMV B-77, функціональний стан, транскрипційні фактори, карцинома Ерліха.