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Research on cancer vaccines on cellular immunity of mice with Lewis lung carcinoma

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Abstract. Antitumor activity of xenogenic and homologous vaccines prepared with the use of protein containing metabolites of Bacillus subtilis B-7025 with molecular weight of 18.5 and 70 kDa were studied on Lewis lung carcinoma model. The relation of tumor growth dynamics in mice that received vaccines injections with cytotoxic activity of lymphocytes and macrophages was revealed. Those results can be used for further studies of the vaccine impact on humoral immunity section and for potential production of cancer vaccines based on these glycoproteins and chicken embryonic proteins.

Keywords: *adjuvants, cancer vaccines, tumor associated antigens, Lewis lung carcinoma, proliferation, apoptosis*

Introduction

Treatment of tumors is a complex, and in malignant forms, not always a solvable problem. One of the main methods of its control is the use of chemotherapy, which provides a direct cytotoxic effect on pathology. Introduction to oncology practice of various schemes of combined and complex treatment with new chemotherapy has enhanced the effectiveness of traditional methods and achieved, in some cases, complete remission, which, however, did not change the overall situation. Indicators of overall mortality from cancer remained almost the same, and metastatic forms of disease take significant proportions of them [1, 4, 6]. Often the death of a patient, regardless of the successfully cured primary tumor node, is caused by metastasis which have already existed at the time of diagnosis. This means that, in most cases, systemic chemotherapy used for prevention and control of metastases does not lead to the desired effect [4,7].

The current situation poses a difficult challenge for researchers to find fundamentally new methods and schemes of malignant tumors treatment. Over recent years there has been growing interest of researchers to methods of biotherapy, such as immunotherapy of cancer patients [2, 4, 5]. The latter has several areas, including the use of cytokines, monoclonal antibodies and specific anti-tumor vaccines. The use of cancer vaccines is very promising, which applies the use of tumor associated antigens (TAA), based on the formation of specific anti-tumor immune responses. It should be noted that most tumor antigens have low immunogenicity, leading to the need to find different ways to improve cancer vaccines [3]. One way to enhance the immune response to antigens is to use adjuvants, the spectrum of which is very broad. However, the effect of adjuvants on the immunogenicity of tumor antigens and the dynamics of nonspecific and specific antitumor responses is still not defined.

Previously, the main task of scientists who studied excipients was to use them for stimulation of humoral immunity. Adjuvants were either included into the anti-infection vaccine or used with animals for the induction of immunoglobulin synthesis. It was found that many of them are able to stimulate cell-mediated immunity and are suitable for use as part of cancer vaccines. However, the effect of excipients on the immunogenicity of TAA and dynamics of nonspecific and specific antitumor responses remains an open question [6].

In previous studies on exposure of vaccines based on chicken embryonic proteins and adjuvants of different biological origin on tumor-bearing animals immunization, it has been shown that all proposed materials of protein-containing metabolites B. subtilis B-7025 with molecular weights of 18.5 and 70 kDa have the best immune modulating properties. Therefore, we selected these components from the filtrate of culture fluid B. subtilis B-7025 for further research on an experimental model of Lewis lung carcinoma.

Paper objective is to study the influence of anti-tumor vaccine based on chicken embryonic proteins and protein-containing metabolites Bacillus subtilis B-7025 on cellular immunity of mice with Lewis lung carcinoma.

Experimental part

Objects of research. Within the experiment, there were used male mice Balb/C, 2-2.5 months old and with average weight of 18-20 g, obtained from the vivarium of Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine. As a model of tumor growth there was used Lewis lung carcinoma.

A series of experiments, namely triple immunization of animals by chicken embryonic proteins (0.1 mg of protein per injection) were carried out in mono or in combination with adjuvant: lipids from cell B. subtilis B-7025 molecular weight 18.5 kDa and 70 kDa (0.006 mg/injections), microbial cell BCG (0,3Ч108 CFC/injections), colloidal silver (Ag) and suspension of iron oxide (Fe3O4) in 2% solution of polidekstran (0.06 mg/injections). For intact control (IC) were used animals injected with NaCl.

