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ИЗМЕНЕНИЕ ФЕНОТИПА Т- И В-ЛИМФОЦИТОВ ПРИ ЛЕЧЕНИИ РАДИОАКТИВНЫМ ЙОДОМ ПАЦИЕНТОВ С БОЛЕЗНЬЮ ГРЕЙВСА

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Резюме. Целью исследования явилось изучение особенностей субпопуляционного состава Т- и В-лимфоцитов и их взаимосвязей в динамике лечения радиоактивным йодом больных с болезнью Грейвса (БГ). Обследовано 36 женщин с верифицированным диагнозом БГ. Определение содержания тиреоидных гормонов осуществлялось методом иммунорадиометрического анализа. Уровень аутоантител к рецептору тиреотропного гормона (рТТГ) оценивался иммуноферментным методом. На основании комплексного предтерапевтического обследования всем пациентам назначалась фиксированная активность ^{131}I от 400 до 700 МБк перорально. В качестве контроля обследовано 56 практически здоровых женщин. Исследование фенотипа Т- и В-лимфоцитов проводили методом проточной цитометрии с использованием прямой иммунофлуоресценции цельной крови. Установлено, что до момента лечения радиоактивным йодом у больных выявляется высокий уровень функциональной активности клеток, который определяется экспрессией CD25-антигена на Т-лимфоцитах и CD23-антигена на В-лимфоцитах. Высокий уровень функциональной активности клеток адаптивного иммунитета у больных с БГ проявляется на фоне повышенного уровня аутоантител к рТТГ. С помощью корреляционного анализа обнаружено, что у больных с БГ в период предтерапевтического обследования состояние тиреоидного статуса определяет стимуляцию функциональной активности Т- и В-лимфоцитов и, соответственно, повышает уровень аутоиммунных процессов. Через 1 месяц после проведения радиойодтерапии (РЙТ) у больных с БГ на фоне транзиторного гипертиреоза (при сохранении повышенной концентрации аутоантител к рТТГ) количество активированных Т-лимфоцитов (включая Т-хелперы и цитотоксические Т-клетки) снижается до контрольных значений. Однако у больных сохраняется низкий уровень в крови цитотоксических Т-лимфоцитов и значительно повышается содержание Treg. При исследовании фенотипа В-лимфоцитов крови у больных с БГ через 1 месяц после РЙТ также обнаружено снижение количества В-клеток и активированных В-клеток

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памяти до уровня контрольного диапазона. При этом выявляется повышение уровней наивных В-лимфоцитов и В2-клеток, а также снижение количества активированных В1-лимфоцитов. Все изменения в субпопуляционном составе Т- и В-клеток и в их фенотипе развиваются на фоне полной потери взаимосвязей между исследуемыми показателями, что характеризует потерю тиреоидного контроля иммунных процессов и кооперативного взаимодействия клеток при развитии иммунного ответа. В целом, изменения фенотипа Т- и В-лимфоцитов в крови у больных с БГ через 1 месяц после лечения радиоактивным йодом отражают тенденцию к снижению функциональной активности клеток адаптивного иммунитета, что может реализовываться и в ингибировании аутоиммунных процессов.

Ключевые слова: Т-лимфоциты, В-лимфоциты, фенотип, субпопуляции, радиойодтерапия, аутоантитела, болезнь Грейвса

CHANGES IN THE T AND B LYMPHOCYTE SUBSET PROFILES UPON TREATMENT OF PATIENTS WITH GRAVES' DISEASE WITH RADIOACTIVE IODINE

