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EXPERIMENTAL STUDY OF THE EFFICACY OF COMBINED USE OF CANCER VACCINE AND INTERFERON

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Aim: **To study in *in vivo* model the efficacy of combined scheme of administration of cancer vaccine (CV) and interferon (IFN).** ***Materials and Methods:*** **Lewis lung carcinoma (LLC) was transplanted to male C57Bl mice. For treatment, CV prepared from LLC cells with the use of cytotoxic lectins of *B. subtilis B-7025*, and preparation of murine IFN-alpha were used. Therapeutic effect was evaluated by measurement of tumor volume and analysis of average life span (ALS) of treated animals. Immunologic study included determination of antitumor cytotoxicity of T-lymphocytes (CTL) and natural killer (NK) cells by radiometric method, functional activity of peritoneal macrophages (MP) — by colorimetric test with nitroazole blue, and evaluation of titers of tumor necrosis factor (TNF) and interleukins-1 and -2 (IL-1, 2).** ***Results:*** **It has been shown that the use of IFN preparation significantly elevated efficacy of vaccine therapy of solid form of LLC: duration of latent period of tumor growth elevated by 25%, ALS — by 28%, index of tumor growth inhibition — by 35–40%. Upon combined use of CV and IFN, significant activation of the cells — effectors of nonspecific immune defense (MP), and specific one (CTL) was observed.** ***Conclusion:*** **The obtained results evidence on perspectiveness of the development of combined schemes of administration of CV and IFN for elevation of the efficacy of vaccine therapy.**

Key Words: **cancer vaccine, murine interferon, Lewis lung carcinoma, immunologic indexes.**

To improve the technology of cancer biotherapy, it is important to understand functions and mechanism of action of cytokines that are involved in attraction of immunocompetent cells to immune reactions and elevate ability of T-lymphocytes to eliminate malignant cells. That's why biological modifiers of immune response are often used in technology of preparation of cancer vaccines (CV) or are used as their components. In the most simple variant, cytokines are administered together with antigen and stimulate immune response against it [1–4].

Interferon (IFN) is one of the key modulators of immune response influencing the processes of antigen recognition, differentiation, and functional activity of immunocompetent cells. The most interesting aspects of studies are effects of IFN related to elevation of tumor immunogenecity and its altered sensitivity to cytotoxic action of T-lymphocytes [5].

The role of IFN in combination with other biological agents or chemopreparations became a subject of intense studies. The research of effects of IFN upon combined use with active specific immunization is of special interest [1, 6, 7]. IFNs, including IFN-α2, -β, -γ, are potent inducers of expression of genes of major histocompatibility complex. Exactly this property, apart from indirect immunomodulating action is one of the main reasons for use of IFN in immunotherapy [8]. The facts on high anticancer acitivity of IFN in the case of metastatic melanoma and the increasing number of reports about positive influence of this cytokine on elongation of relapse-free period in the patients after tumor resection are evidencing the expediency of combined use of IFN with CV [6, 9, 10]. The mechanisms of synergistic action of vaccine and IFN possibly will differ dependent on composition of the vaccine and the immune reactions that provide an effect of immunization. In numerous reports it has been shown that IFN-α possesses the highest anticancer action [5, 6, 8]. The results of experimental and clinical studies are providing the grounds for the use of IFN-α as an element of optimization of combined therapy directed on the decrease of metastasis risk and improvement of quality of life of oncological patients [10, 11].

The aim of present research was to evaluate the possibilities of the use of immunomodulating properties of IFN for elevation of the efficacy of cancer vaccine prepared from autologous tumor cells with the use of cytotoxic lectin (CL) from *B. subtilis B-7025* [12, 13].

MATERIALS AND METHODS

The experiments were carried out on male C57Bl 2.5 months old mice bred in the vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine (Kyiv, Ukraine) with the use of experimental model of Lewis lung carcinoma (LLC). Tumor cells were transplanted in the thigh muscle (by 5.0 x 10^5 vital LLC cells per animal). The animals were treated by therapeutic scheme as follow: 1st group received CV, 2nd – preparation of murine IFN, 3rd — CV + IFN by combined scheme. In this series of experiments, the group of untreated animals (4th group, transplantation control) and intact animals (5th group) served as the controls. All experiments with animals were approved by local ethic committee.

