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INFLUENCE OF TEICHOIC ACID FROM *S. AUREUS* ON METABOLIC ACTIVITY OF MACROPHAGES AND CYTOTOXIC ACTIVITY OF SPLENOCYTES OF MICE BEARING LEWIS LUNG CARCINOMA

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Aim: **To investigate the effect of teichoic acid (TA) from the cell wall of *S. aureus* on some indices of immunological reactivity in mice bearing Lewis lung carcinoma (LLC).** ***Methods:*** **The teichoic acid at the doses of 1, 2 and 4 μg/g of body weight has been administered subcutaneously simultaneously with tumor cells transplantation and in 7 days. The cytotoxic activity of peritoneal macrophages has been assessed by NBT-test. The splenocyte cytotoxic activity against the LLC cells has been tested by flow cytometry. The evaluation of tumor infiltration by lymphoid cells was carried out as well.** ***Results:*** **TA had no significant effect on oxidative metabolism of peritoneal macrophages in tumor bearing mice. Upon TA administration, the cytotoxic activity of splenocytes against the LLC cells has been augmented in a dose-dependent manner (at the TA dose of 4 μg/g, 2-fold decrease of tumor growth and metastasis has been registered) and leads to decreased tumor infiltration by mononuclear cells.** ***Conclusion:*** **TA caused a dose dependent inhibition of growth and metastasis of LLC. It was supposed that TA can influence the tumor grows by activation of splenocytes cytotoxic activity.**

Key Words: **teichoic acid, cell wall of *S. aureus*, Lewis lung carcinoma, oxidative metabolism, peritoneal macrophages, splenocytes, cytotoxic activity.**

Teichoic acids (TA) belong to the main structural components of bacterial cells. By the type of polyol in their content, there are ribitteichoic and glycerolteichoic acids, and by localization — cell wall TA and membrane TA [1, 2]. Membrane TA is linked by covalent bond with glycolipid of cytoplasmic membrane and are named lypoteichoic acids (LTA) [3, 4]. The ability of LTA to initiate proinflammatory immune response is in large part mediated by lipid component and allows consider them as potential therapeutic agents for the treatment of cancer [5–7]. Immunoenhancing and biological properties of cell wall TA are poorly studied yet; it is known that upon microbial infection and upon administration to animals, cell wall TA and their derivatives cause biological effects [8, 9]. By activation of the synthesis of inducible NO-synthase in different organs (liver, kidney etc), they lead to significant elevation of the level of nitrites in blood plasma and to the development of the multiple organ disfunction syndrome [10, 11]. Together with other bacterial components, cell wall TA is able to cause direct toxic on eukaryotic cells [12]. It is necessary to note that structural polymorphism of the TA preparations determines the differences in their biological effects [13, 14].

The recognition of TA and LTA by effector cells of immune system occurs with the involvement of Toll-like receptors, in particular TLR4 and TLR2 [15, 16]. Toll-like receptors play an important role in the induction of antitumor immune reactions. Agonists of these receptors are used as adjuvants in antitumor therapy [17–19]. TLR activation on the surface of antigen presenting cells such as dendritic cells and macrophages leads to activation of their functional maturation which manifests itself in elevation of MHC molecules expression, activation of synthesis and secretion of proinflammatory cytokines required for maturation of Th1 lymphocytes and cytotoxic T-lymphocytes [20, 21]. It is known also that activation of TLRs on the surface of tumor-induced suppressor $CD4^+T_{reg}$ cells leads to abrogation of their suppressor functions.

The aim of present work was to study the influence of cell wall TA from *S. aureus* on the indices of immunologic reactivity of mice bearing Lewis lung carcinoma (LLC).

MATERIALS AND METHODS

Isolation of teichoic acid. Teichoic acid was isolated from the cell walls of *Staphylococcus aureus* Wood 46. The strain was obtained from the Bacterial Culture Museum of the Cathedra of Microbiology and General Immunology of T.G. Shevchenko Kyiv National University (Kyiv, Ukraine). The preparation was isolated as described earlier [20] by the method of Archibald.

Experimental animals and experimental tumor model. In the experiments, female C57/Bl6 mice 2–3 months old weighting 20–25 g bred in the vivarium of biological faculty of T.G. Shevchenko Kyiv National University (Kyiv, Ukraine) were used. All animal procedures were carried out according to the rules of local Ethic Committee.

The strain of metastatic Lewis lung carcinoma was kindly supplied by the Bank of Cell Cultures and Transplantable Experimental Tumor of 3LL of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Ra-

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Abbreviations used: LLC – Lewis lung carcinoma; TA – teichoic acid.

diobiology (Kyiv, Ukraine). LLC cells were transplanted subcutaneously in sacral region by 0.2 ml of 20% cell suspension.

