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T-CELLULAR IMMUNITY AND BACTERIAL ENDOTOXIN CONCENTRATION IN THE TREATMENT OF LARYNGEAL CANCER PATIENTS

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Abstract

Purpose of the study was to explore the state of T-cell immunity and indices of bacterial endotoxin concentration in patients with laryngeal cancer.

Material and methods. *Examination of thirty-one patients with laryngeal cancer ($T_3N_XM_0$ category) was carried out before, during and after the combined treatment. Indicators of T-cell immunity (CD3+, CD4+, CD8+, CD95+) and natural killer cells (CD16+), the apoptosis index (CD95+/CD3+) and the immune-regulatory index (CD4+/CD8+) were counted. The total endotoxin concentration in the blood serum of patients was determined with the help of the limulus amoebocyte lysate test.*

Results. *The initial indices of endotoxin concentration of patients exceeded the control values by 14.7 times. There was no statistically significant decrease in the content of mature T-lymphocytes and T-helpers, but the values of CD8+, CD95+, and CD16+ were increased. The immune-regulatory index was 0.78, with a reference value of 1.13; the apoptosis index was 0.41, with a control value of 0.3. A month after the end of treatment, the endotoxin concentration (2.5 Eu/ml) had no statistically significant differences from its initial values (2.8 Eu/ml) and the values at the end of the combined treatment (2.9 Eu/ml). The dynamics of T-cell immunity parameters was characterized by the absence of differences in the content of mature T-lymphocytes and T-helpers, with respect to the data obtained at the end of the combined treatment, and with an increase in T-suppressors, with a decrease in the number of apoptotic lymphocytes and natural killer cell. The immune-regulatory index did not change at all stages of the observation. The apoptosis index increased, and by the time of completion of treatment it exceeded the initial value (0.41 and 0.8), and a month later it was 0.65.*

Conclusion. *At present, cancer cells are the target of special treatment methods in oncology, but it's likely that in the nearest future, antitumor strategies will take into account another participant in carcinogenesis – the human body microbiota.*

Keywords. T-cell immunity, endotoxin, laryngeal cancer.

Introduction

Conception about the role of immune system in the development of malignant neoplasms, in analogy with anti-infectious immunity, lay in immunological surveillance idea, which resulted in the provision of the immune system failure in tumor disease. A natural continuation of this conclusion was the decision of the need to stimulate the immune system at development of the malignant neoplasm. However, further studies have shown that in some cancer patients the functionality of the immunity system is sufficiently preserved or even activated, but with the inversion of the potentials of the elements of its components. The human innate immune system recognizes pathogen-associated molecular

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patterns conserved among microbes or damage-associated molecular patterns released from tissue injuries to initiate adaptive immune responses during infection and tissue inflammation. But when immune system at the molecular level recognizes and how reacts on tumor cells remains poorly understood until now [*Dunn GP et al.,* 2004; *Germenis AE, Karanikas V.,* 2007; *Savina NP,* 2009; *Schreiber RD et al,* 2011; *Swann JB, Smyth MJ,* 2007; *Zitvogel L et al,* 2006]. At the same time, the possibility to influence the tumor through the immunity system remains the most attractive direction in the fight against malignant neoplasms, as it is known that the immune system possesses a set of functional elements capable of exerting a pronounced antitumor effect by direct destruction of tumor cells or change in their microenvironment.

The term "microenvironment of the tumor" is understood as a set of factors that characterize the events inside tumor, and processes occurring in the immediate vicinity of the neoplasm and factors that cause them, among which inflammation is one of the main [*Berezhnaya NM, 2009; Chekhun VF, 2009; Tan TT, Cousseans LM, 2007 ; Vorontsova LL, et al. 2016*].