To induce tumors, Lewis lung carcinoma cells were injected intramuscularly (i/m) in the right hind limb at a dose of 1×106 cells / mouse in 0.3 ml of the physical solution to all groups of animals other than intact one.

Animals were divided into 10 groups of 10 animals each. Mice of groups II, VII, VIII, IX, X under ether anesthesia had their tumors surgically removed. Vaccination of mice was performed twice with an interval of 2 days in subcutaneous manner in a volume of 0.3 ml of vaccine at a concentration of 0.3 mg / ml. Immunological parameters were measured on the 34th day after tumor cells transplantation.

*Research methods.*Vaccines were produced by the standard method developed by Zatula. The necessary concentration of adjuvants was added to chicken embryo extract of protein with concentration of 0.3 mg / ml. After being mixed, the mixture has been incubated in an incubator for two hours at a temperature of +37 °C.

Immunological researches were carried out using the MTT test. As target cells were used as homologous tumor strains. As sell effectors – allocated lymphocytes and macrophages.

Immunological examination of animals included: identification of cytotoxic activity and antibody-dependent cytotoxic activity of lymphocytes and macrophages,

cooperative cytotoxic activity of effector cells; antibody dependant cooperative cellular cytotoxicity of lymphocytes and macrophages, ELISA detection of generated antibodies specific to chicken embryonic proteins or tumor antigens LLC, enzyme immunoassay of specific antibodies.

Results and discussions

We have evaluated immunological parameters xenogenic antitumor vaccine based on chicken embryonic proteins and adjuvant of different biological origin, in order to establish the most effective adjuvant and determine by which the possible immunological process is the realization of cancer vaccines.

Investigation of cytotoxic activity of lymphocytes plays the main role in blocking tumor process. In assessing the test results to determine cytotoxic activity of lymphocytes can be seen that the introduction of CEP in conjunction with protein containing metabolites of B. subtilis B-7025 (with mol. mass of 18.5 kDa and 70 kDa) decreases lymphocyte cytotoxicity. This indicates a decrease of load on the immune system of animals with the tumor growth. Using lectin B. subtilis B-7025 observed a tendency to increase cytotoxic activity of lymphocytes. Use of other adjuvant not led to significant changes in activity of lymphocytes.

At the researching of antibody depended cytotoxic activity of lymphocytes (Fig. 2) was found that only in the groups treated with CEP and CEP with S. aureus peptidoglycan, it was less than in intact controls. In all other experimental groups have observed a tendency to its increase.

Within the ELISA method there were established that the maximal synthesis of antibodies was observed in groups of animals, which as an adjuvant to CEP got protein containing metabolite B. subtilis B-7025 with mol. weight 18.5 kDa and 70 kDa and peptidoglycan of S. aureus cells. In the group of animals where as adjuvant were used BCG synthesis of antibodies was lower, than in group with chicken embryonic proteins (Fig. 3). According to this we can conclude that BCG activates the cellular immunity and suppress of humoral.

