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THE EFFECT OF *B. subtilis* IMV B-7724 LECTIN ON FUNCTIONAL ACTIVITY OF THE MAIN EFFECTORS OF ANTITUMOR IMMUNITY OF INTACT MICE

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The *purpose* of the study was to evaluate the effect of *B. subtilis* IMV B-7724 lectin on the functional activity of macrophages (Mph) and natural killer cells (NK) of intact Balb/c mice.

Materials and Methods. Balb/c mice were subjected to 10 consecutive administrations of the lectin in a dose of 1 mg/kg of body weight. The functional activity of peritoneal Mph and NK were studied. Statistical analysis of the results was performed according to the widely accepted methods of variational statistics.

Results. Administration of bacterial lectin increased Mph and NK cytotoxic activity; maximal increase was registered after the complete course of injections. A significant increase in the NO production indicates the polarization of peritoneal Mph into pro-inflammatory type M1. The transcription factors of IRF (at the early stage) and STAT (at the latter stages) signalling pathways were involved in the process of Mph polarization.

Conclusion. The ability of *B. subtillis* IMV B-7724 lectin to increase *in vivo* cytotoxic activity of innate immunity effectors and to maintain the long-term Mph M1 polarization urges further studies on the lectin effectiveness.

Key words: *B. subtilis* IMV B-7724 lectin, macrophages, natural killer cells, functional activity, STAT, IRF.

To date, active research into the effectiveness of new immunotherapy agents in the treatment of patients with malignant neoplasms of various nosologies continues, as well as the search for substances that can be used in the construction of such agents is going on. Among natural substances possessing immunomodulatory effects, lectins of various origins are especially interesting. Lectins are a unique group of proteins and glycoproteins possessing significant biological activity. Their antitumor activity has been proved both in *in vitro* and *in vivo* studies. While plant lectins are more investigated, much less information is found in the scientific literature about the properties and medical application of microbial lectins [1, 2]. At Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the National Academy of Sciences of Ukraine, the investigation of the extracellular lectin produced by microorganism *B. subtilis* IMV B-7724 is going on. The lectin's physicochemical and biological properties were characterized; moreover, its cytotoxic activity against tumor cells was demonstrated *in vitro* [3] pointing to the prospects of its application as a means of antitumor immunotherapy. On the other hand, its effects *in vivo* remain to be elucidated.

The effectors of innate immune response, especially macrophages (Mph) and natural killer cells (NK), play significant roles in cancer immune defense. Their

activity is significantly suppressed during tumor growth [4, 5]. The restoration or maintenance of the activity of innate immunity cells during tumor progression can lead to an antitumor and/or antimetastatic effect. Therefore, the aim of our study was to evaluate the effect of *B. subtilis* IMV B-7724 lectin on the functional activity of Mph and NK of intact Balb/c mice.

Materials and Methods

The study has been carried out on females Balb/c mice 2–2.5-month-old, weighting 19.0–21.0 g, bred at the vivarium of IEPOR. The use and care of experimental animals have been performed in accordance with the standard international rules on biologic ethics.

Strain *B. subtilis* IMV B-7724 was used as a source of the lectin. The lectin was isolated from the cultural fluid as described in [3] and was freeze dried at temperatures between +24 °C ...–32 °C. The lectin obtained looks like a brown colored powder, easily soluble in water, buffers (PBS, Tris-HCl); has the higherst sugar-binding specificity towards sialic (N-Acytylneuraminic and N-Glycolylneuraminic) acids. The hemagglutinating activity of lectin (1 mg/ml) is in the range within 1024–2048 titer^{-1} [3].

The lectin was administered to Balb/c mice (n = 15) subcutaneously in doses 1 mg/kg of body weight per 1 administration. The complete course consisted of 10 administrations. Mice in the control group (intact control (IC), n = 5) were injected with the 0.9% NaCl solution following the schedule described above.

The level of NK cytotoxic activity was determined as described in [6]. The functional state of peritoneal Mph was studied by nitric oxide (NO) production, arginase (Arg) and nonspecific cytotoxic activity, as well as by assessing the expression level of STAT-1, -6 and IRF-4, -5 mRNAs by qRT-PCR [6].