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Abstract. The aim of the present study was to evaluate the subpopulation profile of T and B lymphocytes, and their relationships during therapy of the patients with Graves' disease (GD) treated by means of radioactive iodine. We have examined 36 women with verified diagnosis of GD. The contents of thyroid hormones were determined by immunoradiometric analysis. The levels of thyroid-stimulating hormone receptor autoantibodies (rTSH) were evaluated by enzyme-linked immunosorbent assay. On the basis of comprehensive pre-therapeutic examination, all patients were exposed to the fixed-activity therapy with radioactive iodine-131 at a dose of 400 to 700 MBq administered orally in isotonic aqueous solution of sodium iodide. 56 practically healthy women were examined as a control group. The phenotype of T and B cells in whole blood was studied by flow cytometry using direct immunofluorescence. It was shown that the patients, prior to treatment with radioactive iodine, had high levels of cellular functional activity, as determined by expression of CD25 antigen on T cells and CD23-antigen on B lymphocytes. Higher functional activity of the cells responsive for adaptive immunity in the patients with GD manifests in the presence of increased levels of autoantibodies to rTSH. By means of correlation analysis, we found that the patients with GD examined before the therapy had the thyroid status may determine the functional stimulation of T and B cells, thus increasing the levels of autoimmune processes. One month after radioiodine therapy (RIT), the GD patients, along with transient hyperthyroidism with increased concentration of autoantibodies to rTSH, showed a reduction of activated T lymphocyte contents (including T helpers and cytotoxic T cells) to control values. However, the level of cytotoxic T lymphocytes in the blood remained low, and the content of Treg cells was significantly increased in the patients. Decreased contents of B cells activated memory B cell to the control levels were found in patients with GD over 1 month after RIT when studying the phenotype of blood B lymphocytes. In this case, increased levels of naive B lymphocytes and B2 cells were detected, as well as decreased numbers of activated B1 lymphocytes. The observed changes in the subpopulation composition of T and B cells, and in their phenotype developed against the background of complete absence of relationships between the studied parameters, thus suggesting loss of thyroid control of immune processes and cooperative cell interaction during the development of the immune response. Generally, the phenotypic changes of T and B lymphocyte subsets in the blood of patients with GD through 1 month after treatment with radioactive iodine may reflect a trend for decreased functional activity of adaptive cellular immunity which may also account for inhibition of autoimmune processes.

Keywords: T lymphocytes, B lymphocytes, phenotype, subsets, radioiodine therapy, autoantibodies, Graves' disease

Introduction

Graves' disease (GD) is an autoimmune disease of the thyroid gland that develops as a result of the production of autoantibodies to the thyroid-stimulating hormone receptor (TSHR) [4, 21, 25]. The pathogenesis of GD is based on the appearance of class G thyroid-stimulating immunoglobulins (LATS factors: long-acting thyroid stimulator) in the body which interact with TSHR on the thyrocyte membrane and activate the hypersecretion of thyroxine (T_4) and triiodothyronine (T_3) [9]. This immunopathogenetic process leads to the onset of thyrotoxicosis syndrome.

The development of manifest hyperthyroidism in GD is appropriately realized in a change in the state of the immune system. Moreover, the relationships between the parameters of the immune system and the concentration of thyroid hormones in patients with GD are determined not only by the intensity of autoimmune processes but also by the direct influence of thyroid-stimulating hormone (TSH) and thyroid hormones. These thyroid influences on the functional activity of the immune system cells are realized through specific receptors that are expressed both on the outer cytoplasmic membrane and inside the cells [8, 12, 19]. We found earlier that the number of regulatory T cells (Treg) was decreased in the blood of patients with GD while the content of B1 cells was increased [20]. The results of the study by Zhang D. et al. (2019) showed that the blood of GD patients contained a reduced number of Treg with a low level of functional activity [26]. A decrease in the number of B lymphocytes producing IL-10 was also found in the blood of GD patients which in the framework of immunopathogenesis associated with an increase in the concentration of autoantibodies to TSHR [10]. It should be borne in mind that drug treatment with thyreostatic drugs (is the first line of the therapy for GD) can modulate the functional state of the immune system cells. The consequence of this may be a change in the direction of the autoimmune process both in the direction of the development of remission of the disease and relapse of GD.

