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## THE EFFECTS OF IMMUNIZATION WITH XENOGENEIC EMBRYO PROTEINS ON LYMPHOCYTES FUNCTIONS IN MICE BEARING LEWIS LUNG CARCINOMA

**Key Words:** *xenogeneic cancer vaccine, chicken embryo proteins, lymphocytes functioning, Lewis lung carcinoma.*

*Different types of cancer vaccines are elaborated so far and researches in this field are going on. In this experiment, chicken embryo proteins (CEP) as a potential source of antigens for a future xenogeneic vaccine was utilized. Aim: to investigate the effects of CEP on lymphocytes' activity in mice bearing Lewis lung carcinoma (LLC). Materials and Methods: C57Bl male mice were immunized on days 1, 8, and 15 after challenge with LLC cells. The immune response was assessed on days 7, 14, 21 and 28 after tumor transplantation. Cytotoxic activity of natural killer (NK) cells and cytotoxic T-lymphocytes as well as antibody dependent cellular cytotoxicity (ADCC) was estimated in MTT-assay; interferon  $\gamma$ , interleukin (IL)-4, IL-10 levels in the blood serum were detected in ELISA; lymphocyte proliferation was studied in reaction of in vitro blast transformation. Results: in mice bearing LLC tumor, immunization with CEP increased NK, cytotoxic T-lymphocytes and ADCC cytotoxic activities, as well as raised the level of lymphocytes blast-transformation in response to LLC-antigens. Our findings indicate that CEP showed activating effects on lymphocytes, providing an insight into the potential of CEP to elicit anticancer immune response. CEP proved to be a feasible source of antigens which can be utilized in xenogeneic cancer vaccines engineering. Conclusion: it was shown that CEP immunization positively influenced lymphocytes activity in mice bearing LLC tumor eliciting both specific and innate anticancer immune responses. NK cells may play a front role in the activation processes induced by immunization with CEP.*

Immunotherapy can significantly improve treatment of cancer patients. Among different immunotherapeutic modalities, cancer vaccines are considered as relatively safe and effective therapy compared to other methods of cancer treatments [1]. Different kinds of cancer vaccines have been designed by now. Independently of the vaccine type — whether it is whole-cell, peptide/protein, DNA/RNA or dendritic cell based — the main goal of its application is to elicit immune response targeted on tumor antigens.

Predominantly cancer vaccines are designed to elicit immune reactivity to so called tumor associated antigens (overexpressed self-proteins, cancer-testis, cancer-embryo or cancer-differentiation antigens), tumor specific antigens (of viral origin or resulted from somatic mutations in cancer cells) or to (less frequently) proteins involved in tumor progression (i.e. matrix metalloproteinases, angiogenic factors, enzymes). Nevertheless, there is a huge obstacle to this goal: the overwhelming majority of the tumor-associated antigens are self-antigens that are weakly immunogenic to the patient's immune system. One of the possible ways to omit this obstacle is xenogeneic cancer vaccines, which utilize xenogeneic homologous proteins or gens as an antigen part of the vaccine. Minor differences between self and xenogeneic antigens enable the latter to break immune tolerance towards tu-

mor associated antigens through inducing cross-reactive immune reactions [2].

Besides being more immunogenic, xenogeneic cancer vaccines have some other features making them attractive for further investigations. Namely, as long as they are not autologous and therefore are not based on the patient's own antigens, the volume of tumor tissue, which is appropriate for the vaccine preparation, does not limit the volume of vaccine production. As long as xenogeneic cancer vaccines can be manufactured in large-scale, the number of immunization rounds and patients treated are not limited. This type of vaccines can be targeted at virtually any crucial cancer protein; therefore, xenogeneic vaccines can be used in a wider range of patients. Considering abovementioned, xenogeneic vaccines are attractive for developers.

In the previous research, it was shown that immunization with chicken embryo proteins (CEP) (tested as a source of antigens for future xenogeneic anticancer vaccine) has antitumor activity on Ehrlich and Lewis lung carcinoma (LLC) models [3, 4]. On Ehrlich carcinoma model, immunization with CEP induced CEP- and Ehrlich carcinoma specific antibodies and elicited macrophages cytotoxic activity (both direct and antibodies-dependent) [4]. The scope of this research is to examine effects of immunization with CEP on lymphocytes' functions in mice bearing LLC.