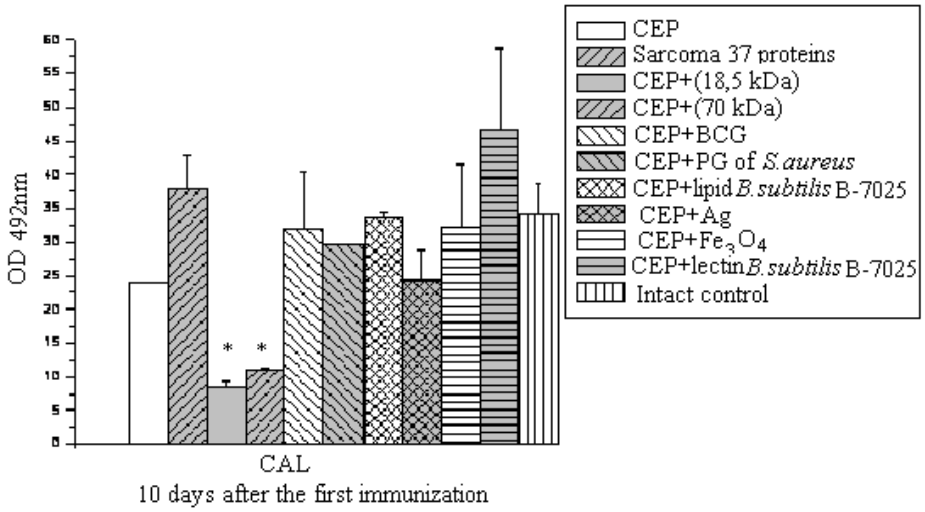

Fig. 1. Cytotoxic activity of lymphocytes of mice provided immunization DEB adjuvant

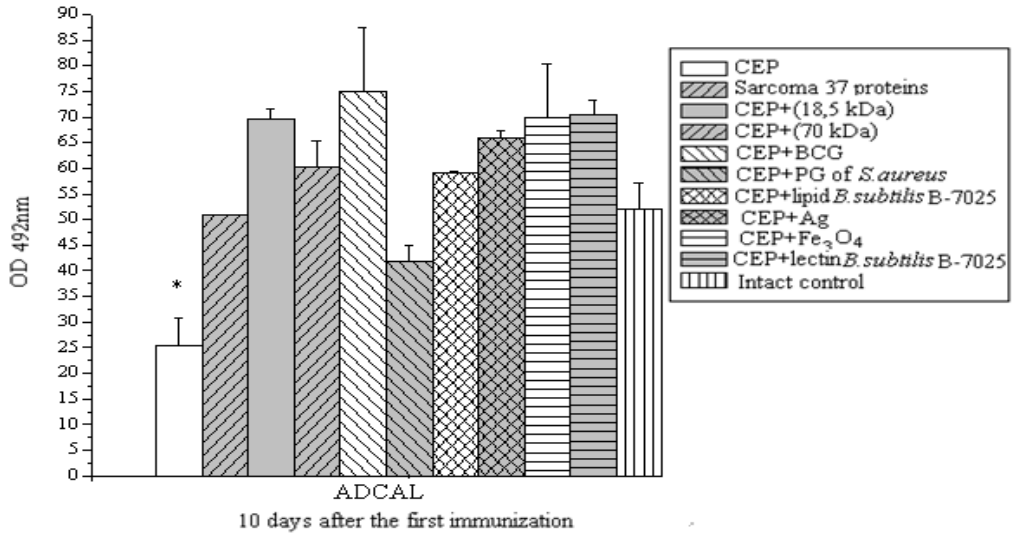

Fig. 2.Antibody depended cytotoxic activity of lymphocytes of mice provided immunization DEB adjuvant

Similar results were also obtained in assessing the accumulation of antibodies to proteins of Sarcoma 37 (Fig. 4). Exploring the dynamics of accumulation of medium molecular immune complexes in the blood of experimental mice injected with the vaccine based on the DEB and B. subtilis metabolites accumulated level of CIC was significantly menshyym compared with other vaccines. It shows their effectiveness.