Radioiodine therapy (RIT) for GD is based on the ability to capture and accumulate radioactive iodine by the cells of the thyroid gland. β-Particles are formed during the decay of the isotope of iodine causing the destruction of thyrocytes and subsequent development of post-radiation hypothyroidism [2]. The compensatory increase in TSH level occurs through a negative feedback mechanism. However, some researchers noted the concentration of autoantibodies to TSHR and/or thyroid peroxidase in the blood serum of GD patients even increased in the first month of RIT [15, 18]. It was assumed that the RIT causes damage to the DNA of thyrocytes as well as the release of new thyroid autoantigens which further stimulate the reactivity of the immune system including the activity of autoimmune process. So, it was shown in the Du W. et al. study (2017) that GD patients 1 week after RIT had a high concentration of IL-6 and CXCL-10 in the blood, the levels of which then decreased but even after 18 months remained elevated relative to the control values [5]. Direct effects of radioactive iodine on the immune system have also been found. For example, it was found that the level of apoptosis of blood leukocytes was increased during RIT [14]. The C t -Bigras S. et al. (2016) study was showed that damage to Treg and invariant NKT cells was observed in GD patients [3]. The authors believed that these results characterized the mechanisms of activation of autoimmune processes during RIT. There is evidence that the ability of T and B lymphocytes to cooperatively interact during the immune response may be impaired when treated GD patients with iodine-131 (^{131}I) [18]. In this regard, the mechanisms of the immune interaction of T and B cells in GD patients during RIT should be determined.

Thus, **the aim of this study** was to investigate the features of the subset composition of T and B cells and their relationships in the dynamics of treatment with radioactive iodine in patients with GD.

Materials and methods

Study participants

36 women (average age was 42.13±15.35) with a verified diagnosis of GD were included in this study: of which 10 women (27.7%) were with overt hyperthyroidism and 26 women (72.2%) were with relapse of the disease. Median thyroid volume was 20.41 ml (15.70-27.55). All patients at the time of inclusion in the study received drug treatment with thiamazole according to the standard scheme (median duration of treatment was 12 months, range: 9-14 months), the drug was canceled 14 days before RIT. The diagnosis of hyperthyroidism and the selection of patients for RIT was carried out according to the federal clinical guidelines for the management of patients with GD [24]. The RIT was carried out on the basis of the department of radionuclide therapy of the FMBA of Russia in Krasnoyarsk. All patients had a fixed ^{131}I activity of 400 to 700 MBq orally in the form of an isotonic aqueous solution of sodium iodide on the basis of a comprehensive pre-therapeutic examination. The examination of patients with GD was repeated 1 month after RIT. A fifty-six apparently healthy women of the same age were examined as a control group. All women in the control group didn't have a history of thyroid diseases in themselves and their blood relatives and also didn't have structural changes in the thyroid gland during ultrasound examination. The exclusion criteria from the control group were pregnancy and lactation. All studies were carried out with the informed consent of the patients and in accordance with the Declaration of Helsinki of

the World Association "Ethical principles of scientific medical research involving human" as amended in 2013 and "Rules of clinical practice in the Russian Federation" approved by the Order of the Ministry of Health of Russia of 19.06.2003 (No. 266).

Assessment of thyroid status and determination of the concentration of autoantibodies to TSHR

The concentration of thyroid hormones in the blood serum was determined by the method of immunoradiometric analysis using standard "Immunotech S.A.S." (Czech Republic) test kits in the hormonal laboratory of the endocrinological center of the Krasnoyarsk Regional Clinical Hospital. The reference values for TSH were 0.17-4.05 mU/L, for free T_4 (fT4) were 11.5-23.0 pmol/L, for free T_3 (fT3) were 2.0-5.7 pmol/L. The level of autoantibodies to TSHR (TSHR-AB) was assessed by the enzyme immunoassay using the "Medizym T.R.A." ("Medipan GmbH", Germany) test kit. The recommended cut-off point for the TSHR-AB level was 1.5 mU/L.

Flow cytometry

The study of the phenotype of T and B lymphocytes was carried out by flow cytometry using direct immunofluorescence of whole peripheral blood and monoclonal antibodies labeled with FITC (fluorescein isothiocyanate), PE (phycoerythrin), ECD (phycoerythrin-Texas Red-X), PC5 (phycoerythrin-cyanin 5), PC7 (phycoerythrin-cyanin 7), AA700 (alexa fluor 700) and AA750 (alexa fluor 750) in the following panels: CD8-FITC/CD127-PE/CD25-PC5/CD4-PC7/CD3-AA700/CD45-AA750 и CD5-FITC/CD23-PE/CD19-ECD/CD45-PC5/CD27-PC7. The distribution of antibodies along the fluorescence channels was carried out in accordance with the principles of forming panels for multicolor cytometric studies [13]. Sample preparation was performed according to the standard procedure [22]. The analysis of stained cells was carried out on a Navios flow cytometer (Beckman Coulter, USA) of the Krasnoyarsk Regional Center of Research Equipment of Federal Research Center "Krasnoyarsk Science Center SB RAS". The processing of the obtained cytometric results was carried out using the Navios Software v. 1.2 and Kaluza v. 2.1.1 (Beckman Coulter, USA) programs. At least 50,000 lymphocytes were analyzed in each sample.