## OBJECT AND METHODS

**Animals.** The study has been carried out on male C57Bl mice 2–2.5-month-old weighting 19–20 g, bred at the vivarium of RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology. The use and care of experimental animals have been performed in accordance with standard international rules on biologic ethics and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [5] and was approved by Institutional Animal Care and Use Committee.

**Preparation of CEP.** CEP was prepared as reported in [6]. Briefly, 7-days chicken embryos were rinsed two times in cold NaCl 0.9% solution, homogenized and then extracted with NaCl 0.9% solution, containing 0.1% EDTA, for 60 min at 4 °C by agitation. Following extraction, chicken embryo tissue was removed by centrifugation at 500 g for 30 min. The resulting supernatant was collected and frozen at -20 °C. Concentration of proteins in the extract was measured by Greenberg and Craddock assay. The same extract was used in all the experiments described in the article.

**Preparation of LLC antigens (LLC-Ag).** Antigens of LLC were prepared by three consecutive cycles of freezing and melting of cells suspension. Following the last melting, cell debris was removed by centrifugation at 500 g for 30 min. The resulting supernatants were collected and frozen at -20 °C. Concentration of proteins in the extract was measured by Greenberg and Craddock assay.

**The scheme of the experiment.** To establish tumors, C57Bl mice (3 mice per one observation point per group, 24 in total) were injected with  $4 \cdot 10^5$  cells/mouse of LLC cells *i.m.* into the right hind leg. Tumor-bearing mice were randomly divided in two groups (12 animals per group), one of which received immunizations with CEP; another group with no immunization is referred as the tumor-bearing control. Immunizations were performed on day 1, 8 and 15 after the tumor challenge. Lymphocytes activity was checked on day 7, 14, 21 and 28 after the tumor cells transplantation.

The data of the immunized mice were compared with the unimmunized tumor-bearing control and with the intact mice of the same strain, sex and age (is referred as the intact control (8 mice per experiment)).

Immunizations were performed *s.c.* with 0.3 ml of CEP solution per mouse (protein concentration 0.3 mg/ml).

**Cytotoxic activity (CTA) assay.** CTA of spleen lymphocytes was determined by MTT-assay [7]. K-562 cells were used as targets for the examination of natural killer (NK) cells' CTA, while LLC cells were used as targets for cytotoxic T-lymphocytes (CTL) and antibody dependent cellular cytotoxicity (ADCC).

In brief, target cells ( $2 \cdot 10^4$  cells/well) and immune cells ( $1 \cdot 10^5$  lymphocytes/well), in RPMI medium supplemented with 10% fetal bovine serum (all reagents from Sigma, USA) and antibiotics, were placed in a flat-

bottom 96-well plate and incubated for 18 h in a 100% humidity atmosphere with 5% CO<sub>2</sub> at 37 °C. After that, 0.01 ml of MTT solution/well (5 mg/ml, Sigma, USA) was added, and incubation continued for 2 h. Then the plates were centrifuged (500 g for 15 min) and washed twice with 0.9% NaCl solution. After all, 0.12 ml of KOH (2 mole/liter) and 0.14 ml of DMSO (50% solution) were added into each well. Optical density was measured at  $\lambda = 545$  nm vs  $\lambda = 630$  nm with a micro ELISA reader (StatFax-2100, USA). Each sample was measured in triplicate.

CTA index (CTAI, %) was calculated by the formula:

$$CTAI = [1 - (OD_{lc+tc} - OD_{lc}) / (OD_{tc} - OD_{blank})] \cdot 100\%$$

where OD<sub>lc</sub> — optical density of wells in which only lymphocytes were incubated; OD<sub>tc</sub> — optical density of wells in which only target cells were incubated; OD<sub>lc+tc</sub> — optical density of wells in which tumor cells together with lymphocytes were incubated; OD<sub>blank</sub> — optical density of wells with the culture medium only.