CV was prepared from grounded LLC tissue samples treated with cytotoxic lectins of *B. subtilis B-7025* (1 g of tumor tissue per 10 ml CL solution (0.5 mg/ml)) and stored at –18 °C [12]. Preparation of CL was isolated from culture medium of *B. subtilis B-7025* by the method described earlier [14]. CV was

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Abbreviations used: CTL – cytotoxic lymphocytes; CV – cancer vaccine; IFN – interferon; IL-1 – interleukin-1; LI – labeling index; LLC – Lewis lung carcinoma; MP – peritoneal macrophages; NK – natural killer cells; TNF – tumor necrosis factor.

injected subcutaneously (s. c.) by 0.3 ml at the days 1, 4, 8, 11, 15 after LLC transplantation.

Preparation of IFN-alpha was prepared according to [15]. IFN solution was prepared *ex tempore* and administered 5 times intraperitoneally (i.p.) by 1000 units in 0.5 ml of physiologic solution 24 h prior to CV administration. Such scheme for IFN administration was chosen of the base of results of studies on combined use of CV with other immunomodulating preparations [16, 17]. Efficacy of treatment was evaluated by tumor growth inhibition index and average life span (ALS) of experimental animals.

Immunologic research included determination of cytotoxic activity of T-lymphocytes (CTL) and natural killer (NK) cells in cytotoxic tests *in vitro* [18], cytochemical activity of peritoneal macrophages (MP) using test with nitroazole blue (NBT) [19], the level of production of tumor necrosis factor (TNF) by MP *in vitro* using TNF-sensitive L-929 cells [20], the levels of production of IL-1 by adherent fraction of spleen lymphocytes, and IL-2 — by nonadherent one using standard radiometric methods [21, 22]. These studies were carried out at the days 28 and 45 after tumor cells transplantation.

Statistical analysis was carried out using Student's *t*-criterium; the values $p < 0.05$ were considered significant [23].

RESULTS AND DISCUSSION

The study of LLC growth evidenced on significant increase of the duration of latent period and pronounced inhibition of tumor growth upon combined use of CV and murine IFN compared with these indexes in the groups of transplantation control or upon separate use of CV and IFN (Fig. 1). Analogous peculiarities have been registered during analysis of ALS of experimental animals (Table 1). In the group of LLC-bearing mice that received CV at monoregimen (group 1), insignificant increase of the duration of latent period has been observed as well as tumor growth inhibition by 24–45%, that lead to significant increase of ALS. Administration of CV with IFN (group 3) led to significant increase of its efficacy: the duration of latent period elevated by 25%, whilst tumor growth inhibition increased by 35–40%. ALS indexes in groups 1 and 3 were 53.71 ± 4.0 and 65.0 ± 2.9 days respectively ($p < 0.05$). It should be stressed that upon separate use murine IFN also was effective compared to control indexes: the duration of latent period elevated by 39%, ALS — by 38% (54.8 ± 3.75 and 39.8 ± 1.8 days respectively, $p < 0.05$). So, the obtained results have shown that aadministration of IFN to LLC-bearing mice at monoregimen or in combination with vaccine elevates vaccine's efficacy.

The study of immunologic indexes allowed to analize their changes in treated animals (Table 2). In control group of LLC-bearing mice at the day 28 the cytotoxic activity of NK cells and CTL has been restored at relatively high level (labeling index (LI) was +322.6% and +196.0%, respectively compared to intact control). These indexes as well as MP activity (LI = +79.4%) were significantly higher than these of intact animals ($p < 0.05$).

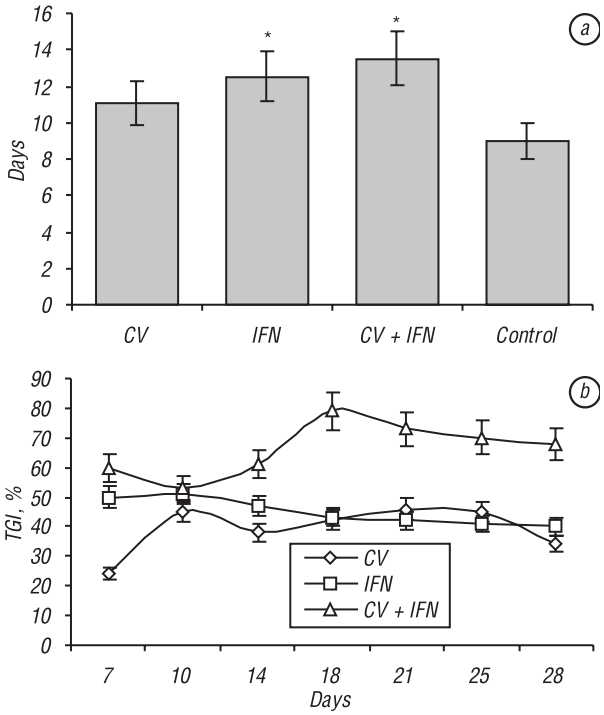

Fig. 1. Duration of latent period (*a*) and tumor growth inhibition of experimental animals

*$p < 0.05$ compared to control of transplantation.