Statistical analysis

Statistical description was performed by counting the median (Me) and the inter-quarter span in the form of 25 and 75 percentiles ($Q_{0.25}$-$Q_{0.75}$). The significance of differences between the indices of independent samples was assessed by the nonparametric Mann–Whitney U test. The significance of the differences in indicators of GD patients before and after RIT was determined by the Wilcoxon matched pairs test. The Spearman rank correlation coefficients were calculated to characterize the strength of the relationship between the studied indicators. Statistical analysis was performed in an application package Statistica 8.0 (StatSoft Inc., 2007).

Results

Study of thyroid status and the level of TSHR-AB

The functional state of the pituitary-thyroid system of GD patients before RIT corresponded to subclinical hyperthyroidism with a persistent high level of TSHR-AB (Figure 1). The state of subclinical thyrotoxicosis was confirmed by a low level of TSH and normal fT4 concentration, respectively: in GD Me = 0.01 mU/L ($Q_{0.25}$ = 0.005; $Q_{0.75}$ = 0.04) and Me = 15.25 pmol/L ($Q_{0.25}$ = 11.97; $Q_{0.75}$ = 19.81), in control group Me = 1.17 mU/L ($Q_{0.25}$ = 0.88; $Q_{0.75}$ = 1.54), $p < 0.001$) and Me = 15.25 pmol/L ($Q_{0.25}$ = 11.97; $Q_{0.75}$ = 19.81). The level of fT3 before RIT corresponded to the reference range of normal values, but remained significantly increased relative to the values established in control group: in GD Me = 4.25 pmol/L ($Q_{0.25}$ = 3.05; $Q_{0.75}$ = 5.51), in control group Me = 1.82 pmol/L ($Q_{0.25}$ = 1.49; $Q_{0.75}$ = 2.18), $p < 0.001$.

An increase in the concentration of TSH to control values was observed in patients 1 month after RIT while the content of TSHR-AB remained almost at the initial level. Also, the concentration of fT3 in GD patients increased 1 month after therapy (in patients – Me = 6,27 pmol/L, $Q_{0.25}$ = 5,59, $Q_{0.75}$ = 6,42; in persons of the control group – Me = 1,82 pmol/L, $Q_{0.25}$ = 1,49, $Q_{0.75}$ = 2,18; $p < 0,001$), while the level of fT4 during this period of the examination corresponded to the reference range.

Features of the subpopulation composition of T and B lymphocytes in GD patients before and after RIT

An increase in the percentage of $CD3^+CD25^+$, $CD3^+CD4^+$, $CD3^+CD4^+CD25^+$ and $CD3^+CD8^+CD25^+$ cells but with a decrease in the amount of $CD3^+CD8^+$ lymphocytes relative to the control values was found in GD patients before RIT (Table 1). The percentage of $CD3^+CD25^+$, $CD3^+CD4^+CD25^+$ and $CD3^+CD8^+CD25^+$ lymphocytes in patients 1 month after RIT decreased to the control range. The relative number of $CD3^+CD8^+$ cells remained almost at the initial level. At the same time, an increase in the number of $CD3^+CD4^+CD127^{Low}CD25^{High}$ cells in the peripheral blood was found in GD patients 1 month after RIT.