In order to determine ADCC activity, 0.01 ml/well of autologous blood serum was added to target containing wells and preincubated for 30 min. After that, lymphocytes were added to the wells and all the other steps were the same as is described above.

**Lymphocytes blast-transformation assay.** Lymphocytes were obtained from aseptically removed lymph nodes by homogenizing with Potters homogenizer. Aliquots of lymphocytes ( $2 \cdot 10^6$  cells/ml) in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 1% gentamicin were transferred to flat-bottom plates 200  $\mu$ l/well and stimulated by 15  $\mu$ g protein/well of the LLC cells extract (LLC-Ag) or by the 10  $\mu$ g protein/well of concanavalin A (ConA, Sigma, USA) or left without stimulation (spontaneous reaction). The plates were incubated for 2 days at 37 °C with 5% CO<sub>2</sub> atmosphere. The degree of response was determined by the percentage of transformed cells counted per 100 cells. LLC-Ag was prepared as described above.

The stimulation index (SI) was calculated as following:

SI = percentage of transformed cells induced by LLC-Ag or ConA stimulation / percentage of transformed cells in wells without stimulation.

**Cytokines detection in blood serum.** Interleukin (IL)-4, IL-10 and interferon- $\gamma$  (IFN- $\gamma$ ) concentration in the blood serum was analyzed with the help of appropriate BD OptEIA (BD Biosciences, USA) kit according to the manufacturer's recommendations.

**Statistics.** The data are presented as mean value  $\pm$  standard error ( $M \pm m$ ). The statistical analysis was made using Student t-test. The difference was considered as significant when  $p < 0.05$ ;  $p$  value higher 0.05 but lower 0.1 ( $0.05 < p < 0.1$ ) was treated as a tendency. The correlation analysis was made by Pearson correlation coefficient.

## RESULTS AND DISCUSSION

To examine how immunization with CEP influences lymphocytes' activity in a tumor-bearing host, C57Bl

mice were injected with LLC cells on day 0 of the experiment. On days 1, 8 and 15, half of the LLC bearing mice were immunized with CEP. Lymphocytes functions of immunized, control tumor-bearing and intact mice were checked on days 7, 14, 21 and 28 after the tumor challenge.

Immunization with CEP affected the lymphocytes activity of both natural and adaptive immune responses.

NK CTA of both the CEP immunized and the tumor-bearing control groups was higher than that of the intact mice on days 7–28 of tumor growth (Fig. 1). However, on day 7 of the observation, NK CTA of the immunized mice was even 1.6 times higher compared to the tumor-bearing control mice ( $p < 0.05$ ).

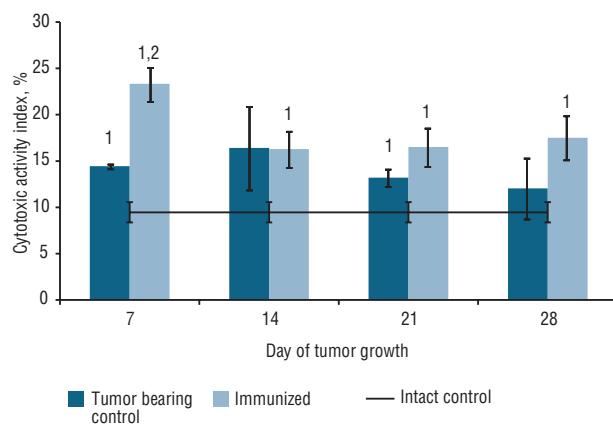
Specific anticancer immune response of the unimmunized tumor-bearing mice was evidently suppressed. CTA of CTL in this group of mice (Fig. 2) was lower than that in the intact control group ( $p < 0.05$  on day 14 and 28 of tumor growth). For example, on day 28 of tumor growth CTL CTA was by 3.3 and by 2.6 times lower in this group than in the intact and the CEP-immunized groups respectively ( $p < 0.05$  in both cases). ADCC in the group (Fig. 3) was lower than in the intact group during almost the entire experiment ( $p < 0.05$  on days 7, 14 and 28).

