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THE INFLUENCE OF PSYCHOEMOTIONAL STATUS ON METASTASIS OF LEWIS LUNG CARCINOMA AND HEPATOCARCINOMA-29 IN MICE OF C57BL/6J AND CBA/LAC STRAINS

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Aim: **To study the influence of psychoemotional status on the development of experimental lung metastases of strain-specific murine Lewis lung carcinoma in C57BL/6J mice and hepatocarcinoma-29 in CBA/Lac male mice.** ***Materials and Methods:*** **Sensory contact model was used for generating animals with repeated experience of social victories or defeat in daily agonistic interactions. Tumor cells were injected into the tail vein after 20 days of agressive confrontations and the number of metastases in the lung was calculated 16 days later.** ***Results:*** **The experimental metastasis is shown to develop differently in mice with opposing social experience: the winners of both strains had significantly less metastases in the lung than the losers.** ***Conclusion:*** **The results obtained indicate that psychoemotional status affects Lewis lung carcinoma and hepatocarcinoma-29 metastasis in male mice.**

Key Words: **psychoemotional status, sensory contact model, Lewis lung carcinoma, hepatocarcinoma-29, mice, metastasis.**

As it is known, oncological diseases promote a negative emotional background in humans and therefore it is important to know what effect this background may have on tumor growth and metastasis. To address the problem, we proposed in the early 1990s an experimentally developed model, which yields mice with alternative psychoemotional status — winners and losers [12]. It was shown that repeated experience of social victories or defeat in daily agonistic interactions leads to the formation of aggressive or submissive behaviors in male mice, which are accompanied by the positive and negative psychoemotional state, respectively [4, 12]. Experiments performed earlier on the murine strain-nonspecific transplantable Krebs-2 carcinoma showed that tumor grew differently in the losers and winners [9]. Later, using syngeneic Lewis lung adenocarcinoma it had been shown that when the tumor was transplanted to the mice after the termination of confrontations, the tumor growth rate was the highest in the animals-losers, which metastasis rate was 1.5 times higher than in the controls and nearly two times higher than in the winners [10]. In the present research, to study the influence of psychoemotional status on the development of experimental lung metastases, tumor cells (hepatocarcinoma-29 (H-29) for CBA/Lac mice and Lewis lung carcinoma (LLC) for C57BL/6J mice) were injected into the tail vein of mice with already established social status of either being the winner or the loser.

MATERIALS AND METHODS

Experimental animals. Mice of C57BL/6J (C57) and CBA/Lac (CBA) strains were bred and kept under the standard vivarium conditions at the Institute of Cytology and Genetics, Siberian Division, Russian Academy of Sciences. Mice were housed on a 12 h/12 h light/dark regimen and received the standard food (pellets) and water *ad libitum.* Weaned at the age of one month, the males were housed before the experiment in single litter groups of 8–10 individuals in 36 x 23 x 12 cm cages. Mice used in experiments were 10–12 weeks old. All experimental procedures were in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Technique for generation of aggressive and submissive behaviors in male mice. Winners and losers were generated using the sensory contact model as described in [13]. In breef, pairs of animals were placed in steel cages divided in two compartments by a perforated transparent partition allowing the animals to see, hear and smell their neighbour, but not to contact them physically. Test sessions commenced 2 days after adaptation of the animals to these new housing conditions (sensory contact). Every afternoon the steel cover of the cage was replaced by a transparent one, and 5 min later (the period needed for adaptation to the lighting condition) the partition was removed for 10 min to allow agonistic interactions. Superiority of one of the partners was evident within 2–3 daily test sessions. Agonistic interactions were discontinued if intensive attacks lasted more than 3 min. Every day after the test session, each defeated mouse was placed in another two compartment cage with a partition, in which another winner was present in the other compartment. The winners remained in their own compartments. The procedure yielded equal numbers of males with an opposite social experience of aggression, evidenced by victories (aggressors, winners) and defeats (defeated mice, losers) in agonistic interactions. In this experiment, the control group was represented by the males that lived together for a long time in groups with stable formed dominant–subordinate relationships. It was shown that, irrespective of the psychoemotional status (dominant or subordinate), animals in such groups are not stressed [2].