Changes in the phenotypic composition of blood B lymphocytes were also detected in GD patients before treatment with radioactive iodine: a decrease in the percentage of $CD19^+$ cells and an increase in the relative number of $CD19^+CD27^+CD23^+$ lymphocytes (Table 2). $CD19^+$ and $CD19^+CD27^+CD23^+$ cell levels were normalized 1 month after therapy. However, additional changes in the phenotypic composition

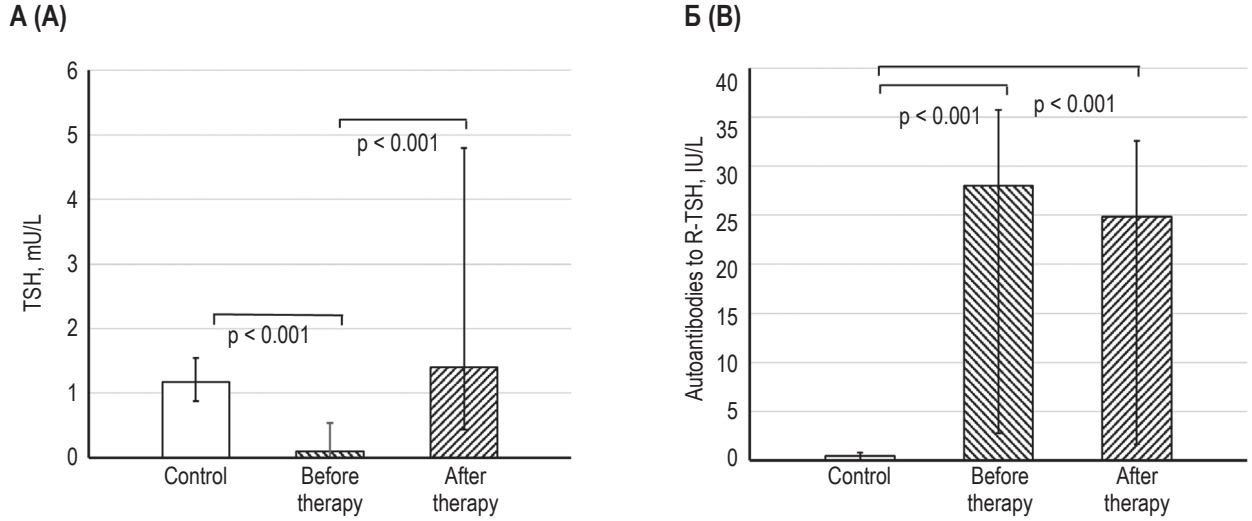

Figure 1. Concentration of TSH (A) and autoantibodies to TSH receptor (B) in the blood serum before and after radioiodine therapy of patients with Graves' disease

TABLE 1. PHENOTYPE OF T LYMPHOCYTES IN THE BLOOD OF PATIENTS WITH GRAVES' DISEASE BEFORE AND AFTER 1 MONTH OF RADIOIODINE THERAPY, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control n = 56		Patients with Graves' disease n = 36			
			Before radioiodine therapy		1 month after radioiodine therapy	
	1		2		3	
	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$
T lymphocytes, 10^9/L	1.51	1.19-1.65	1.71	1.24-2.26	1.14	0.78-1.49
					$p_2 = 0.029$	
$CD3^+$, %	72.0	68.3-76.0	74.6	71.0-77.1	66.3	63.6-76.7
$CD3^+CD25^+$, %	0.21	0.03-1.20	1.42	1.21-1.94	0.19	0.09-0.70
			$p_1 < 0.001$		$p_2 < 0.001$	
$CD3^+CD4^+$, %	41.4	37.8-46.1	47.2	39.9-54.1	43.4	38.9-51.1
			$p_1 = 0.045$			
$CD3^+CD8^+$, %	30.0	26.2-35.0	24.5	18.3-28.5	19.3	12.8-29.9
			$p_1 = 0.024$		$p_1 = 0.017$	
$CD3^+CD4^+CD25^+$, %	0.12	0.03-0.48	0.84	0.64-0.94	0.32	0.08-0.57
			$p_1 = 0.005$		$p_2 = 0.002$	
$CD3^+CD8^+CD25^+$, %	0.06	0.03-0.17	0.31	0.29-0.45	0.05	0.03-0.08
			$p_1 = 0.036$		$p_2 = 0.034$	
$CD3^+CD4^+CD8^+$, %	0.38	0.30-0.85	0.51	0.38-0.98	0.55	0.38-1.08
$CD3^+CD4^+CD127^{Low}CD25^{High}$, %	1.8	0.9-2.8	2.3	1.8-3.5	4.7	2.7-5.9
					$p_1 = 0.004$ $p_2 = 0.048$	

Note. p_1, statistical significant differences with the control group; p_2, -//- with parameters of the patients before radioiodine therapy.