In the group of immunized mice, CTA of CTL (Fig. 2) followed a completely different pattern: it did not differ from the intact control level on day 7 after the tumor injection but sharply increased on day 14 ( $p < 0.05$  as compared to the intact control and the data on day 7 of the investigation). On day 21 and 28 of tumor growth, CTA of CTL in the immunized mice slightly decreased but remained significantly higher than that in the control tumor-bearing group. Lymphocytes' ADCC (Fig. 3) of the immunized mice was higher compared to both the intact ( $p < 0.05$  on day 21) and the tumor-bearing control ( $p < 0.05$  on days 14, 21 and 28) groups.

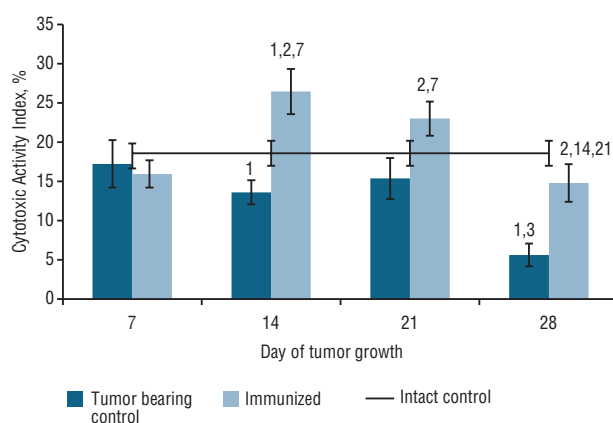
Lymphocytes proliferative response on LLC-Ag was elevated in the group of immunized mice. On day 14 after the tumor transplantation, lymphocytes blast-transformation in response to LLC-Ag as well as the SI of LLC-Ag induced to spontaneous blast-transformation increased and were significantly higher than that in the intact control group and in the previous time point (table 1). On the contrary, lymphocytes of the control tumor-bearing mice did not respond to LLC-Ag and the SI of LLC-Ag induced to spontaneous blast-transformation was lower ( $p < 0.05$  on day 7) or did not differ significantly from the intact control data. These findings go in line with the suppression of specific anticancer immune response in this group of mice.

Lymphocytes blast-transformation in response to ConA (as a general measure of functional T-cell competence [8]) in the group of immunized mice was preserved throughout the entire time of observation, while in the control group it significantly decreased on day 28 of tumor growth (as compared to the intact control data and to all the previous time points of observation).

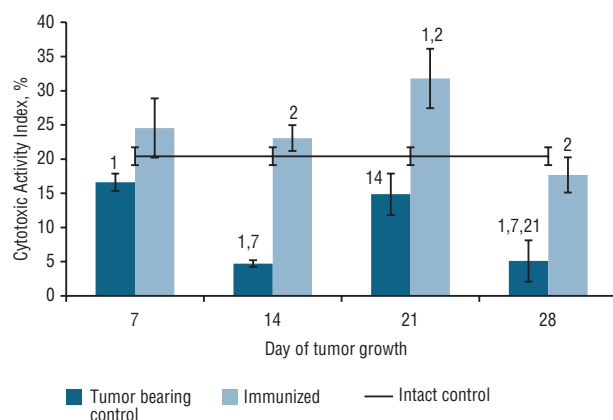
The spontaneous lymphocytes blast-transformation of the immunized and the control tumor-bearing mice



**Fig. 1.** Natural killer cells' cytotoxic activity of the immunized with CEP and the control LLC-bearing C57Bl mice: 1 —  $p < 0.05$  compared to the intact control; 2 —  $p < 0.05$  compared to the control tumor-bearing group



**Fig. 2.** T-lymphocytes' cytotoxic activity of the immunized with CEP and control LLC-bearing C57Bl mice: 1 —  $p < 0.05$  compared to the intact control; 2 —  $p < 0.05$  compared to the control tumor-bearing group; 3 —  $p < 0.05$  compared to all the previous results in the group; 7 —  $p < 0.05$  compared to the data on day 7 in the group; 14 —  $p < 0.05$  compared to the data on day 14 in the group; 21 —  $p < 0.05$  compared to the data on day 21 in the group