TA at the doses of 0.5, 1, or 2 μg/g of body weight at the volume of 0.2 ml was administered subcutaneously in the sacral region simultaneously with LLC transplantation. Introduction of TA was repeatedly given in a 7 days. Thus, total doses were 1, 2, or 4 μg/g of body weight respectively. Results haven been registered on the 35th day after the tumor cells transplantation.

Animals were housed in 5 groups (n = 10 per group) as follow: 1) intact animals (group A); 2) control LLC-bearing mice (group B); 3) LLC-bearing animals that received TA at a dose of 1 μg/g (group C); 4) LLC-bearing animals that received TA at a dose of 2 μg/g (group D); 5) LLC-bearing animals that received TA at a dose of 4 μg/g(group E).

Metabolic activity of peritoneal macrophages was determined by reduction of nitroblue tetrazolium (NBT) by [21]. For stimulation of oxygen-dependent metabolism PMA solution (Sigma) was used.

Cytotoxic activity of splenocytes was determined by the method of flow cytometry [22]. LLC cells served as the target cells. Effector cells and target cells were mixed at the ratio of 25 : 1 in RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum, 2 mM L-glutamine (Sigma) and 40 μg/ml gentamycine for 6 h at 37 °C in CO_2 incubator. Then cell mixture was placed in cytometric tubes (Falcon) and mixed with 2,5 μg/ml propidium iodide (Sigma). The samples were analyzed on flow cytometer FacsCalibur (Becton Dickinson) using CellQuest program. The percent of lyzed target cells was calculated by a formula: X = E – C, where E — % of lyzed target cells in experimental sample, and C — % of spontaneous lysis of target cells.

Isolation of mononuclear cells infiltrating tumor. Tumor tissues were mechanically disintegrated by scissors, homogenized in Potter's homogenizer, and treated by trypsin solution (1 : 250) in citrate buffer, pH 7.8, containing 150 мл NaCl [23]. Cell suspension was triply washed with cold Henks solution. Mononuclear cells were isolated from cell suspension by centrifugation in ficoll-verografin gradient (ρ = 1.077). For standartization of the results, the data were presented as specific cell content (C1) calculated by the formula:

CI = (total number of mononuclear cells / tumor weight) x 10^5.

Statistical analysis of obtained data was performed with the use of Students *t*-test.

RESULTS AND DISCUSSION

Administration of TA to LLC-bearing mice resulted in different influence on tumor growth dependently of the TA dose (Table). In animals that received TA at the doses of 1 μg/g and 2 μg/g, stimulation of tumor growth has been observed and the volume of primary tumors 2.5 fold higher than those in group B, while the number of metastases was by 65 and 28% higher, respectively. It is necessary to note that metastasis level in experimental animals was characterized by notable individual variability. In mice that received TA at a dose of 4 μg/g, at the moment of the experiment cessation the volumes of primary tumors were 4-fold lower compared to the control, and lung metastases were absent.

Table. Influence of teichoic acid of *S. aureus* on the growth parameters of LLC in mice

TA-treated animals, TA doses	Volume of primary tumor (cm^3)	Number of metastases per animal (M ± m)	IMI, %*
4 μg/g, (n = 10)	0.230 ± 0.025**	0**	100
2 μg/g, (n = 10)	2.301 ± 0.098**	17.92 ± 6.04	–
1 μg/g, (n = 10)	2.461 ± 0.122**	23.15 ± 5.85	–
Untreated animals (n = 10)	0.941 ± 0.051	14.0 ± 6.15	

Notes: *IMI (index of metastasis inhibition, %) was calculated by the formula IMI = [1 – E/C] x 100%, where C and E – average number of metastases per animal from control and experimental groups respectively; **$P < 0.05$ compared with untreated tumor-bearing animals.

The influence of TA on tumor growth may be mediated by two ways: by direct toxic influence on tumor cells [12]; and via Toll-like-dependent activation of antitumor immunity. For evaluation of the impact of immune system mediating TA influence on tumor growth, we have studied some indices of immunologic reactivity of experimental animals that play an important role in antitumor immunity, namely metabolic activity of peritoneal macrophages and cytotoxic activity of splenocytes and the relative content of mononuclear cells in tumor tissue.

Mononuclear phagocytes are an important component of antitumor resistance. Proinflammatory activation of macrophages directed toward elimination of tumor cells as well, is accompanied by so-called "oxygen burst" [24, 25]. According to our data, spontaneous metabolic activity of macrophages in control LLC-bearing animals was similar to that of intact animals (Fig. 1), and administration of TA at the doses of 1 μg/g, 2 μg/g did not cause statistically significant alteration of this index. TA at a dose of 4 μg/g caused insignificant decrease of oxygen-dependent metabolism of macrophages.