Tumor models and animal's treatment. Hepatocarcinoma-29 (H-29) originally arose spontaneously in a CBA mice and after a series of s. c. transplanta-

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Abbreviations used: H-29 – hepatocarcinoma-29; LLC – Lewis lung carcinoma.

tions was transferred in ascites form. It has since been maintained in this form by i.p. passages on CBA mice. For i. v. inoculation, the malignant ascites were diluted with physiological solution to a final concentration of 1 x 10^5 cell/ml. The Lewis lung carcinoma (LLC) was maintained by i.m. passages on C57 mice. To produce the tumor cell inocula, tumor transplants were removed, cut up with scissors; the suspension was filtered through a stainless steel mesh and spun two times at 500 g. The pellet was washed in physiological solution and resuspended to a final concentration of 5 x 10^5 cell/mL. Both H-29 and LLC were transplanted to mice via inoculation into tail vein of 0.5 ml of the corresponding cell suspension. In each experiment, the tumor cells were grafted simultaneously to all the mice, experimental (winners and losers after 20 days of confrontations) and the controls: tumor cell administration to a mouse was alternated between the experimental groups and the control group.

After tumor transplantation, the animals were kept in their compartments without agonistic interactions for 16 days until the end of the experiment. On day 16, the mice were decapitated, the lungs were fixed in 10% formalin and tested for the presence of metastases using a binocular magnifier (magnification 8 ×).

Statistical analysis. The data on number of metastases were statistically processed using one-way ANOVA with the factor "social groups" for every strain. Then, paired comparisons of groups were performed using Student's *t*-test. Differences between experimental groups were considered to be significant if $p < 0.05$.

RESULTS AND DISCUSSION

The data for the control animals, losers and winners of CBA and C57 strains are presented in the Figure. One-way ANOVA showed that the number of metastases was reliably determined by the social group of animals as for C57 [$F(2.47) = 21.95$; $p < 0.001$] and CBA [$F(2.45)_4 = 45.75$; $p < 0.005$] strain. Paired comparisons of data for C57 mice using Student's *t*-test did not show differences in the number of metastases in lung between the control and winners ($p > 0.05$). However, the winners and losers, as well as the losers and controls differed significantly by this parameter (for both comparisons $p < 0.001$). In CBA mice, the control animals and losers did not differ significantly ($p > 0.05$) in the number of metastasis in the lung. However, the winners had significantly lower number of metastases than the losers ($p < 0.01$) and controls ($p < 0.05$).

Data obtained earlier indicate expressed and replicable effects of the psychoemotional status of mice on the growth rate of the primary tumors (Krebs-2 and LLC) transplanted in muscles and the lung metastasis [9, 10]. This study has demonstrated that the experimental metastases also develop differently in mice with negative and positive experience: the winners of both strains had significantly lower number of lung metastases than the losers. Thus, LLC and H-29 metastasis in male mice was affected by psychoemotional status, but in different mode for the strains: whilst in C57 strain repeated defeat experience was the factor provoking a more pronounced metastasis of LLC in the losers than in the controls and winners, the CBA winners had a markedly reduced number of lung metastases of H-29 tumor than the control mice and losers. That means that the effect of the positive or negative psychoemotional experience of recipients on tumor metastasis depends on either the mouse strain or the tumor model, or both.

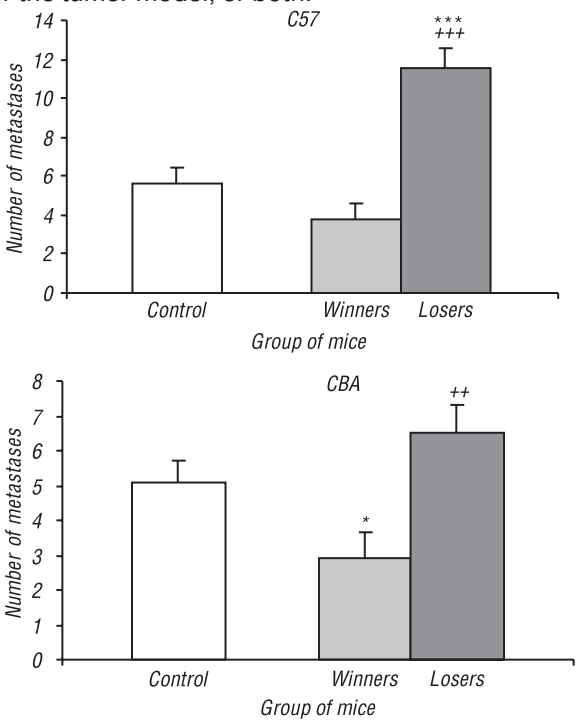

Figure. Number of LLC metastases in lung of C57 mice and H-29 metastases in lung of CBA mice
*$p < 0.05$; ***$p < 0.001$ *vs* control; $^{++}p < 0.01$; $^{+++}p < 0.001$ *vs* winners.