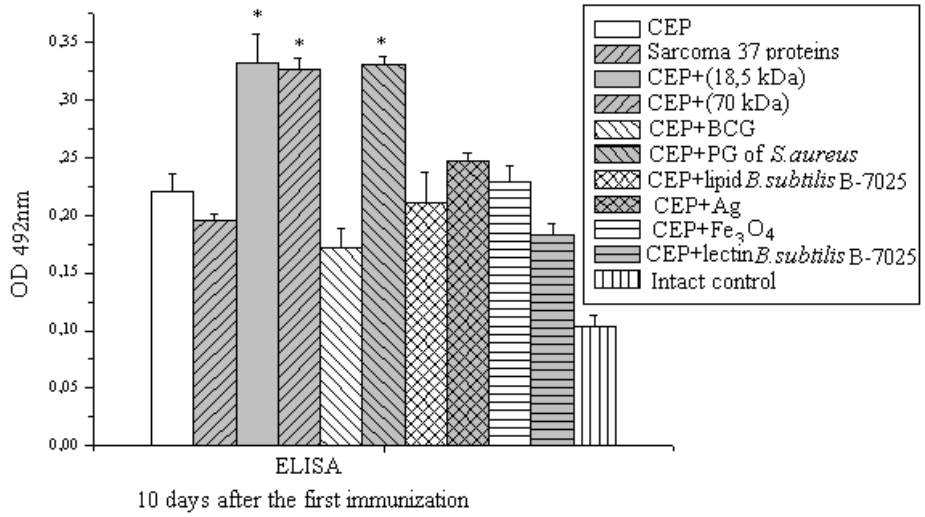

Fig. 3. ELISA detection of serum in experimental groups specific to chicken embryonic proteins provided immunization CEP with adjuvants

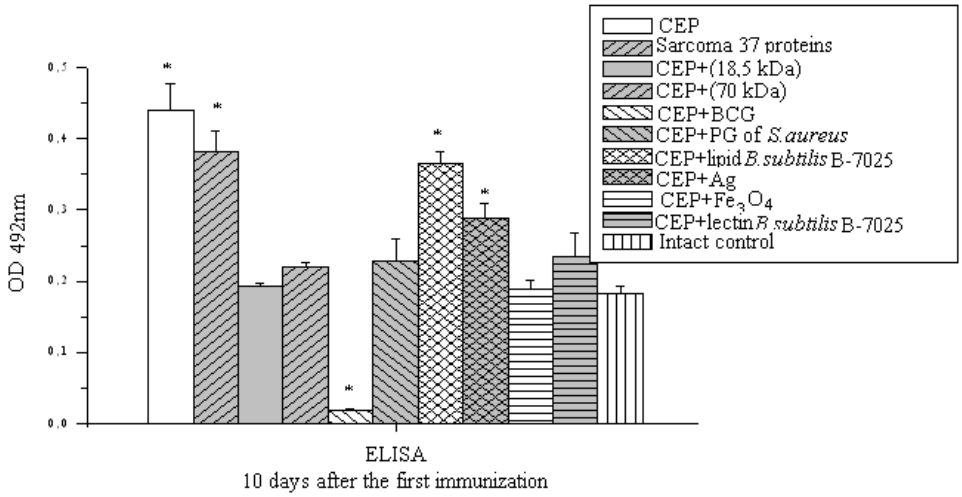

Fig. 4. ELISA detection of serum proteins in experimental groups against Sarcoma 37

It is shown that a mixture of metabolites of B. subtilis B-7025 has immunotoxic effects on the mice Balb/C and does not cause inflammatory reactions. Introduction of CEP with adjuvant, mainly with protein containing metabolites of B. subtilis B-7025, induces the formation of specific Ig G in the serum of animals. These data suggest the feasibility study of lipids as potential immunomodulating agents for their further use in oncology practice.

While determining the cytotoxic activity of lymphocytes (Fig. 5) it was found that applicable to mice with Lewis lung carcinoma, the use of vaccines based on CEP in conjunction with protein-containing metabolites B. subtilis B-7025 with a molecular mass of 70 kDa (group VI) increases this figure up to 11,19 ± 0,26%, compared to tumor growth control (24,84 ± 0,0642%). Increased cytotoxic activity of lymphocytes is also observed in mice of groups III and V by 3,67 ± 3,638 and by 4,25 ± 3,505%, respectively. During immunization of mice which underwent surgery by vaccines based on tumor cells and protein-containing metabolite B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, CEP in conjunction with protein-containing metabolites B. subtilis B-7025 with a molecular mass of 18.5 kDa have showed positive impact on this immunity section compared to performed surgery control.