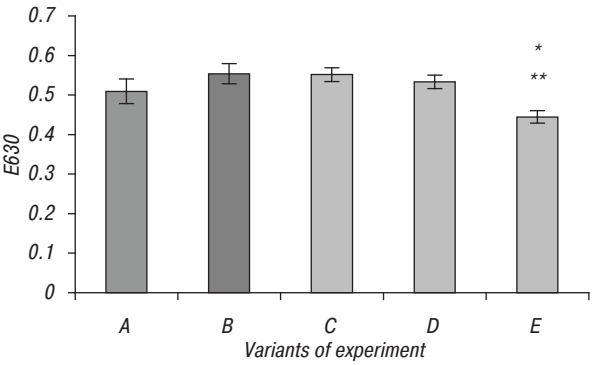

Fig. 1. Influence of teichoic acid from *S. aureus* on spontaneous metabolic activity of peritoneal macrophages from LLC-bearing mice. *A* — intact animals, n = 10; *B* — untreated tumor-bearing animals, n = 10; *C* — tumor-bearing animals that received TA at a dose of 1 μg/g, n = 10; *D* — tumor-bearing animals that received TA at a dose of 2 μg/g, n = 10; *E* — tumor-bearing animals that received TA at a dose of 4 μg/g, n = 10

*$P < 0.05$ compared with intact animals; **$P < 0.05$ compared with untreated tumor-bearing animals.

The level of stimulated metabolic activity of peritoneal macrophages that characterizes the state of their metabolic reserve was by 25% higher in group B than that in group A (Fig. 2). In TA-treated animals this index did not differ significantly from that in control LLC-bearing animals and was moderately higher than in group A. It's necessary to note that in total TA has no prolonged (up to 30 days) influence on metabolic activity of peritoneal macrophages.

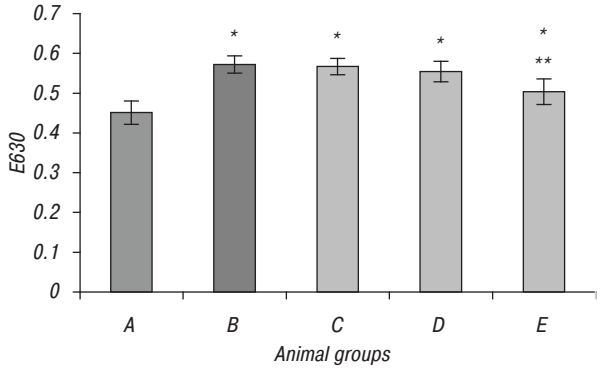

Fig. 2. Influence of teichoic acid from *S. aureus* on stimulated metabolic activity of peritoneal macrophages of LLC-bearing mice. *A* — intact animals, n = 10; *B* — untreated tumor-bearing animals, n = 10; *C* — tumor-bearing animals that received TA at a dose of 1 μg/g, n = 10; *D* — tumor-bearing animals that received TA at a dose of 2 μg/g, n = 10; *E* — tumor-bearing animals that received TA at a dose of 4 μg/g, n = 10
**P* < 0.05 compared with a intact animals; ***P* < 0.05 compared with untreated tumor-bearing animals.

Splenocytes may control an appearance and perform unspecific elimination of tumor cells [26]. It may be mentioned (Fig. 3) that in group B the level of cytotoxic activity of splenocytes was twice lower than in group A. Administration of TA caused a dose-dependent stimulation of the activity of these cells, and upon administration of TA at a dose of 4 μg/g the level of this index reached the values characteristic for intact animals.

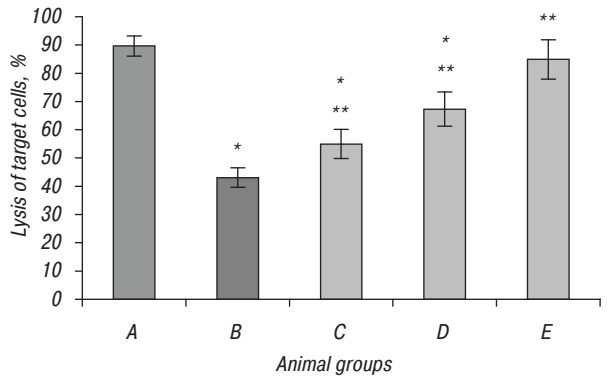

Fig. 3. Influence of teichoic acid from *S. aureus* on the indices of cytotoxic activity of splenocytes of LLC-bearing mice. *A* — intact animals, n = 10; *B* — untreated tumor bearing animals, n = 10; *C* — tumor-bearing animals that received TA at a dose of 1 μg/g, n = 10; *D* — tumor-bearing animals that received TA at a dose of 2 μg/g, n = 10; *E* — tumor-bearing animals that received TA at a dose of 4 μg/g, n = 10
**P* < 0.05 compared with intact animals; ***P* < 0.05 compared with untreated tumor-bearing animals.