It appears as though tumor growth and metastasis are affected by the psychoemotional status of individuals via modification of their immune reactivity. There is a wealth of data confirming the development of immune deficiency in defeated animals as a result of stress produced by fighting, housing conditions, subordinate status, repeated social defeat etc. [for review, 1, 5, 16, 18–23]. Thus, a possible explanation for an increased number of lung metastases in the losers in our experiments could be psychogenic immune deficiency. In this case tumor metastasis will decrease in victorious animals, for which the enhancement of immune reactivity has been shown [5]. This is observed in the CBA winners with transplants of H-29, but not in the C57 winners with LLC [10, present report]. Moreover, as was mentioned above, solid transplants of LLC in the winners grew as rapidly as they did in losers and more rapidly than in controls [10]. Therefore, it is unlikely that the state of immunity is the main factor that determines the processes of metastasis in our experiments.

Different rates of tumor metastasis in the winners and losers may result from the differences in their neuroendocrine status, because brain activities change differently under the influence on repeated experience of victories or defeat in daily agonistic interactions

[12]. Neurotransmitters may trigger hormone release, leading to different changes in adrenal-corticoid and androgen functions in the male mice with opposite types of social behaviors [11]. Some neuroendocrine factors are peculiar growth factors and may modify tumor growth and metastasis by both direct action on tumor cells and indirectly, via influence on the vascular bed of the target organs for metastasis. A large number of physiological features, such as the activity of tissue macrophages and NK cells, the permeability of endothelial capillaries, the tonus and rate of vascularization etc, which undergo neuroendocrine regulation, may influence colonization by the tumor cells of target organs and the growth rate of metastases [7, 14, 18, 19]. There is a number of papers indicating that enhanced tumor metastasis in defeated animals is reversed by antagonists of some neurotransmitters receptors [3, 6, 14, 15].

It is obvious that further research is required. However, it is also obvious that chronic negative psychoemotional status and stress can enhance tumor growth and metastasis. It appears reasonable to find out if pharmacological correction of the emotional status of tumor-bearing individuals can influence tumor growth and metastasis development.

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ВЛИЯНИЕ ПСИХОЭМОЦИОНАЛЬНОГО СОСТОЯНИЯ НА МЕТАСТАЗИРОВАНИЕ КАРЦИНОМЫ ЛЕГКОГО ЛЬЮИС И ГЕПАТОКАРЦИНОМЫ-29 У МЫШЕЙ ЛИНИЙ C57BL/6J И CBA/LAC

Цель **работы — исследование влияния психоэмоционального состояния на метастазирование линейноспецифических опухолей — карциномы легкого Льюис у мышей линии C57BL/6J и гепатокарциномы-29 у мышей линии CBA/Lac.** ***Материалы и методы:*** **для получения самцов мышей с повторным опытом социальных побед и поражений в ежедневных агрессивных взаимодействиях была использована модель сенсорного контакта (на протяжении 20 дней). Клетки опухолей вводили в хвостовую вену животных. Количество метастазов в легких подсчитывали через 16 дней после перевивки опухоли.** ***Результаты:*** **показано, что метастазирование в легкие протекает неодинаково у мышей с различным психоэмоциональным состоянием: у мышей с опытом побед обеих линий количество метастазов в легких было существенно меньше, чем у животных с опытом психоэмоциональных поражений.** ***Выводы:*** **психоэмоциональное состояние влияет на процессы метастазирования карциномы легкого Льюис и гепатокарциномы-29 у самцов мышей линий C57BL/6J и CBA/Lac.**

Ключевые слова: **психоэмоциональное состояние, модель сенсорного контакта, карцинома легких Льюис, гепатокарцинома-29, мыши, метастазирование.**