**Fig. 3.** Lymphocytes' antibody dependent cellular cytotoxicity in the immunized with CEP and control LLC-bearing C57Bl mice: 1 —  $p < 0.05$  compared to the intact control; 2 —  $p < 0.05$  compared to the control tumor-bearing group; 7 —  $p < 0.05$  compared to the data on day 7 in the group; 14 —  $p < 0.05$  compared to the data on day 14 in the group; 21 —  $p < 0.05$  compared to the data on day 21 in the group

followed the same pattern: statistically significantly increased (as compared to the intact control mice) on day 7, returned to the intact control level on days 14 and 21, and increased again on day 28 after the tumor challenge. Increase in spontaneous blast-transformation is characteristic for tumor-bearing hosts [9]. However, in the group of control tumor-bearing mice, the increase in spontaneous blast-transformation was accomplished with significant decrease in SI that may indicate lymphocytes exhaustion; in the immunized group of mice, this effect was seen only on day 28 of tumor growth. Moreover, the lymphocytes blast-transformation is one of the early steps beginning formation of the immune response. Indeed, in the group of immunized mice CTL CTA correlated with the level of LLC-Ag induced blast-transformation ( $r = 0.58$ ,  $p = 0.07$ ) and with the SI of LLC-Ag induced to spontaneous blast-transformation ( $r = 0.67$ ,  $p = 0.03$ ). In the control group of mice, there were no such relations.

The levels of some cytokines in the blood serum of the immunized with CEP and the control tumor-bearing mice are presented in the table 2.

On day 7 after the tumor challenge, the level of IFN- $\gamma$  in both the immunized and the control tumor-bearing groups was higher than in the intact control, but due to high intragroup individual variability the difference be-

tween the groups was not statistically significant. It worth mentioning, nonetheless, that the IFN- $\gamma$  level in the group of immunized mice exceeded the intact mice IFN level by 9.4 times ( $p = 0.08$ ), and IFN level in the control tumor-bearing group by 1.9 times. What is more, in the immunized group of mice not only the IFN level was increased, but its ratio to IL-4 as well. It reached 81.7 in the CEP group, whereas in the control tumor-bearing group IFN/IL-4 ratio made 36.9, and in the intact group it was only 10.2.

IL-4 level in both treatment groups did not differ significantly from the intact control data. IL-10 level in both the immunized and the control tumor-bearing mice was significantly higher than that in the intact control group over the entire time of the experiment (see table 2). There was no significant difference between IL-10 level in the immunized and the control tumor-bearing group. However, in both groups IL-10 level was highly variable among individuals and correlated closely with IL-4/IFN- $\gamma$  ratio:  $r = 0.80$  ( $p = 0.007$ ) and  $r = 0.86$  ( $p = 0.002$ ) for the control tumor-bearing and the immunized groups respectively. Moreover, in the control tumor-bearing group the IL-10 level inversely correlated with spontaneous lymphocytes blast-transformation ( $r = -0.56$ ,  $p = 0.08$ ).

Table 1

Lymphocytes blast-transformation in *in vitro* reaction of the immunized with CEP, unimmunized LLC-bearing control and intact C57Bl mice