Existing data on the interdependence of carcinogenesis and inflammation are quite contradictory: the phenomenon of hemorrhagic necrosis of tumors is known with a pronounced concomitant infection, but on the other hand chronic inflammation promotes the development all forms of cancer. And, if in some cases inflammation processes precede tumor transformation of cells, in others – tumor cells induce local inflammation, attracting cells of the immune system to the tumor growth zone [*Cruz SM, Balkwill FR, 2015; Mantovani A et al., 2008; Ryabichenko YV et al, 2005*]. Moreover, the most powerful chemoattractants are lipopolysaccharides (LPS) – major outer surface membrane components present in almost all Gram-negative bacteria (Gram-positive with LPS is Listeria monocytogenes only), which act as extremely strong stimulators of innate or natural immunity. LPS consist of a poly- or oligosaccharide region that is anchored by a specific carbohydrate lipid moiety termed lipid A. Lipid A is a trigger numerous physiological immune-stimulatory effects, but in higher doses can also lead to pathological reactions such as septic shock induction [*Fomin AA et al. 2009; Lui AH, Redmon AH, 2001;* Wexler H, Oppenheim JD, 1979; *Yakovlev MY, 2003*].

During the past decade the elucidation of structure activity correlations in LPS and lipid A has not only contributed to a molecular understanding of both immune-stimulatory and toxic septic processes but has also re-animated the development of new pharmacological and immune-stimulatory strategies for the prevention and therapy of infectious and malignant diseases.

Purpose of the study: The T-cellular immunity status learn and significations of bacterial endotoxin concentration on treatment stagesin patients on laryngeal cancer.

Material and methods

Studies were performed in 31 patients with laryngeal cancer categories T2–3N0M0 (group A) before, during and after the combined treatment (observation period 2010 – 2017, personal observation period of at least five years). The age of the patients ranged from 34 to 65 years. The morphological characteristics of neoplasms are keratinizing squamous cell carcinoma. The control group consisted of 25 practically healthy people (group C) of the same age.

Indicators of T-cell immunity (mature T-lymphocytes – CD3+, subpopulations of T-lymphocytes with helper and suppressor activity – CD4+ and CD8+, natural killers – CD16+ and Fas/APO-1 induced T-lymphocytes – CD95+) were determined by phenotyping with monoclonal antibodies of the SPA "Granum+ (Kharkov). The apoptosis index (CD95+/CD3+) and the immune-regulatory index (CD4+/CD8+) were calculated. The total concentration of endotoxin of gram-negative bacteria (CE) in serum of patients was determined using the Micro-LAL test [*Bondarenko VM et al., 2002*].

Comparisons of the studied parameters were carried out using the tests of Wald-Wolfowitz and Wilcoxon (with Bonferroni correction), with a significance level of 0.05. The analyzed data are presented as "median and interquartile interval": *Me* (*RQ = UQ – LQ*). The causal relationship between the indicators was evaluated using multiple logistic regression analysis. Statistical processing of the received data was made using computer programs of the STATISTICA package (Stat Soft Statistica v.7.0) [*Borovikov V, 2001*].

Results and discussion

The levels data of the concentrations of bacterial endotoxin (EC) and some parameters of T-cell immunity in peripheral blood of patients, before the beginning of treatment in table 1 are presented.

The EC baseline values in patients (group A) exceeded the control values by 14.7 times. At the same time, there was no statistically significant decrease in the content of mature T-lymphocytes and T-helpers (CD4+) in patients with laryngeal cancer, but the values of CD8+, CD95+, and CD16+ values were increased. The immunoregulatory index (IRI) in patients with laryngeal cancer was 0.78, at the reference value of 1.13; the apoptosis index (AI) was 0.41 with the control value of 0.3.

Thus, in case of tumor disease is not the absolute submission on the insolvency of the immune system. Rather, and these observations, in particular, support the assumption that in some cancer patients the functionality of the immunity system is sufficiently preserved or even activated, but with the inversion of the potentials of elements its constituent. Obviously, this also depends on the localization, degree of prevalence, morphological characteristics and features of the metabolism of the tumor process [*Chizhikov NV, Oparina NV, 2007*; *Huang L, Mellor AI, 2014; Kovalev OO, Khorolets OV, 2017*].

