

EVALUATION OF EFFECTIVENESS OF ANTITUMOR VACCINES OBTAINED WITH THE USE OF METABOLIC PRODUCTS OF *BACILLUS SUBTILIS* AB-56

G.P. Potebnya*, S.V. Khutornoi, G.V. Didenko

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
NAS of Ukraine, Kyiv 03022, Ukraine

ОЦЕНКА ЭФФЕКТИВНОСТИ ПРОТИВООПУХОЛЕВЫХ ВАКЦИН, ПОЛУЧЕННЫХ ПРИ ПОМОЩИ ПРОДУКТОВ МЕТАБОЛИЗМА *BACILLUS SUBTILIS* AB-56

Г.П. Потебня*, С.В. Хуторной, Г.В. Диденко

Институт экспериментальной патологии, онкологии и радиобиологии им. Р.Е. Кавецкого
НАН Украины, Киев, Украина

Antitumor activity of homologous vaccines prepared with the use of metabolic products of *B. subtilis* AB-56 were studied on sarcoma-37 model. The relation of tumor growth dynamics in mice that received vaccine injections with production of antibodies to homologous vaccine antigens was revealed. Those results may be used for production of antitumor vaccines on the base of glycoprotein isolated from culture fluid of *B. subtilis* AB-56 and tumor cells, their standardization and evaluation of their effectiveness.

Key Words: *B. subtilis* AB-56, autovaccine, antitumor activity, glycoprotein, *B. megaterium* H, sarcoma-37.

На модели саркомы-37 изучали противоопухолевую активность гомологичных вакцин, приготовленных с помощью продуктов жизнедеятельности *B. subtilis* AB-56. Обнаружена связь динамики роста опухоли у мышей, получавших инъекции вакцинных препаратов, с продукцией антител к гомологичным антигенам вакцин. Результаты исследования являются основой для создания противоопухолевых вакцин на основе гликопротеида, выделенного из культуральной жидкости *B. subtilis* AB-56 и опухолевых клеток, их стандартизации и оценки их эффективности. **Ключевые слова:** *B. subtilis* AB-56, аутовакцина, противоопухолевая активность, гликопротеид, *B. megaterium* H, саркома 37.

Experimental and clinical data have evidenced the perspective of the application of immunotherapy with the use of vaccines prepared on the base of autologous (in experiment – homologous) tumor cells (TC) and metabolic products of *B. subtilis* AB-56 [1–3]. For promotion of the antitumor effectiveness of those vaccines and for standardization of their production, from the filtrate of the culture fluid (FCF) of *B. subtilis* the active fractions possessing cytotoxic properties toward TC and free from ballast compounds were isolated. It has been shown that one of those fractions is presented by glycoprotein [4]. Earlier we have shown that upon interaction of *B. subtilis* AB-56 FCF with TC the heterogeneity of TC plasma membranes is increasing as well as their immunogenicity [5, 6].

The present research was aimed on the comparative study of the efficacy and immunogenicity of vaccines prepared from sarcoma-37 cells with the use of FCF of *B. subtilis* AB-56 or glycoprotein isolated from FCF (GP-FCF).

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*Correspondence.

Abbreviations used: FCF – filtrate of culture fluid of *B. subtilis* AB-56; GP-FCF – glycoprotein isolated from filtrate of culture fluid of *B. subtilis* AB-56; GP-H – glycoprotein isolated from *B. megaterium* H; TC – tumor cells.

MATERIALS AND METHODS

In experiment Balb/c male 2 months old mice weighting 18–20 g obtained from vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine (Kyiv, Ukraine) were used. Sarcoma-37 cells possessing low immunogenicity in autologous system [7] were used as a model and were transplanted subcutaneously ($1 \cdot 10^6$ per animal). Vaccines were prepared on a base of homologous cells by standard method [1] (TC + FCF of *B. subtilis* AB-56, GP-FCF (10 μ mg/ml) + TC of *B. subtilis* AB-56). On the 2-nd day after tumor transplantation the treatment of experimental animals began. Vaccine preparations were administered triply (one injection per week) subcutaneously in the dose 0.3 ml (0.5 mg of protein) per animal. The control group of tumor-bearing animals received FCF, GP-FCF, or glycoprotein, isolated from *B. megaterium* H culture medium (GP-H) [2, 7]; *B. megaterium* H possesses properties of natural antagonist of *B. subtilis* AB-56 [1, 8]. Antitumor effectiveness of vaccines was evaluated by dynamics of tumor growth and medium longevity of life (MLL) of experimental animals. Modulation indexes (MI) were calculated with the use of equation:

$$MI = \frac{(TV_E(MLL_E) - TM_C(MLL_C))}{TV_C(MLL_C)} \cdot 100 \%$$

where TV_E — tumor volume (MLL_E) in experimental group;

TM_C — tumor volume (MLL_C) in control group.

