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THE PROBLEM OF FINDING ADJUVANTS FOR THE CONSTRUCTION ANTICANCER VACCINES BASED ON CHICK EMBRYONIC PROTEINS

Adjuvants are used in vaccines to enhance the immune response for more than 80 years. Development of adjuvants and adjuvant systems has evolved from the first empirical experiments to create targeted systems, and is caused mainly by achievements in the study of the immune system and improvement of analytical, chemical and immunological methods. Such a rapid development of this technology allows us to hope for clinical success of new adjuvant vaccines for diseases for which it was has impossible to develop effective preventive measures, such as malaria, tuberculo-

sis and HIV [1,2]. The aim of vaccination is the establishment of effective immunity in humans, providing long-lasting protection against infection. Most of the current vaccine is developed on the basis of certain antigens (AG), in contrast to a fully inactivated or attenuated pathogens. AG microorganisms, tumor cells, or allergens are introduced into the human body in the form of purified proteins. But such «molecular vaccine» may in some cases, have low immunogenicity, and to enhance the immune response by the human immune system, it's necessary to add adjuvants [3, 4].

The work was aimed on the selection of potential adjuvant for designing antitumor vaccines and study their effects on the immune system in animal experiments with Lewis lung carcinoma (LLC).

In experiment were used male Balb/c line 2-2.5 months old and average weight 18 - 20 g mice obtained from vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine. The Lewis lungs metastatic epidermoid carcinoma was used as a model of tumor growth.

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 (0,3 x 10^s CFC/injections), colloidal silver (Ag) and suspension of iron oxide (Fe_30_4) in 2% solution of polidekstran (0.06 mg/injections). For intact control (1C) were used animals injected with NaCl.

Immunological examination included: determination of cytotoxic activity and antibody-dependent cytotoxic activity of lymphocytes and macrophages, cooperative cytotoxic activity of effecter cells, cooperative antibody-depended cellular cytotoxicity of lymphocytes and macrophages, ELISA detection of generated antibodies specific to chicken embryonic proteins or tumor antigens LLC.

As the results of investigations evidenced, the introduction of chicken embryonic proteins by themselves independently, so do in combining with adjuvant caused inhibition of growth of LLC in experiments on animals. Stability of this effect remains at all stages of growth of experimental tumors. The comparative analysis of the size of primary tumors in animals from different groups at the end of the experiment (34th day) showed that in animals, who received the vaccine based on CEB with glycoproteins *B. subtilis* B-7025 tumor volume was 13% lower than in the IC . It is necessary to note that the degree of inhibition of tumor growth in terms of different tumor process was uneven.

The dynamic of growth of the LLC after interruption to the animals studied vaccines was different. In the primary stages of tumor process the interruption of all the studied substances resulted inhibition of tumor growth. Through the development of the tumor suppressive effect of the studied preparations has been gradually decreased. The most expressive effect was in animals that received the vaccine based on CEP and glycoproteins as adjuvant.

Within the immunological experiments there were established that the maximal synthesis of antibodies was observed in groups of animals, which as an adjuvant to

CEP got 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. 1). According to this we can conclude that BCG activates the cellular immunity and suppress of humeral.

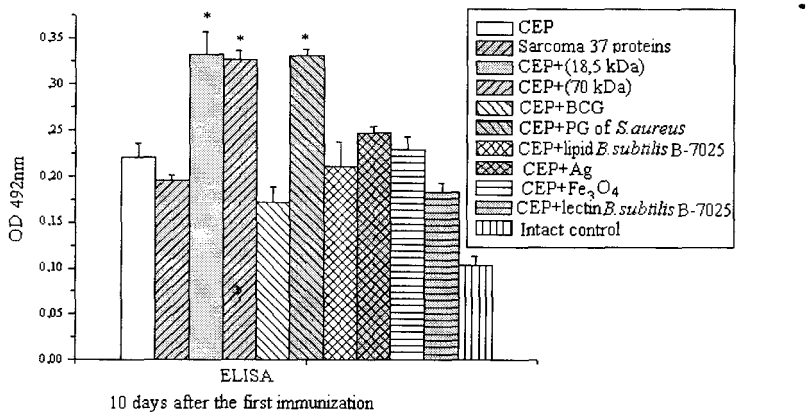

Figure 1. ELISA detection of serum in experimental groups specific to chicken embryonic proteins provided immunization CEP with adjuvants.

Similar results were also obtained in assessing the accumulation of antibodies to proteins of Sarcoma 37 (Fig. 2).