During its growth, malignant tumor became infiltrated by lymphoid cells that dependent on their subpopulations could either suppress or promote tumor growth. According to our results, TA administration at all studied doses resulted in the suppression of tumor infiltration by mononuclear cells compared to group B (Fig. 4), but there was a reverse dependence between the dose and the degree of such suppression. Possibly, such phenomenon could be explained by a dose-dependent influence of TA on chemokine production [27, 28].

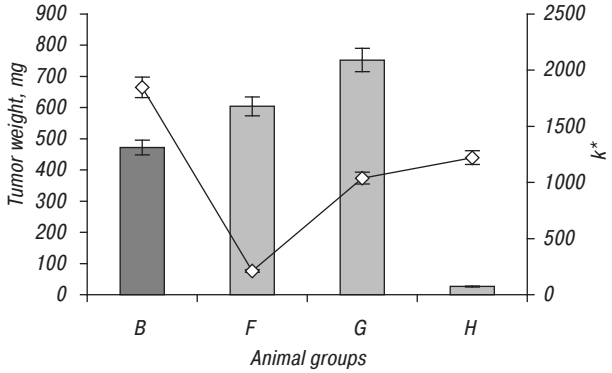

Fig. 4. Influence of teichoic acid from *S. aureus* on the content of mononuclear cells in tumor tissue of LLC-bearing mice. *B* — untreated tumor-bearing animals, n = 10; *F* — tumor-bearing animals that received TA at a dose of 1 μg/g, n = 10; *G* — tumor-bearing animals that received TA at a dose of 2 μg/g, n = 10; *H* — tumor-bearing animals that received TA at a dose of 4 μg/g, n = 10
*k** — number of leucocytes per 1 mg of tumor tissue.

Inhibition of growth and metastasis of LLC upon the influence of TA may be due to a combined action of the preparation on tumor cells as well as on effector cells of immune system. Taking into account the ability of TA to stimulate iNOS production by normal and malignant transformed cells, one may propose the accumulation of significant amount of NO causing cell damage in the place of injection of the preparation (region of tumor cell transplantation). Because of cell destruction, the local inflammation manifesting itself in point ulceration of animal's skin was observed. The development of local inflammation promoted inhibition of tumor growth and metastasis of LLC.

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ВЛИЯНИЕ ТЕЙХОЕВОЙ КИСЛОТЫ *S. AUREUS* НА МЕТАБОЛИЧЕСКУЮ АКТИВНОСТЬ МАКРОФАГОВ И ЦИТОТОКСИЧЕСКУЮ АКТИВНОСТЬ СПЛЕНОЦИТОВ МЫШЕЙ С КАРЦИНОМОЙ ЛЕГКОГО ЛЬЮИС

Цель: **исследовать влияние тейхоевой кислоты *Staphylococcus aureus* Wood 46 (ТК) на иммунологические параметры у мышей с карциномой легкого Льюис.** ***Методы:*** **в качестве модели использовали перевиваемую карциному легкого Льюис. Тейхоевую кислоту *S. aureus* Wood 46 получали по методу Арчибальда. Кислородзависимый метаболизм перитонеальных макрофагов оценивали по восстановлению нитросинего тетразолия (НСТ-тест). Цитотоксическую активность спленоцитов определяли методом проточной цитофлуориметрии.** ***Результаты:*** **ТК оказывала дозозависимое влияние на рост и метастазирование карциномы Льюис. У животных, получивших ТК в дозе 4 мкг/г, размеры первичной опухоли были на момент окончания эксперимента в 4 раза меньше по сравнению с контролем, метастазы в легких отсутствовали. ТК незначительно стимулировала кислородзависимый метаболизм перитонеальных макрофагов. Отмечали дозозависимую активацию цитотоксической активности спленоцитов: при использовании ТК в дозе 4 мкг/г цитотоксическая активность спленоцитов у животных опытной группы в 2 раза превышала показатели контрольных животных-носителей опухолей.** ***Выводы:*** **ТК оказывает дозозависимое ингибиторное влияние на рост и метастазирование карциномы легкого Льюис. Одним из механизмов, опосредующих супрессорное действие ТК на опухолевый рост, можно считать активацию цитотоксической активности спленоцитов.**

***Ключевые слова*: карцинома легкого Льюис, тейхоевая кислота, кислородзависимый метаболизм, перитониальные макрофаги, спленоциты, цитотоксическая активность.**