Serum activity against antigens of FCF + TC vaccine was determined by solid phase immuno enzyme assay (SPIEA) according to [2]. SPIEA was carried out in 96–well plates (Dynatech, USA). In preliminary experiments by cross–titer of vaccine FCF + TC and antiserum against it (the sera of rabbits immunized with lyophilized vaccine preparation), sera of mice without tumors and those which received injections of all studied preparations, the antigen concentrations optimal for sensitization of solid phase were determined (50 μ g per well) as well as the appropriate titer of the used sera. Peroxidase–labelled antibodies against immunoglobulins of rabbit or mice (Amersham, UK) were used in dilution 1 : 2000; orthophenildiamin was used as a substrate. The evaluation of the reaction intensity was carried out by photometry with the use of immunoenzyme analyzer Titertek Multiskan (Finland) at the wave length 492 nm.

RESULTS AND DISCUSSION

The results of determination of growth dynamics of sarcoma–37 cells transplanted to the different groups of mice have shown nearly equal effectiveness of GP–FCF + TC vaccine in comparison with standard FCF + TC vaccine (Fig. 1, 2). Although on the 16–th day more pronounced inhibition of tumor growth was recorded in FCF + TC treated group (MI = –62% against MI = –37,5% in GP–FCF + TC treated group), on the 24–th day in GP–FCF + TC treated group MI was –78%, against MI = –49 in FCF + TC group. Such dynamics was preserved till the end of experiment (80–th day). It's necessary to note that components of FCF and GP–FCF were also able to inhibit tumor growth (on the day 32 MI were –41 and –29%, respectively). In the case when GP from *B. megaterium H* was used, up till day 28 the stimulation of tumor growth was registered (MI = 8%); later the partial tumor regression was observed (on day 40 MI = –42%), which may be explained

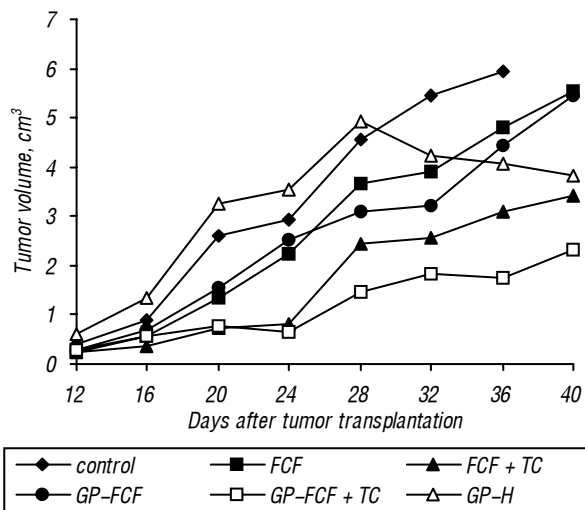


Fig. 1. Growth dynamics of sarcoma–37 in the studied groups of experimental animals

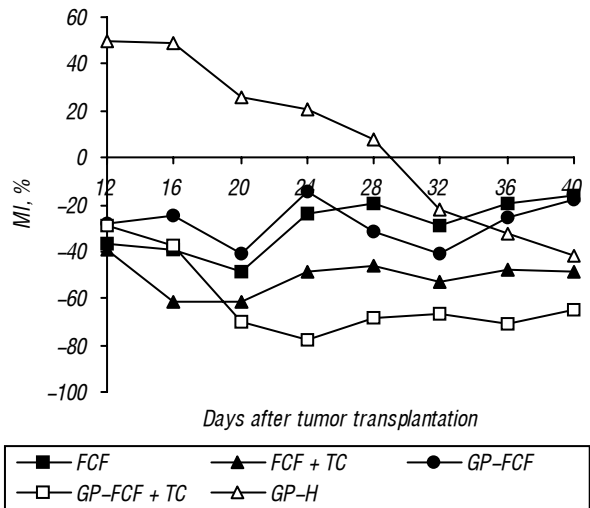


Fig. 2. Modulation index of the tumor volume in the studied groups of experimental animals

by possible existence of similar antigenic determinants in tumor cells and GP–H preparation [8].