TABLE 2. PHENOTYPE OF B LYMPHOCYTES IN THE BLOOD OF PATIENTS WITH GRAVES' DISEASE BEFORE AND AFTER 1 MONTH OF RADIOIODINE THERAPY, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control n = 56		Patients with Graves' disease n = 36			
			Before radioiodine therapy		1 month after radioiodine therapy	
	1		2		3	
	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$
B lymphocytes, 10^9/L	0.24	0.18-0.33	0.26	0.19-0.35	0.24	0.12-0.35
$CD19^+$, %	11.4	10.7-16.0	9.7	9.1-12.3	13.9	11.8-15.2
			$p_1 = 0.043$		$p_2 = 0.048$	
$CD19^+CD27^-$, %	8.8	6.6-15.8	7.4	6.0-11.8	12.1	8.7-14.9
					$p_2 = 0.044$	
$CD19^+CD27^+$, %	2.3	1.2-2.5	2.2	1.4-3.2	2.6	1.6-2.9
$CD19^+CD5^+$, %	2.0	0.8-5.4	2.8	2.0-3.6	3.1	1.9-4.0
$CD19^+CD5^-$, %	9.0	7.0-14.1	7.6	7.3-10.5	11.1	9.9-12.2
					$p_2 = 0.039$	
$CD19^+CD23^+$, %	5.5	2.1-8.5	5.6	3.8-6.2	4.3	3.7-5.9
$CD19^+CD27^-CD23^+$, %	1.29	0.63-3.72	1.86	1.28-2.19	1.41	1.18-4.00
$CD19^+CD27^+CD23^+$, %	0.17	0.06-0.53	0.44	0.29-0.84	0.24	0.10-0.37
			$p_1 = 0.027$		$p_2 = 0.040$	
$CD19^+CD5^+CD23^+$, %	1.91	0.94-3.59	1.60	0.84-1.91	1.20	0.61-1.41
					$p_1 = 0.035$	
$CD19^+CD5^-CD23^+$, %	2.99	1.83-5.34	3.85	2.99-4.60	3.19	2.35-4.90

Note. As for Table 1.

of B lymphocytes were found in GD patients during this period of the examination: an increase relative to the initial values of the levels of $CD19^+CD27^-$ and $CD19^+CD5^-$ cells, a decrease in the percentage of $CD19^+CD5^+CD23^+$ lymphocytes relative to the control values.

Correlation analysis results

Features of the relationship between indicators of thyroid status and the phenotypic composition of T and B cells in the blood as well as between individual subsets of lymphocytes were investigated using correlation analysis. Examination of the control group showed that the relative amount of $CD3^+CD25^+$ lymphocytes was negatively correlated with the percentage of $CD19^+CD5^+$ cells ($r = -0.68$, $p = 0.008$) and the concentration of TSH also negatively correlates with the percentage of $CD3^+CD4^+CD127^{Low}CD25^{High}$ cells ($r = -0.75$, $p < 0.001$). It was revealed in GD patients before the start of RIT that the relative content of $CD3^+$ and $CD3^+CD8^+CD25^+$ lymphocytes negatively correlated with the percentage of $CD19^+CD23^+$ ($r = -0.82$, $p = 0.009$ and $r = -0.89$, $p < 0.001$, respectively) and $CD19^+CD5^-CD23^+$ cells ($r = -0.83$, $p = 0.008$ and $r = -0.91$, $p < 0.001$, respectively). The concentration of TSH in GD before the start of treatment was positively correlated with $CD3^+CD4^+CD127^{Low}CD25^{High}$ ($r = 0.94$, $p < 0.001$) and $CD19^+CD5^+$ cells ($r = 0.76$, $p = 0.014$) while the level of TSHR-AB in the blood serum was positively correlated with the percentage of $CD3^+CD25^+$ cells ($r = 0.65$, $p = 0.012$). Correlations between the studied parameters were completely absent in GD patients after 1 month of RIT.