Table 1. Average life span (ALS) and duration of latent period in mice bearing Lewis lung carcinoma

Group	Treatment	Number of animals	ALS, days			Latent period, days		
			X ± m	t	LI, %	X ± m	t	LI, %
I	CV	14	53.7 ± 4.0	2.8*	35.0	11.1 ± 1.3	1.4	13.5
II	IFN	15	54.8 ± 3.8	3.1*	37.7	12.5 ± 1.1	2.5*	28.3
III	CV + IFN	11	65.0 ± 3.9	5.8*	63.3	13.5 ± 1.6	2.7*	38.2
IV	Control	10	39.8 ± 1.8	–	–	9.0 ± 0.8	–	–

*$p < 0.05$ compared to control of transplantation.

Table 2. Activity of effectors of cellular antitumor immunity in mice bearing Lewis lung carcinoma (day 28 of tumor growth)

Group	Treatment	CI, %		Activity of MP in NBT-test
		NK	CTL	
I	CV	43.2 ± 2.3*,**	7.8 ± 4.5***	0.176***
II	IFN	9.4 ± 4.7***	28.0 ± 4.3*	0.243
III	CV + IFN	10.6 ± 5.2***	34.6 ± 2.4*	0.600*,***
IV	Control of transplantation	35.5 ± 2.6*	24.5 ± 13.8*	0.393*
V	Intact control	6.4 ± 2.0	2.0 ± 1.2	0.219

*$p < 0.05$ compared to intact control;

**$0.05 < p < 0.1$ compared to control of transplantation;

***$p < 0.05$ compared to control of transplantation.

Administration of CV to mice resulted in the marked NK cells activation (43.2 ± 2.3 *versus* 35.5 ± 2.6% for group of transplantation control, $p < 0.05$); at the same time the indexes of activities of CTL and MP remained at the level of intact control and were significantly lower than these of transplantation control group. Upon the use of IFN, the situation was different — the preparation acted mainly via activation of CTL. Combined treatment with both agents (CV + IFN) along by increased specific response was accompanied with elevation of MP activity (compared to transplantation control group, LI was +41% and +52,7%, respectively, $p < 0.05$), whilst NK cells cytotoxicity remained at the level of that of intact animals.

It is known that along with effectors of specific and natural resistance, cytokines (IL-1, IL-2, TNF, IFN) play

an important role in anticancer defense of the body. As biologic modifiers of immune response, they are often used in technology of preparation of cancer vaccines or are used as their components. That's why one may suppose that positive effects of vaccine therapy combined with IFN could be in part explained by its influence on the level of production at least some of mentioned cytokines, in particular, TNF, IL-1, IL-2.

At the day 28 of tumor growth, production of TNF in animals from transplantation control group was decreased compared with intact control: this is evidenced by relatively low titer of TNF (LI = –51.9%, $p < 0.05$) (Table 3). It should be noted that at this time point in experimental groups (groups I–III) production of TNF remained at the level of intact animals only in mice that received IFN (the titer of TNF activity in this group was 2.55 ± 0.25 log2 and was twice higher than such indexes of transplantation control group as well as other experimental groups. At this period no alterations in IL-1 activity have been observed in all studied groups.

Level of IL-2 production in the supernatants of cultured lymphocytes isolated at day 28 from the animals of transplantation control group was significantly lower than that of intact animals (LI = –56.8%, $p < 0.05$). At this period in mice from groups I and II the titer of IL-2 activity was practically equal to that of tumor-bearing control mice (LI was –35% and –16%, respectively, compared to transplantation control group). The titers of IL-2 activity in mice that received IFN + CV were close to these of intact control (LI = –36% compared to intact control, and +46.8% compared to transplantation control group) (Table 4). It's interesting that exactly in this group the highest percent of animals (25%) that didn't develop tumors has been registered.