Group	Days of tumor growth	Blast-transformation, (number of blasts,%)			Stimulation Index	
		Spontaneous	In response to:		ConA/ spontaneous	LLC-Ag/ spontaneous
			ConA	LLC-Ag		
Intact control		16.63 $\pm$ 0.7	41.0 $\pm$ 1.8	24.5 $\pm$ 2.8	2.6 $\pm$ 0.1	1.5 $\pm$ 0.2
Tumor bearing control	7	<b>28.7 <math>\pm</math> 4.1<sup>1</sup></b>	37.0 $\pm$ 4.6	20.3 $\pm$ 4.9	<b>1.4 <math>\pm</math> 0.4<sup>1</sup></b>	<b>0.7 <math>\pm</math> 0.1<sup>1,3</sup></b>
	14	<b>15.7 <math>\pm</math> 1.8<sup>7</sup></b>	49.3 $\pm$ 3.5	28.3 $\pm$ 6.0	3.2 $\pm$ 0.3	1.9 $\pm$ 0.7
	21	<b>14.0 <math>\pm</math> 2.0<sup>7</sup></b>	41.1 $\pm$ 1.5	27.0 $\pm$ 2.0	2.3 $\pm$ 0.9	1.8 $\pm$ 0.4
	28	<b>21.8 <math>\pm</math> 0.8<sup>1,14,21</sup></b>	<b>31.4 <math>\pm</math> 1.4<sup>1,3,14,21</sup></b>	22.8 $\pm$ 1.4	<b>1.4 <math>\pm</math> 0.04<sup>1</sup></b>	1.1 $\pm$ 0.1
Immunized	7	<b>26.7 <math>\pm</math> 2.9<sup>1</sup></b>	46.3 $\pm$ 3.6	28.0 $\pm$ 2.6	1.8 $\pm$ 0.4	1.1 $\pm$ 0.2
	14	15.3 $\pm$ 1.8	49.0 $\pm$ 3.1	<b>40.0 <math>\pm</math> 5.2<sup>1,7</sup></b>	<b>3.2 <math>\pm</math> 0.2<sup>1*</sup></b>	<b>2.6 <math>\pm</math> 0.2<sup>1,7</sup></b>
	21	19.7 $\pm$ 3.3	39.7 $\pm$ 3.5	32.7 $\pm$ 4.6	2.1 $\pm$ 0.5	1.7 $\pm$ 0.4
	28	<b>24.7 <math>\pm</math> 2.9<sup>1</sup></b>	39.7 $\pm$ 1.2	24.5 $\pm$ 3.4	<b>1.5 <math>\pm</math> 0.2<sup>1</sup></b>	1.0 $\pm$ 0.1

1 –  $p < 0.05$  compared to the intact control;

1\* –  $0.05 < p < 0.1$  compared to the intact control;

3 –  $p < 0.05$  compared to the immunized group;

7 –  $p < 0.05$  compared to the data obtained on day 7 in the group;

14 –  $p < 0.05$  compared to the data obtained on day 14 in the group;

21 –  $p < 0.05$  compared to the data obtained on day 21 in the group.

Table 2

Level of some cytokines in the blood serum of immunized with CEP and control LLC-bearing C57Bl mice

Group	Cytokine, (pkg/ml)	Days of tumor growth			
		7	14	21	28
Intact control	IL-4	13.60 $\pm$ 4.3			
	IL-10	0 $\pm$ 0			
	IFN- $\gamma$	139.4 $\pm$ 47.2			
Tumor-bearing control	IL-4	18.6 $\pm$ 2.9	16.8 $\pm$ 2.1	8.7 $\pm$ 1.8	14.6 $\pm$ 2.1
	IL-10	<b>8.3 <math>\pm</math> 4.1<sup>1*</sup></b>	<b>56.4 <math>\pm</math> 8.6<sup>1</sup></b>	37.4 $\pm$ 26.0	<b>45.4 <math>\pm</math> 19.2<sup>1*</sup></b>
	IFN- $\gamma$	684.0 $\pm$ 300.1	199.1 $\pm$ 12.3	186.6 $\pm$ 67.2	312.6 $\pm$ 118.6
Immunized	IL-4	16.0 $\pm$ 5.9	13.3 $\pm$ 2.6	13.4 $\pm$ 2.3	16.4 $\pm$ 4.7
	IL-10	<b>38.9 <math>\pm</math> 16.1<sup>1*</sup></b>	<b>54.0 <math>\pm</math> 12.2<sup>1</sup></b>	73.9 $\pm$ 44.7	<b>13.6 <math>\pm</math> 11.1<sup>1</sup></b>
	IFN- $\gamma$	<b>1303.0 <math>\pm</math> 484.0<sup>1*</sup></b>	<b>140.4 <math>\pm</math> 14.1<sup>2</sup></b>	164.9 $\pm$ 17.2	194.4 $\pm$ 31.9

1 –  $p < 0.05$  compared to the intact control;

1\* –  $0.05 < p < 0.1$  compared to the intact control;

2 –  $p < 0.05$  compared to the control tumor-bearing group;

2\* –  $0.05 < p < 0.1$  compared to the control tumor-bearing group.