Discussion

The character of the GD course before the start of ROT in patients is determined by the state of subclinical hyperthyroidism of autoimmune genesis which is manifested by a high concentration of TSHR-AB, a low TSH level and increased content of fT3. An increase in the percentage of T lymphocytes expressing

the CD25 marker ($CD3^+CD25^+$, $CD3^+CD4^+CD25^+$ and $CD3^+CD8^+CD25^+$) is observed in GD patients in the peripheral blood against this background. CD25 was defined as the α-subunit of the IL-2 receptor (IL-R2α) which in complex with the β (CD122, IL-2Rβ) and γ (CD132, IL-2Rγ) subunits formed the high-affinity IL-2 receptor expressed on activated cells [17, 27]. Accordingly, it can be stated that the GD patients before the start of RIT had an increase in the number of activated T lymphocytes (including helper T cells and cytotoxic T cells) but with a decrease in the level of cytotoxic T cells non-expressing CD25.

When studying the phenotype of B lymphocytes, it was found that GD patients at the stage of pre-RIT examination had a low content of total B lymphocytes against the background of an increase in the relative number of memory B cells expressing the CD23 receptor. It is known that CD23 is a low-affinity IgE receptor whose expression level is increased on activated B lymphocytes [1, 6, 23]. The immunopathogenesis of GD is based on the exit to the periphery of the "forbidden" clone of B cells, which can form a cell clone producing of LATS-factor [4, 21, 25]. It can be assumed that an increase in the number of activated memory B cells with a decrease in the level of total B lymphocytes characterizes these processes of GD immunopathogenesis.

We characterized the dependence of the subset composition of T and B lymphocytes on the state of thyroid status in GD using the methods of correlation analysis. The only relationship between the studied parameters was found in the control group – a negative relationship between the concentration of TSH in serum and the percentage of Treg. Patients with GD at the stage of pre-therapeutic examination had an inversion of this relationship, that is the relationship between the concentration of TSH and the amount of Treg became positive. It can be concluded that the regulatory effect of the thyroid status in GD is focused on the stimulation of immune and, accordingly, autoimmune reactions since patients had a positive relationship between the TSH concentration and the number of activated B lymphocytes as well as between the level of TSHR-AB and activated T cells at this stage of the examination.

The relationship between the subpopulation composition of T and B lymphocytes was also investigated using correlation analysis. The only relationship revealed in the control group was characterized by the presence of a negative relationship between the number of activated T lymphocytes and the level of B1 cells in the blood. Negative relationships between the percentage of T lymphocytes and activated (expressing the CD25 antigen) cytotoxic T cells with activated (expressing the CD23 antigen) B lymphocytes and B2 cells have been found in GD. These relationships characterize the presence of competitive processes in the cooperative interaction of T and B lymphocytes against the background of impaired thyroid status and the development of autoimmune processes in GD.

The serum TSH concentration in GD patients increased significantly and reached the control range in one month after RIT. However, the high level of TSHR-AB and the concentration of fT3 remained in the patients examined at this stage. A similar condition in the early post-radiation period with GD can be associated with both transient hyperthyroidism and relapse of the disease (due to the destructive effect of ^{131}I on thyroid gland) as well as the ingress of intrathyroid autoantigens into the peripheral bloodstream [18, 24]. However, the number of activated total T lymphocytes in GD patients with transient hyperthyroidism was normalized due to a decrease in the levels of activated T helpers and cytotoxic T cells to control values. At the same time, the reduced content of cytotoxic T cells in the examined patients remained but the amount of Treg increased (more than 2 times). Similar changes in the phenotypic composition of blood T lymphocytes in GD patients characterize a decrease in functional activity (due to a decrease in the number of CD25 expressing T cells) as well as, accordingly, in autoreactivity (due to an increase in the number of Treg).

The number of B lymphocytes and activated memory B cells returned to normal in GD patients one month after RIT. But at the same time, an increase in the content of naive B lymphocytes and B2 cells relative to the initial level as well as a decrease in the number of activated B1 cells were found in GD patients relative to the control range. It is known that the development and maintenance of certain autoimmune processes in the body is associated with B1 cells [7, 11, 16]. Therefore, the changes in the subset composition of B lymphocytes in patients with GD one month after RIT also determine the trend towards normalization of the functional activity of B cells including from the standpoint of the implementation of autoimmune aggression.