Table 4. Production of IL-2* by T-lymphocytes from spleen of $C^{57}Bl$ mice after transplantation of Lewis lung carcinoma cells

Group of animals	Activity of CTL, CI, %	Terms of observation (days after tumor cell transplantation)	
		28	45
CV	7.8 ± 4.5[2]	< 1 log_2[1, 2]	–
CV + IFN	34.6 ± 2.4[1]	2.26 ± 0.01[1, 2]	2.68 ± 0.07[1, 3]
IFN	28.0 ± 4.3[1]	1.29 ± 0.01[1, 2]	–
Control of transplantation	24.5 ± 3.8[1]	1.54 ± 0.1[1]	–
Intact control	2.0 ± 1.2	3.57 ± 0.08	

*Production of IL-2 is expressed in titres of activity, log_2;
[1]$p < 0.05$ compared to intact control; [2]$p < 0.05$ compared to control of transplantation; [3]$p < 0.05$ compared to previous observation point.

That's why it looks reasonable to analyze the state of effectors of anticancer defense (CTL, NK, MP) (Fig. 2) in these animals. Immunologic research has been carried out at day 45 after LLC transplantation (at this term all animals from transplantation control group died, ALS = 39.8 ± 1.8 days). The results have shown significant activation of effector cells of specific and unspecific defense ($p < 0.05$). At this term of observation the highest CTL activity has been registered in mice that received IFN separately or in combination with CV (LI was +993.0% and +615.6% respectively, $p < 0.05$, compared to intact animals). The indexes of NK and MP activity in mice of experimental groups were similar and were significantly higher than control ones.

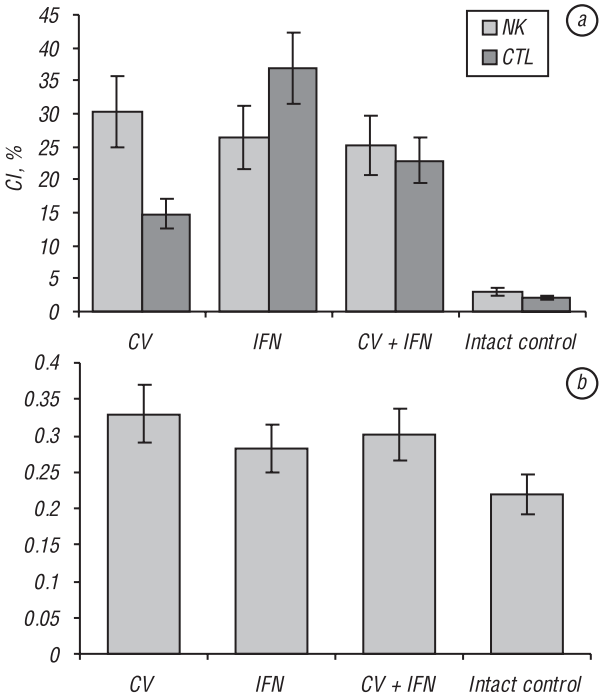

Fig. 2. Activity of effectors of cell immunity in mice with undeveloped tumors (day 45 after LLC transplantation), that received CV + IFN by combined scheme: *a* — cytotoxicity of NK and CTL, *b* — activity of MP in NBT-test.

In conclusion, the presented data allow suppose that the use of IFN at monoregimen or in combination with CV favors restoraton of the functional reserve of all effectors of antitumor defense of the body, and that combined administration of CV and IFN could be considered as a promising tool. Further exploration of synergistic action of these preparations could be useful for the development of effective schemes of biotherapy of oncologic diseases.

REFERENCES

1. **Dillman RO, Wiemann M, Nayak SK, De Leon C, Hood K, DePriest C.** Interferon-gamma or granulocyte-macrophage colony-stimulating factor administered as adjuvants with

Table 3. Production of TNF and IL-1 *in vitro* by adherent splenocytes of mice bearing Lewis lung carcinoma that received CV + IFN by combined scheme