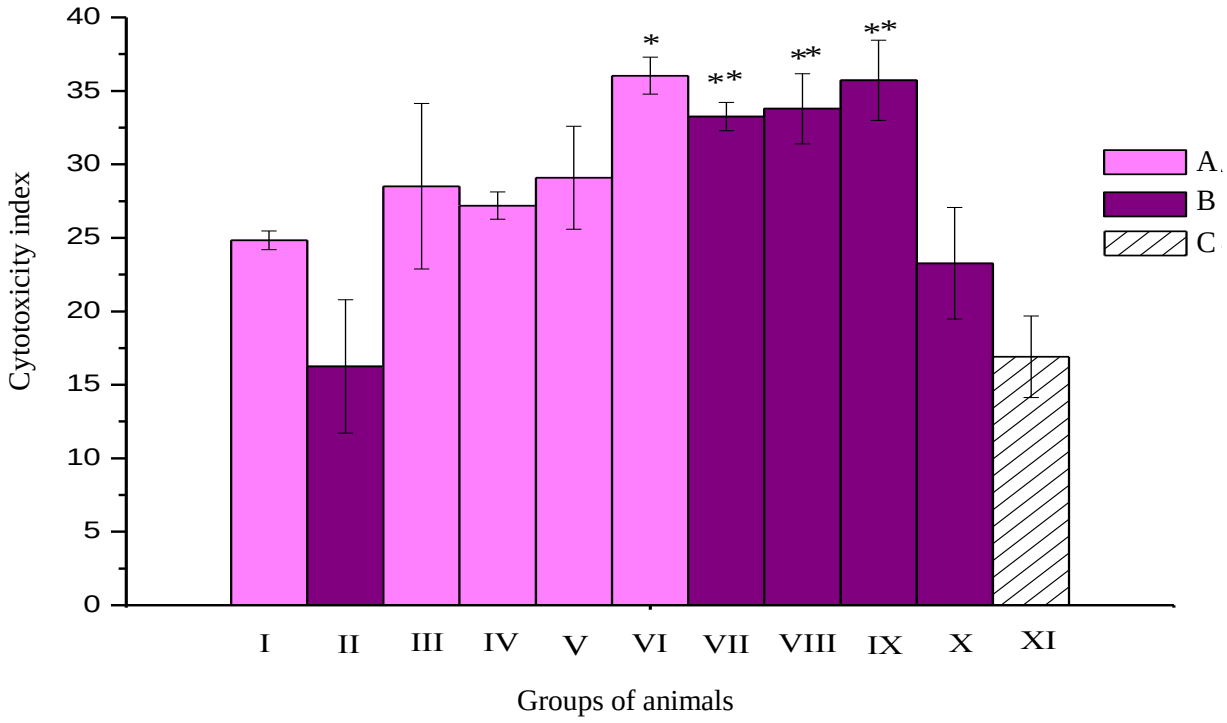

Fig 5. Effect of immunization by cancer vaccine on the performance of the cytotoxic activity of lymphocytes in immunized mice with Lewis lung carcinoma as of 34th day after tumor cells (TC) transplantation (n = 10 in each group).

A. Animals with no surgical removal of the tumor: I – unvaccinated animals; III, IV – groups of animals immunized with vaccine based on tumor cells (TC) and protein-containing metabolites (PM) B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively; V, VI – groups of animals immunized with a vaccine based on CEP with PM B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively.

B. Animals which underwent surgical removal of the tumor: II – the control group of animals with a surgery done; VII, VIII – groups of animals immunized with vaccine based on TC and PM B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively; IX, X – groups of animals immunized with a vaccine based on CEP with PM B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively.