The statistical processing of the results was performed according to generally acsepted rules of the variation statistics using Prism software Version 8.0. The difference was concidered significant at $P < 0.05$.

Results and Discussion

The administration of *B. subtilis* IMV B-7724 lectin to intact mice had an activating effect on peritoneal Mph and NK which was the most evident after the completion of the full course of lectin administration. There was noted a statistically significant increase on the cytotoxic activity of peritoneal Mph and NK (by 1.1 and 1.2 times on day 21 and 28 respectively, $P < 0.05$) as compared to the IC (Fig. 1).

There is an evidence that some lectins (namely, GalNAc/Gal-specific lectin produced by sea mussel *Crenomytilus grayanus*) act as an immune modulatory reagent by inducing cytokines production in macrophages that leads to increased bactericidal activity of these cells [7]. We hypothesized that *B. subtilis* lectin can increase Mph and NK cytotoxic activity

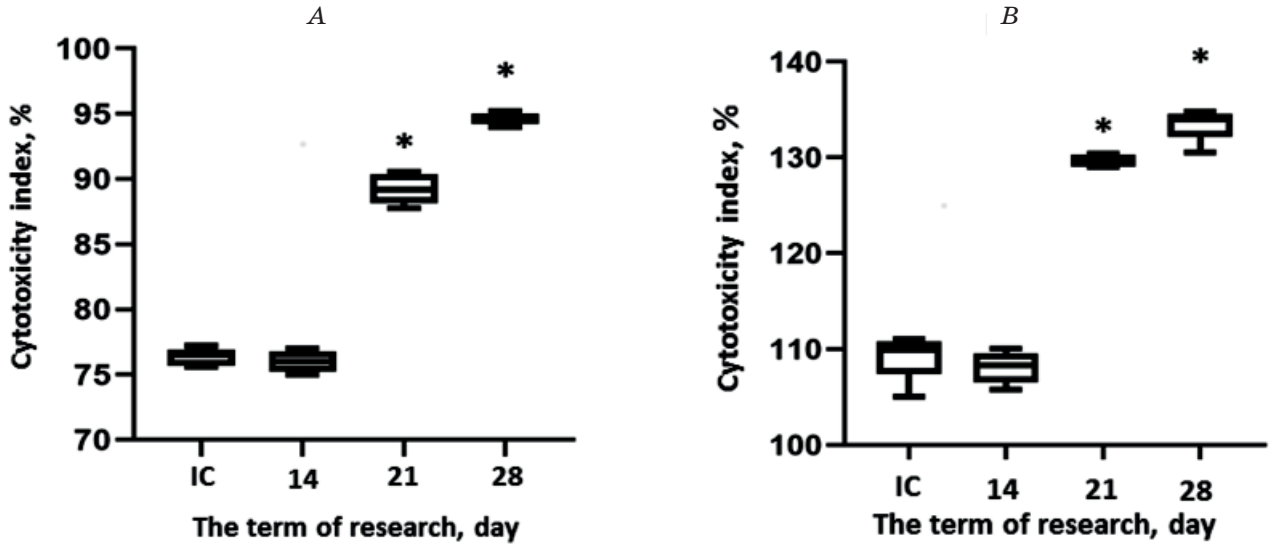

Fig. 1. **Cytotoxic activity (%) of peritoneal Mph (*A*) and NK cells (*B*)**
* — $P < 0.05$ compared with IC.

acting similarly — by inducing cytokines production after binding to certain surface carbohydrates.

Mph can perform opposite functions depending on the signals they receive from the microenvironment. In the antitumor response, the polarization state of Mph is important: M1 — possess antitumor properties, M2 — promote tumor progression. Such a property as plasticity enables Mph to change a polarization state (M1↔M2) in response to external stimuli making them interesting targets for antitumor therapy. Mph production of NO and Arg activity point to the Mph polarization state. After the last lectin administration (day 21), the NO/Arg ratio increased by 1.5 times compared to the control group ($P < 0.05$) and remined elevated on day 28. That is, after the full course of lectin injections, cells with the M1 phenotype prevailed in the peritoneal Mph.