Analysis of MLL in experimental groups of animals cured with antitumor vaccines (Table) demonstrated the equal efficacy of GP–FCF + TC and FCF + TC vaccines (MI were +124, +117%, respectively).

Table. Medium longevity of life of sarcoma 37–bearing mice from different groups

N _e	Preparation	Number of animals	MLL, days	t	p	MI for MLL, %
1	FCF	9	50.1 + 7.3	2.56	< 0.05	+ 51.8
2	FCF + TC	9	71.5 + 12.4	3.06	< 0.01	+ 116.7
3	GP–FCF	8	52.4 + 7.8	2.01	> 0.05	+ 58.8
4	GP–FCF + TC	8	74.0 + 11.9	3.39	< 0.01	+ 124.2
5	GP–H	10	44.0 + 12	1.69	> 0.05	+ 29.4
6	Control	10	33.0 + 2.2			

The increase of MLL in the groups of animals cured with FCF and GP–FCF preparations was registered, too, but MI were significantly lower (+51.8% ($p < 0.05$) and +58.8% ($p > 0.05$), respectively). It may be explained by non–specific adjuvant action of carbohydrate–containing biopolymers, according to our previous data [5].

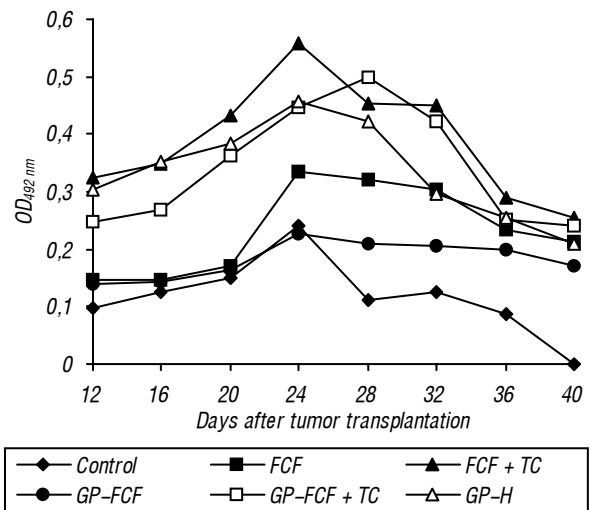


Fig. 3. Medium values of sera activity in IEA toward vaccine antigens

So one may suppose that GP–FCF–based vaccine promotes antitumor resistance of animals in the way as FCF–autologic vaccine does. In comparison with FCF of *B. subtilis* AB–56 GP–FCF + TC vaccine preparation contains smaller amount of ballast components. The results of immunoenzyme assay are presented on Fig. 3. In the control group of tumor–bearing animals that received the injections of physiologic solution of sodium chloride the activity of the sera toward vaccine antigens was significant only on the day 24 (optical density (OD) is 2–fold higher that OD of the samples in control group without tumors). On the later stages of tumor growth the antibodies level is decreasing (data are not significant).

In the group that received GP–H injections the serum activity was high beginning from 12–th day (OD = 0.302), continued to raise till day 24 (OD = 0.458) and then tended to decrease. Possibly, that effect may be caused by antigenic similarity between GP–H and blastoma cells [8]. In the groups which were cured by FCF and GP–FCF injections only light increase in the sera activity was observed; only at the 28–th day the OD reached significant values (0.311 and 0.243, respectively) with the next slow decrease. This effect may be explained by adjuvant activity of those compounds [5].

In the groups which received FCF + TC and GP–FCF + TC vaccines the increase of the OD was significant from 12–th till 24–th day (on the day 24 was 0.558 and 0.445, respectively). Upon stabilization of tumor growth at the days 28–32, the sera activity was relatively high, but later (36–40 days) tend to decrease; anyhow it's OD was 2.5–fold higher than OD of the sera of the intact animals.

In conclusion, our results point to the possibility of production of effective antitumor vaccine on the base of glycoprotein from culture medium filtrate of *B. subtilis* AB–56 and autologic tumor cells. The results of the study

of the sera activity pointed to the possibility of IEA application for evaluation of effectiveness of antitumor vaccines, especially on early stages of vaccination.

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