Until now there has been no consensus among oncoimmunologists on which immune assay most accurately predicts clinical effectiveness of immunotherapy and the cancer vaccinotherapy in particularly [10, 11], but it became evident that functional assessments are needed to fully characterize the effects of the vaccine. «In the past 10 years an increasing number of trials have included well designed and carefully performed immunologic monitoring, including multiple functional assessments of CD<sup>8+</sup> and CD<sup>4+</sup> T cells, NK cells, and antibody responses» [10]. Therefore, we considered whether immunization with CEP effects three major functions of lymphocytes — proliferation, CTA and cytokine production.

As it was elucidated, immunization with CEP positively affected all tested functions of lymphocytes. First of all, in the immunized group, NK CTA was increased during the whole experiment ( $p < 0.05$  compared to the intact control level), with the peak on day 7 after the tumor challenge ( $p < 0.05$  compared to both the intact control and the tumor-bearing control). The maximum NK CTA synchronized with the maximum IFN- $\gamma$  concentration in blood serum which exceeded the intact mice IFN level by 9.4 times ( $p = 0.08$ ). It is broadly accepted that early IFN- $\gamma$  is necessary for Th1 polarization [12]. Even more, for an effective immune response to be induced, NK and dendritic cells (DC) cooperation is of crucial importance [13] as long as activated NK cells induce maturation and type-1 polarization of DC, which results in greatly enhanced levels of IL-12 production and superior inducing of functional tumor-specific CTLs [14]. This NK cells' helper role in DC maturation depends on cell-to-cells interactions together with IFN and tumor necrosis factor production [15]. Moreover, activated NK cells can directly interact with T-lymphocytes, co-stimulate proliferation of Ag-specific T-lymphocytes and production of IFN- $\gamma$  by CD<sup>4+</sup> lymphocytes [16]. At least some of these interactions could influence induction of specific antitumor response in the immunized group of mice. Indeed, specific anticancer immune reactions were elicited only in the immunized group. The activation of specific anti-LLC immune response became evident since day 14 of tumor growth and included increase in lymphocytes proliferation in response to LLC antigens and specific cytotoxic reactions towards LLC cell. Compared to the unimmunized tumor-bearing mice, lymphocyte proliferation induced with LLC-Ag was by 94.4, 49.7, and 163.4% higher on day 14, 21 and 28 respectively; CTL CTA was by 94.9, 49.4 and 164.3% higher (on day 14, 21 and 28 respectively,  $p < 0.05$ ); ADCC was by 388.9, 114.0 and 246.5% higher on day 14, 21 and 28 respectively.

On the other hand, in the unimmunized tumor-bearing mice, specific anticancer immune response was evidently suppressed because CTL CTA in this group of mice was lower than that in the intact control group ( $p < 0.05$  on day 14 and 28 of tumor growth) and lymphocytes ADCC was lower than in the intact group during almost the entire experiment ( $p < 0.05$  on days 7, 14 and

28). Moreover, spontaneous blast-transformation of the control mice's lymphocytes was negatively linked with the increase in IL-10 in blood serum ( $r = -0.56$ ,  $p = 0.08$ ), that points to the formation of immunosuppressive milieu in this group of mice. Furthermore, the NK cells CTA and IFN- $\gamma$  production were significantly less pronounced than that in the immunized group of mice.