Table 1

The concentration of bacterial endotoxin and the T-cell immunity indicators in the control group and group A patients

Test	Control Group (n = 25)	Group A (n = 31)	*p-level*
EC (*EU/ml)*	0.19 0.21 – 0.18 = 0.03	2.8 3.04 – 2.12 = 0.92	< 0.05
CD3+ (%)	64.0 65.0 – 62.0 = 3.0	59.0 64.0 – 55.0 =9.0	0.096
CD4+ (%)	38.0 38.0 – 36.0 = 2.0	36.0 40.0 – 34.0 = 6.0	0.923
CD8+ (%)	32.0 34.0 – 32.0 = 2.0	46.0 48.0 – 42.0 = 6.0	< 0.05
CD95+ (%)	19.0 22.0 – 18.0 = 4.0	26.0 30.0 – 22.0 = 8.0	< 0.05
CD16+ (%)	18.0 22.0 – 16.0 = 6.0	28.0 32.0 – 24.0 = 8,0	< 0.05
CD4+/CD8+ (IRI)	1.13 1.2 – 1.06 = 0.14	0.81 0.9 – 0.68 = 0.22	< 0.05
CD95+/CD3+ (AI)	0.32 0.35 – 0.28 = 0.07	0.41 0.52 – 0.35 = 0.17	0.046

At the end of the combined treatment, there was a decrease in the content of both mature T-lymphocytes (CD3+) and their subpopulations (CD4+ and CD8+) in peripheral blood of patients (group A1), with $p < 0.05$ for each of the indicators, but the content of natural killer (NK) cells (CD16+, $p = 0.376$) and the value of the IRI ($p = 0.056$) did not statistically significantly change. At the same time, the levels of EC (2.9 *EU/ml*, $p < 0.05$) and the apoptotic lymphocytes content (CD95+, $p = 0.003$) and IA ($p < 0.05$) are increased.

It should be noted that regardless of the step-by-step methods of combination therapy: surgical–radiotherapy (subgroup A1a) or radiotherapy–surgery (subgroup A1b), there were no statistically significant differences in the studied parameters (except for the content of mature T-lymphocytes: subgroup A1a – 38.5%, subgroup A1b – 46.0%, $p = 0.018$), which however can be explained by the difference in the number of observations in subgroups (table 2).

One month after the treatment end, the EC in the blood of the patients (2.5 *Eu/ml*, group A2) did not have statistically significant differences from its initial values (2.8 *Eu/ml*, group A) and EC values immediately upon completion combined treatment (2.9 *Eu/ml*, group A1). Dynamics of the T-cell immunity parameters was characterized by the absence of significant differences in the content of mature T-lymphocytes (CD3+) and T-helpers (CD4+) with respect to data obtained at the end of the combined treatment and statistically significant increase in T suppressors (CD8+), with a decrease in the number of apoptotic lymphocytes. But all the studied indicators (CD95+ exception) had statistically significant differences with the initial data. The IRI did not change at all stages of monitoring. The AI index of lymphocytes increased and by the end of the combined treatment it exceeded the initial value (0.41 and 0.8, respectively), and a month later it was 0.65, which in each case had statistically significant differences

Table 2

The bacterial endotoxin concentration and T-cell immunity indices in patients undergoing combined treatment

Test	Group A1 (*n* = 31)	Subgroup A1a (*n* = 24)	Subgroup A1b (*n* = 7)	*p-level*
EC *(Eu/ml)*	2.9 3.5 – 2.4 = 1.1	2.63 3.22 – 2.27 = 0.95	3.7 4.4 – 2.9 = 1.5	0.073
CD3+ (%)	40.0 44.0 – 29.0 =15.0	38.5 42.0 – 27.5 = 14.5	46.0 55.0 – 42.0 = 13.0	0.018
CD4+ (%)	28.0 36.0 – 24.0 = 12.0	26.0 34.0 – 24.0 = 10.0	28.0 42.0 – 28.0 = 14.0	0.554
CD8+ (%)	34.0 36.0 – 32.0 = 4.0	34.0 36.0 – 32.5 = 3.5	32.0 34.0 – 32.0 = 2.0	0.893
CD95+ (%)	32.0 38.0 – 26.0 = 12.0	32.0 40.0 – 27.0 = 13.0	34.0 36.0 – 24.0 = 12.0	0.933
CD16+ (%)	27.5 32.0 – 22.0 = 10.0	26.0 30.0 – 22.0 = 8.0	30.0 32.0 – 22.0 = 10.0	0.398
IRI	0.82 1.17 – 0.71 = 0.46	0.8 1.07 – 0.71 = 0.36	0.88 1.31 – 0.81 = 0.5	0.612
AI	0,41 1.1 – 0.62 = 0.48	0,8 1.25 – 0.64 = 0.61	0.65 0.78 – 0.49 = 0.29	0.398