It was found that in all experimental groups, the level of medium molecular circulating immune complexes (CIC) in serum was higher compared with intact animals. In mice immunized with CEP and Fe_30_4, CIC level exceeded the same index in animals that received no adjuvant chicken embryonic proteins. Add to CEP almost all investigated adjuvant (except a mixture of lipids *B. subtilis* B-7025) led to a decrease in titer of antibodies against protein S-37. As a result of the test to determine cytotoxic activity of lymphocytes against cells S-37, demonstrates that the introduction of CEP is not likely led to its change.

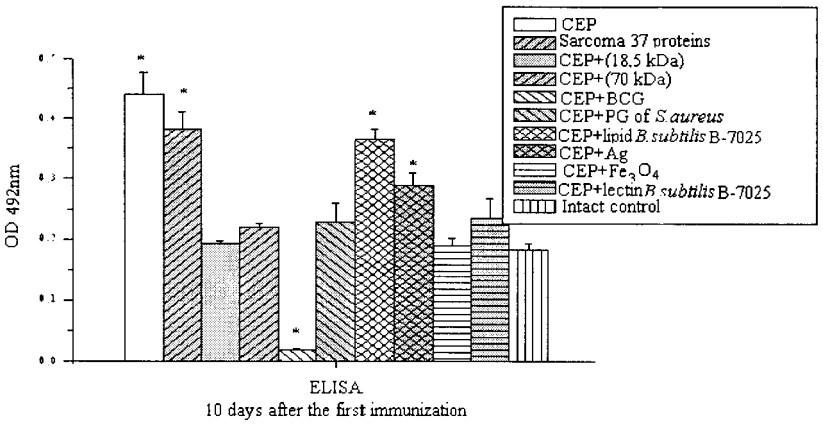

Figure 2. ELISA detection of serum proteins in experimental groups against Sarcoma 37.

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

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Проведённые исследования показали, что микрофлору влагалища у пациенток с ПТО характеризовало значительное многообразие факультативных микроорганизмов, причём в 75% случаев отмечено формирование 3-5 компонентных ассоциаций. Среди представителей условно-патогенной микрофлоры наиболее часто высевались E.Coli в 29,2+1,8% при интенсивности колонизации 5,1+0,3% IgKOE/r, что превышает нормальные значения. В 25+0,1% случаев выявлен Strep.faecalis гр. D в концентрации 5,0±0,9 IgKOE/r. Несколько реже (в* 4,2+0,2 %) высевали Staph.aureus, Staph. Epidermidis, Corynebacter.spp. и Candida., которые встречались в 12,5% случаев, причём в количестве, превышающем нормальные популяции (4,7+0,7 IgKOE/r.). В высокой концентрации высевались Enterococc.spp. и Staph. Heamolyticus (интенсивность колонизации (6,0 + 0,2 IgKOE/r.). Наряду с увеличением колонизации факультативной микрофлоры у пациенток было отмечено снижение частоты и интенсивности колонизации Lactobacill.sp. (8,3+1,7 IgKOE/r.), что объясняется изменением гормонального фона у пациенток в пери- и постменопаузе, что может приводить к увеличению обсеменения §лагапищааэробной и анаэробной микрофлорой. Микробиоценоз влагалища характеризуется большой вариабельностью количественных и качественных показателей микроорганизмов у данной группы пациенток. В результане проведённых исследований идентифицированы такие штаммы условно-патогенной микрофлоры как грибы рода Candida, кишечные стафилококки, которые потенциально могут быть причиной развития инфекционной патологии. После проведённого лечения препаратом Полижинакс зафиксировано значительное улучшение данных микробиоценоза влагалища, характеризующееся снижением уровня микробной контаминации в 2,6 раза. Причём, если до лечения интенсивность колонизации всех микроорганизмов (за исключением Citrobacter freundii) превышала нормальные значения для данных микроорганизмов и колебалась от 5,0 до 6,0 IgKOE/r., то после проведённого лечения в 50% случаев концентрация микроорганизмов не превышала 2,0-3,0 IgKOE/r (рис.1). Следует подчеркнуть, что стрептококк, стафилококк и кишечная палочка после проведённой терапии не высевались.

Эффективность проводимой терапии также оценивали по характеру течения послеоперационного периода: температурной реакции, качественной и количественной оценке отделяемого из влагалища, макроскопической оценки послеоперационных швов, длительности послеоперационного койко-дня. Нормальная температура в послеоперационном периоде была у 43,5% (30) женщин, у остальных 56,5% (38) пациенток - субфебрильная температура в течении, в среднем, 2,1+1,1 дней. Макроскопически швы в области промежности с признаками слабо выраженного перифокального отёка (что соответствовало операционной травме тканей промежности и стенок влагалища), который, однако, не выявлялся уже к 3-м суткам послеоперационного периода. Следует также отметить, что в течение первых двух суток мы оставляем во влагалище, введённый сразу после операции, тугой марлевый тампон с левомеколем с целью профи-