Considering the interferonogenic properties of bacterial lectins [2], it can be assumed that the lectin of *B. subtilis* IMV B-7724 activates Mph indirectly through inducing IFNγ production by lymphocytes. In this process, the transcription factors (TF) of the STAT and IRF signaling pathways may be involved. STAT1 and IRF5 participate in M1 polarization; STAT6 and IRF4 — in M2 [8]. To test our hypothesis we evaluated the mRNA expression level of these TFs. Changes in STAT1/STAT6 and IRF5/IRF4 ratio in Mph coincide with changes in functional activities of these cells. A significant increase in mRNA expression of these TFs was noted after the complete course of lectin administration (Fig. 2). At the initial stages, the polarization of Mph M1 occurs due to the activation of TF of the IRF signaling pathway. Later, the polarization of M1 Mph was enabled probably due to the activation of the STAT signaling pathway.

Thus, the analysis of the obtained results allows us to conclude that the administration of *B. subtilis* IMV B-7724 lectin in a dose of 1 mg/kg (course dose of 10 mg/kg) to intact Balb/c mice lead to Mph and NK activation which both are potent players of anticancer immune response. The TFs of the STAT and IRF signaling pathways are involved in the polarization of peritoneal Mph M1 with antitumor properties. The ability of lectin to guide *in vivo* Mph polarization towards M1 type and to maintain the induction of a significant number of cells with pro-inflammatory properties for a long time can be used in designing a new antitumor immunotherapeutic agent.

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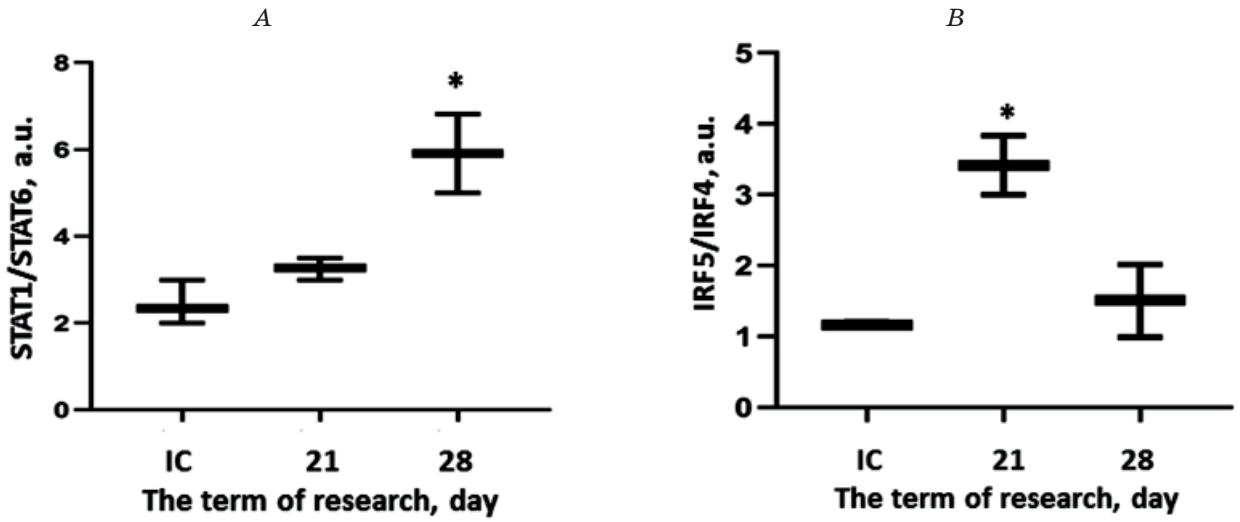

Fig. 2. **STAT1/STAT6 (*A*) and IRF5/IRF4 (*B*) mRNA expression levels ratio in peritoneal Mph**
* — $P < 0.05$ compared with IC.

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