Note: *p-level for A1a vs A1b subgroups*

Table 3

Dynamics of bacterial endotoxin concentration and indicators T-cell immunity at the stages of observation and treatment

Test	Group A (*n* = 31)	Group A1 (*n* = 31)	Group A2 (*n* = 31)	*p-level*
EC *Eu/ml*	2.8 3.04 – 2.12 = 0.92	2.9 3.5 – 2.4 = 1.1	2.5 3.2 – 1.9 = 1.3	0.922* 0.134**
CD3+ (%)	59.0 64.0 – 55.0 =9.0	40.0 44.0 – 29.0 =15.0	40.0 52.0 – 33.0 = 19.0	< 0.05* 0.131**
CD4+ (%)	36.0 40.0 – 34.0 = 6.0	28.0 36.0 – 24.0 = 12.0	32.0 38.0 – 28.0 = 10.0	0.002* 0.167**
CD8+ (%)	46.0 48.0 – 42.0 = 6.0	34.0 36.0 – 32.0 = 4.0	36.0 38.0 – 32.0 = 6.0	< 0.05* 0.041**
CD95+ (%)	26.0 30.0 – 22.0 = 8.0	32.0 38.0 – 26.0 = 12.0	28.0 36.0 – 24.0 = 12.0	0.061* 0.028**
CD16+ (%)	28.0 32.0 – 24.0 = 8.0	27.5 32.0 – 22.0 = 10.0	24.0 28.0 – 18.0 = 10.0	0.011* 0.024**
IRI	0,81 0.9 – 0.68 = 0.22	0.82 1.17 – 0.71 = 0.46	0.94 1.07 – 0.65 = 0.42	0.102* 0.727**
AI	0.41 0.52 – 0.35 = 0.17	0.76 1.1 – 0.62 = 0.48	0.75 0.91 – 0.44 = 0.47	< 0.05* 0.037**

Note: **- p-level for A vs A2 groups; **- p-level for A1 vs A2 groups*

and could be explained by the aggressiveness of the treatment methods. It should be noted that the dynamics of the IRI values emphasize the fact that each of such important subpopulations of T-lymphocytes, as CD4+ and CD8+ includes clones with helper, suppressor and cytotoxic activity, so what level of regulation reflects IRI, the ratio of which cells it is determined in each case, as evidenced by its changes in one direction or another – questions that often remain unanswered [*Berezhnaya NM, 2006*]. The NK (CD16+) content index was increased relative to the baseline values at the end of the combined treatment and slightly decreased a month after their completion, but also had statistically significant differences in each case (table 3).

The presented data testify to the presence of the endotoxin excess in the peripheral blood of patients and the increase in its content, as a result of stress factors of special treatment, can be a complex inducer of changes in the multilevel immune status of the organism. A sharp variation in the microbiota, at the ability of intestinal microbes to influence systemic inflammation, is a risk factor for cancer recurrence or development of another tumor, and also in tissues outside the gastrointestinal tract [3, 13]. In our observations, it was precisely in 9 patients with elevated (≥ 3.1 *Eu/ml*) or sharply reduced (≤ 0.11 *Eu/ml*) endotoxin concentration (EC: *Me* 2.5, *min* 0.09, *max* 5.3 *Eu/ml*), relapses (metastases) of laryngeal cancer or development of the second primary neoplasm were diagnosed.

It should be noted that at the final stage of the observations, the odds ratio (OR) in the probability of relapses (metastasis) of the neoplasm was 1.4 for EC; 0.99 for CD3+; 0.97 for CD4+; 0.98 for CD8+; 1.18 for CD95+; 0.16 for CD16+ (the greater the unit odds ratio, the more likely to expect events from the factor with the largest OR).

CONCLUSION

Endogenous (intestinal primarily) microflora and dysbiosis affect the numerous functions of the host organism. In recent decades, the elucidation of the correlations, structure, activity of LPS and lipid A has contributed not only to a better understanding of the development of immune-stimulating and toxic processes but has also served as the basis for the development of new pharmacological approaches in the prevention and treatment of infectious and oncological diseases. At present, in oncology the target of special treatment methods are cancer cells and their microenvironment, but it's likely that in the near future, antitumor strategies will take into account another participant in carcinogenesis – the human body microbiota.

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