C. XI – intact animals

Note: * - p <0.05 compared to tumor growth control, ** - p <0,05 compared to surgery control
PM – protein-containing metabolite, TC – tumor cells of Lewis lung carcinoma.

Thus, according to the values of cytotoxic activity of lymphocytes it can be concluded that the specified index of tumor-bearing mice was stimulated to the greatest extent by the vaccine which consists of CEP and protein-containing metabolite B. subtilis B-7025 with molecular weight of 70 kDa. Applicable to mice with tumors removed the use of vaccines with CEP and protein-containing metabolites B. subtilis B-7025 with a molecular mass of 18.5 kDa was the most effective. These results are of particular importance for tumor blocking.

The research on cytotoxic activity of macrophages has showed that the mice of almost all groups happened to stimulate cytotoxic activity of macrophages. Especially the increased cytotoxicity of macrophages compared to tumor growth control (25,72 ± 0,11%), was observed in mice of groups III and V by 9,53 ± 0,08% and by 8,64 ± 0,13% respectively, where vaccines based on protein-containing metabolite B. subtilis B-7025 with molecular weight of 18.5 kDa were used, both with tumor cells and chicken embryonic proteins involved. In the case of surgical removal of the tumor, the largest values were observed in group X (42,46 ± 1,66%). After removal of the tumor and the use of vaccines based on tumor cells and protein-containing metabolite B. subtilis B-7025 with a molecular mass of 18.5 kDa, there was recorded a decrease of cytotoxic activity of macrophages compared to control animals after surgery by 3,45 ± 0,13%. Thus, the group X has showed the highest index of cytotoxicity.

According to the indices of cytotoxic activity of macrophages, it can be stated that the use of vaccines, which include protein-containing metabolite B. subtilis B-7025 with a molecular mass of 18.5 kDa, both with tumor cells and chicken embryonic proteins involved, leads to significant positive modulation of macrophages. With the surgical removal of the tumor applied, the use of vaccines based on CEP in conjunction with protein-containing metabolite B. subtilis B-7025 with molecular weight of 70 kDa (group X) was the most effective, whereas a negative effect on this index was observed when using vaccines consisting of tumor cells and protein-containing metabolite B. subtilis B-7025 with a molecular weight of 18.5 kDa.