Cytokine	Group	Titer of activity, log_2	Cytotoxicity index, %					
			1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64
TNF	CV	1.13 ± 0.29	127.5 ± 20.2	23.6 ± 8.8	0	0	0	0
	IFN	2.55 ± 0.25**	279.2 ± 33.7	133.1 ± 20.8	57.3 ± 14.0	9.5 ± 2.43	0	0
	CV + IFN	1.50 ± 0.19	102.2 ± 17.9	16.2 ± 12.5	0	0	0	0
	Control of transplantation	1.25 ± 0.31	75.3 ± 15.4	49.4 ± 23.3	0	0	0	0
	Intact control	2.6 ± 0.4	119.1 ± 20.4	107.9 ± 7.7	62.9 ± 6.8	6.7 ± 2.1	0	0
IL-1	CV	1.3 ± 0.3	2.6 ± 0.1	1.8 ± 0.4	1.3 ± 0.2	1.1 ± 0.04	0,8 ± 0,04	0,8 ± 0,2
	IFN	2.0 ± 0.0	2.8 ± 0.4	2.6 ± 0.3	1.6 ± 0.2	0.9 ± 0.2	0,6 ± 0,1	0,5 ± 0,1
	CV + IFN	1.0 ± 0.6	2.3 ± 0.3	1.9 ± 0.3	1.2 ± 0.2	1.1 ± 0.1	1,0 ± 0,1	1,1 ± 0,03
	Control of transplantation	2.0 ± 0.0	3.2 ± 0.3	2.4 ± 0.4	1.6 ± 0.3	1.2 ± 0.1	1,6 ± 0,02	1,1 ± 0,04
	Intact control	2.4 ± 0.4	2.5 ± 0.04	2.3 ± 0.07	1.9 ± 0.05	1.6 ± 0.3	0,8 ± 0,1	1,1 ± 0,06

*$p < 0.05$ compared to intact control; **$p < 0.05$ compared to control of transplantation; ***p<0,05 $p < 0.05$ compared to previous observation point.

a vaccine of irradiated autologous tumor cells from short-term cell line cultures: a randomized phase 2 trial of the cancer biotherapy research group. J Immunother 2003; **26**: 367–73.

2. **Hunger RE, Brand CU, Streit M, Eriksen JA, Gjertsen MK, Saeterdal I, Braathen LR, Gaudernack G.** Successful induction of immune responses against mutant ras in melanoma patients using intradermal injection of peptides and GM-CSF as adjuvant. Exp Dermatol 2001; **10**: 161–7.

3. **Dillman R, Selvan S, Schiltz P, Peterson C, Allen K, Depriest C, McClay, Barth N, Sheehy P, De Leon C, Beutel L.** Phase I/II trial of melanoma patient-specific vaccine of proliferating autologous tumor cells, dendritic cells, and GM-CSF: planned interim analysis. Cancer Biother Radiopharm 2004; **19**: 658–65.

4. **Slingluff CL Jr, Petroni GR, Yamshchikov GV, Hibbitts S, Grosh WW, Chianese-Bullock KA, Bissonette EA, Barnd DL, Deacon DH, Patterson JW, Parekh J, Neese PY, Woodson EM, Wiernasz CJ, Merrill P.** Immunologic and clinical outcomes of vaccination with a multiepitope melanoma peptide vaccine plus low-dose interleukin-2 administered either concurrently or on a delayed schedule. J Clin Oncol 2004; **22**: 4474–85.

5. **Vorontsova AL, Kudryavets YuI.** Interferon as important element for optimization of therapy of oncological patients. Oncologiya 2000; **2**: 16–20 (In Russian).

6. **Vaishampayan U, Abrams J, Darrah D, Jones V, Mitchell MS.** Active immunotherapy of metastatic melanoma with allogeneic melanoma lysates and interferon alpha. Clin Cancer Res 2002; **8**: 3696–701.

7. **Belotskiy SM, Spivak NYa.** Interferons: biological and clinical effects. Kyiv, 2006; 287 p (In Russian).

8. **Kirkwood J.** Cancer immunotherapy: The interferon-α experience. Semin Oncol 2002: **29**: 18–26.

9. **Zhilchuk VE, BЄ, Vorontsova AL, Kudryavets YuI, Zadorozna TD, Arakova TN, Stoika RS.** Influence of neoadjuvant interferon therapy on pathomorphosis and immunochemical patterns of breast cancer. Oncologiya 2005; **7**: 157–60 (In Ukrainian).

10. **Kirkwood JM, Ibrahim JG, Sosman JA, Sondak VK, Agarwala SS, Ernstoff MS, Rao U.** High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial E1694/S9512/ C509801. J Clin Oncol 2001; **19**: 2370–80.

11. **Vorontsova AL.** Immunotropic action of interferons and perspectives of its clinical application. Immunologigiya&Allergologiya 2004; **1**: 69–70 (In Russian).