Thus, immunization with CEP elicited specific anticancer immune reactions, which could be induced due to the early activation of NK cells. Which pathways could underlay NK activation? NK cells are known to express a number of surface receptors interacting with embryonic or xenogeneic antigens. For example, receptor Nkp46 recognizes xenogeneic target cells [17], receptor DNAM-1 recognizes human melanoma cells with characteristics of cancer stem cells [18], ligands for receptor NKG2D are highly expressed, among others, in embryonic tissues [19]. Nevertheless, there is no information these receptors trigger NK cell's activation after cross-linking of soluble ligands (which the CEP are). Another route of NK activation consists in their Fc-receptors (mainly CD16) cross-linking of antibody-antigen complexes. Even more, in some conditions, as parallel stimulation of NKG2D and CD16 receptors or combined IL-2/IL-18 stimulation, NK cells can gain APC-like properties [16, 20], and therefore can serve as a bridge between innate and adaptive immunity. It is known that LLC-cells naturally express ligands for NKG2D receptor [21]. On the other hand, in our previous experiments, it was shown that there are CEP-reacting antibodies in the blood serum of unimmunized mice bearing different tumor strains [22]. Thus, the assumption that NK cells were activated through interaction with CEP-antibodies complexes sounds plausible. For now, it cannot be definitely concluded which way (or combinations) elicited NK cells' response to CEP immunization and this issue remains to be elucidated. The results of the research may have practical applications. If the activation depends on CEP-antibodies complexes, the level of CEP-specific antibodies can be used for screening of responsive and unresponsive patients to CEP-based immunotherapy.

## CONCLUSION

In general, it was shown that CEP immunization positively influenced lymphocytes activity in mice bearing LLC tumor eliciting both specific and innate anticancer immune responses. It seems that NK cells may play a front role in the activation processes induced by immunization with CEP. Although by now the precise ways of NK cells' activation with CEP are not clear. The answer to this issue could have impact on ways of developing xenogeneic anticancer vaccines based on CEP.

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## ВПЛИВ ІМУНІЗАЦІЇ КСЕНОГЕННИМИ ЕМБРІОНАЛЬНИМИ ПРОТЕЇНАМИ НА АКТИВНІСТЬ ЛІМФОЦИТІВ У МИШЕЙ З КАРЦИНОМОЮ ЛЕГЕНІ ЛЬОЇС

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**Резюме.** Нині вже розроблено різні типи протипухлинних вакцин, та дослідження в цьому напрямку тривають. У нашому дослідженні в якості джерела антигенів для конструювання потенційної ксеногенної вакцини було використано ембріональні протеїни курки (ЕПК). **Мета:** дослідити вплив ЕПК на активність лімфоцитів у мишей з карциномою легені Льоїс. **Об'єкт і методи:** мишам-самцям лінії C57Bl/6 на 1-шу, 8-му та 15-ту доби після перещеплення клітин карциноми легені Льоїс (КЛЛ) вводили ЕПК. Імунну відповідь оцінювали на 7-му, 14-ту, 21-шу та 28-му доби після перещеплення пухлини. Цитотоксичну активність натуральних кілерних клітин (НKK), цитотоксичних T-лімфоцитів та антитіло-залежну клітинну цитотоксичність лімфоцитів досліджували в МТТ-тесті; рівень інтерферону- $\gamma$ , інтерлейкіну (ІЛ)-4 та ІЛ-10 в сироватці крові визначали за допомогою імуноферментного аналізу; проліферацію лімфоцитів вивчали за допомогою реакції бласт-трансформації *in vitro*. **Результати:** введення ЕПК мишам з КЛЛ сприяло підвищенню цитотоксичної активності НKK, цитотоксичних T-лімфоцитів та антитіло-залежної клітинної цитотоксичності, а також посилювало бласт-трансформацію лімфоцитів у відповідь на антигени КЛЛ. Наші результати вказують, що введення ЕПК мало активуючий вплив на лімфоцити, що засвідчує здатність ЕПК викликати протипухлинну імунну відповідь. Було показано, що ЕПК є потенційним джерелом антигенів, які можуть бути використані під час розробки ксеногенних протипухлинних вакцин. **Висновок:** імунізація ЕПК мала позитивний вплив на активність лімфоцитів у мишей з КЛЛ, викликаючи як специфічну, так і вроджену протипухлинну імунну відповідь. Провідну роль у процесах активації, які були індуковані введенням ЕПК, можуть відігравати НKK.

**Ключові слова:** ксеногенна протипухлинна вакцина, ембріональні протеїни курки, функціональна активність лімфоцитів, карцинома легені Льоїс.

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