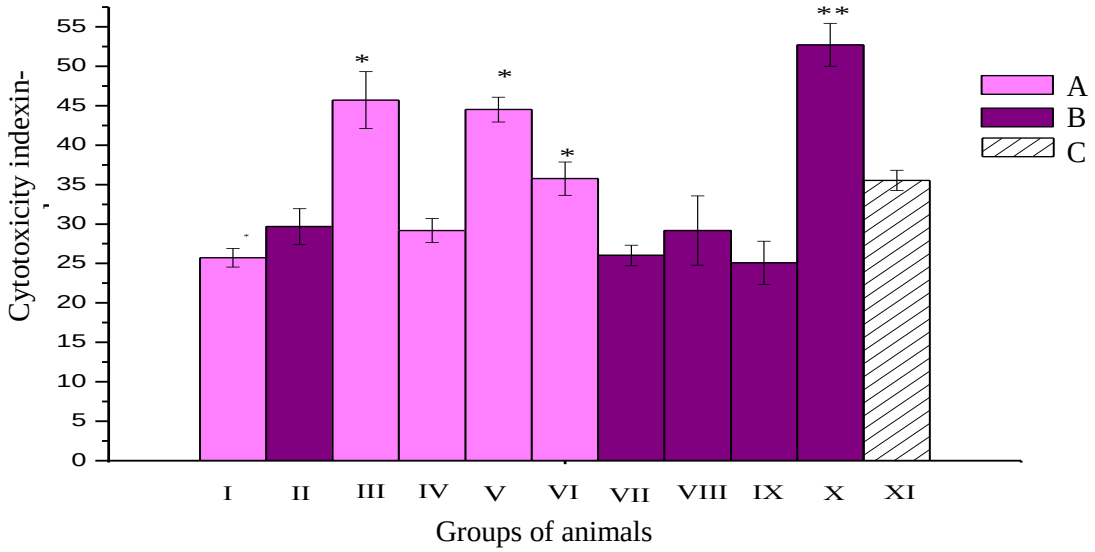

Fig 6. Effect of immunization by cancer vaccine on the performance of the cytotoxic activity of macrophages of immunized mice with Lewis lung carcinoma as of 34th day after tumor cells transplantation (n = 10 in each group).

A. Animals with no surgical removal of the tumor: I – unvaccinated animals; III, IV – groups of animals immunized with vaccine based on tumor cells (TC) and protein-containing metabolites (PM) B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively; V, VI – groups of animals immunized with a vaccine based on CEP with PM B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively.

B. Animals which underwent surgical removal of the tumor: II – the control group of animals with a surgery done; VII, VIII – groups of animals immunized with vaccine based on TC and PM B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively; IX, X – groups of animals immunized with a vaccine based on CEP with PM B. subtilis B-7025 with a molecular mass of 18.5 or 70 kDa, respectively.

C. XI – intact animals.

Note: * - p <0.05 compared to tumor growth control, **-p <0,05 compared to surgery control.

PM – protein-containing metabolite, TC- tumor cells of Lewis lung carcinoma.

Conclusions

1. It was established that the most immunologically active adjuvants for a xenogenic antitumor vaccines are glycoproteins B. subtilis B-7025 with mol. weight 18.5 kDa and 70 kDa.

2. The introduction intact mice sarcoma 37 antigenic washings cause the synthesis of antibodies that cross-react with chicken embryonic proteins. The most severe reaction observed for antigens immunized colloidal silver and lipid B. subtilis B-7025.

3. The lowest level of antibody accumulation was found in groups of animals, which as adjuvant to CEP got BCG antibody, which indicates the activation of cellular immunity and humoral suppression.

4. The range of immunological effects studied vaccines includes activating effect on the cellular and humoral response of adaptive immunity and on the reaction of natural antitumor resistance (cytotoxic activity of LF and MF).

5. Applicable to mice with Lewis lung carcinoma, the use of vaccine based on chicken embryonic protein and protein-containing metabolite B. subtilis B-7025 with a molecular mass of 18.5 kDa enhances cell-mediated cytotoxicity in lymphocytes in 2 times and macrophages in 1.5 times, compared to corresponding control animals with tumors.

6. It was shown that the vaccine based on chick embryonic protein and protein-containing metabolite B. subtilis B-7025 with molecular weight of 70 kDa was the most effective in terms of influencing the cellular fraction of the immune system of the mice with Lewis lung carcinoma which underwent surgery. This is reflected in the growth of the cytotoxic activity of macrophages by 2 times compared to those of control animals with surgically removed tumor.

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Грегирчак Н.Н., Лыч И.В. Влияние противоопухолевых вакцин на клеточное звено иммунитета у мышей с карциномой легкого Льюис

Аннотация. На модели карциномы легкого Льюиса изучали противоопухолевую активность ксеногенных и гомологичных вакцин изготовленных на основе белок содержащих продуктов жизнедеятельности Bacillus subtilis B-7025 с молекулярной массой 18,5 и 70 кДа. Была обнаружена связь между динамикой роста опухолей у мышей, иммунизированных препаратами вакцин, и цитотоксической активностью лимфоцитов и макрофагов. Результаты работы являются основой для дальнейших исследований влияния вакцины на гуморальное звено иммунитета и возможности создания противоопухолевых препаратов на основе данных гликопротеидов и куриных эмбриональных белков.

Ключевые слова*: адьюанты, противоопухолевые вакцины, опухоль ассоцированые антигены, Карцинома легких Левиса, пролиферация, апоптоз*