12. **Potebnya GP, Tanasienko OA, Lysovenko GS, Titova GP.** Efficacy of anticancer vaccines on the base of cytotoxic lectines of bacterial origin. Ukr Khimiother Zh 2001; **11**: 29–32 (In Russian).

13. **Potebnya GP, Khutornoi SV, Didenko GV.** Evaluation of effectiveness of antitumor vaccines obtained with the use of metabolic products of *Bacillus subtilis* AB-56. Exp Oncol 2002; **24**: 225–8.

14. **Potebnya GP, Tanasienko OA, Titova GP, Kovalenko EA, Getman EI.** Specifity and biological activity of cytotoxic lectins synthesized by *Bacillus subtilis* B-7025. Exp Oncol 2002; **24**: 150–2.

15. **Vorontsova AL, Kudryavets YuI.** Interferon as an important element for optimization of oncological patients therapy. Oncologiya 2000; **2:** 16–20.

16. **Potebnya GP, Safronova LA, Cheremshenko NL, Lysovenko GS, Sorokulova IB, Prikhod'ko VO, Trokhimko VO, Tanasienko OA, Bombin AV.** Influence of probiotic subalin on the efficacy of cancer vaccine. Microb Zh 2006; **68**: 51–8 (In Ukrainian).

17. **Potebnya GP, Lysovenko GS, Mosienko VS, Lysovenko VG.** Efficacy of combined use of cancer vaccine and immunomodulating preparation blastolen. Ukr Chemother Zh 2002; **2**: 60–4 (In Ukrainian).

18. **Rykova MP, Spiradze IV, Zedgenidze MS, Lyakhov VV, Fuks BB.** New highly sensitive technique for testing normal killer cells. Immunologiya 1981; **3**: 88–90 (In Russian).

19. **Perederiy VG, Zemskov AM, Bychkova NG, Zemskov VM.** Immune state, principles of its evaluation and correction of immune disturbance. Kyiv: Zdorov'ya, 1995: 61–2 (In Russian).

20. **Fisch H, Gifford G.** In vitro production of rabbit macrophage tumor cell cytotoxin. Int J Cancer 1983; **32**: 105–12.

21. **Haskova V, Kaslik J, Matl I, Matejckova M.** New technique of circulating immunocomplex estimation in human sera. Cas Lek Cesk 1977; **116**: 436–7.

22. **Lymphocytes.** Methods. Klaus J, ed. Moskow: Mir, 1990; 395 p (In Russian).

23. **Lakin GF.** Biometry. Moskow: Vishaya Shkola, 1980; 293 p (In Russian).

ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ ЭФФЕКТИВНОСТИ КОМБИНИРОВАННОГО ИСПОЛЬЗОВАНИЯ ПРОТИВООПУХОЛЕВОЙ ВАКЦИНЫ И ИНТЕРФЕРОНА

Цель: **исследовать в эксперименте эффективность комбинированной схемы введения противоопухолевой вакцины (ПВ) и интерферона (ИФН).** ***Материалы и методы:*** **карциному легкого Льюис (КЛЛ) трансплантировали мышам-самцам C57Bl. Для лечения использовали ПВ, приготовленную из клеток КЛЛ с помощью цитотоксических лектинов *B. subtilis B-7025*, и препарат мышиного ИФН. Терапевтический эффект оценивали путем измерения объема солидной опухоли и анализа средней продолжительности жизни опытных животных. Иммунологическое исследование включало определение противоопухолевой цитотоксичности Т-лимфоцитов (ЦТЛ) и природных киллерных клеток (ПКК) радиометрическим методом; функциональной активности перитонеальных макрофагов (Мф) в колориметрическом НСТ-тесте; определение титров фактора некроза опухоли (ФНО), интерлейкинов-1 и -2.** ***Результаты:*** **показано, что использование препарата ИФН существенно повышает эффективность вакцинотерапии солидной формы модельной КЛЛ: на 25% повышается продолжительность латентного периода, на 28% — средняя продолжительность жизни мышей, на 35–40% — индекс торможения опухолевого роста. При комбинированном применении ПВ и ИФН отмечают существенную активацию клеток-эффекторов как неспецифической (Мф), так и специфической (ЦТЛ) иммунной защиты.** ***Выводы:*** **полученные результаты свидетельствуют о перспективности разработки комбинированных схем введения ПВ с ИФН, позволяющих повысить эффективность вакцинотерапии.**

Ключевые слова: **противоопухолевая вакцина, интерферон, карцинома легкого Льюис, иммунные показатели.**