The complete absence of correlations between the studied indicators of thyroid status and the immune system as well as between different subsets of T and B cells was revealed in GD patients a month after therapy against the background of characterized changes in the phenotypic composition of T and B lymphocytes. This condition is determined by an imbalance in the thyroid regulation of immune processes which develops against the background of the breakdown of thyrocytes due to the action of ^{131}I. It can also be assumed that in this case, the different sensitivity of T and B lymphocytes to 131I in long-term period after RIT in GD take revenge and ultimately may realized as disorders of cooperative interaction during the development of the immune response.

Conclusion

Thus, it was found that the GD patients before the treatment with radioactive iodine had a high level of functional activity of the immune system cells which was determined by the expression of the CD25 antigen on T lymphocytes and the CD23 antigen on B lymphocytes. A high level of functional activity of the adaptive immunity cells in patients with GD was manifested against the background of an increased level of TSHR-AB. It was found with the help of correlation analysis that the state of the thyroid status in GD patients during the pre-therapeutic examination determined the stimulation of the functional activity of T and B lymphocytes and accordingly increased the level of autoimmune processes. At the same time, based on the analysis of the features of the relationships between the subset composition of cells, it can be assumed that there are competitive relationships in the cooperative interaction of T and B lymphocytes in GD patients. The number of activated T lymphocytes (including T helpers and cytotoxic T cells) decreased to control values in GD patients 1 month after RIT against the background of transient hyperthyroidism (while maintaining an increased concentration of TSHR-AB). However, a low level of cytotoxic T lymphocytes in the blood and a significant increase in the Treg content in GD patients persisted during this period of the examination. A decrease in the number of B cells and activated memory B cells to the level of the control range was found in GD patients one month after RIT in the study of the phenotype of blood B lymphocytes. At the same time, an increase in the levels of naive B lymphocytes and B2 cells as well as a decrease in the number of activated B1 lymphocytes were detected in GD. All changes in the subset composition of T and B cells and in their phenotype developed against the background of a complete loss of relationships between the studied parameters, which characterized the loss of thyroid control of immune processes and the cooperative interaction of cells during the development of an immune response. In general, changes in the phenotype of T and B lymphocytes in the blood of patients with GD 1 month after treatment with radioactive iodine reflect a tendency towards a decrease in the functional activity of adaptive immunity cells which can also be realized in the inhibition of autoimmune processes.

References

1. Belenyuk V.D., Savchenko A.A., Borisov A.G., Kudryavtsev I.V. Features of peripheral blood B-cell subset phenotype are associated with clinical outcome of widespread purulent peritonitis. *Russian Journal of Infection and Immunity, 2021, Vol. 11, no. 3, pp. 454-462.* (In Russ.). doi: 10.15789/ 2220-7619-CBC-1397.

2. Corvilain B., Hamy A., Brunaud L., Borson-Chazot F., Orgiazzi J., Bensalem Hachmi L., Semrouni M., Rodien P., Lussey-Lepoutre C. Treatment of adult Graves' disease. *Ann. Endocrinol. (Paris), 2018, Vol. 79, no. 6, pp. 618-635.*

3. Côté-Bigras S., Tran V., Turcotte S., Rola-Pleszczynski M., Verreault J., Rottembourg D. Impaired immune regulation after radioiodine therapy for Graves' disease and the protective effect of Methimazole. *Endocrine, 2016, Vol. 52, no. 3, pp. 587-596.*

4. Davies T.F., Andersen S., Latif R., Nagayama Y., Barbesino G., Brito M., Eckstein A.K., Stagnaro-Green A., Kahaly G.J. Graves' disease. *Nat. Rev. Dis. Primers., 2020, Vol. 6, no. 1, 52.* doi: 10.1038/s41572-020-0184-y.

5. Du W., Dong Q., Lu X., Liu X., Wang Y., Li W., Pan Z., Gong Q., Liang C., Gao G. Iodine-131 therapy alters the immune/inflammatory responses in the thyroids of patients with Graves' disease. *Exp. Ther. Med., 2017, Vol. 13, no. 3, pp. 1155-1159.*

6. Engeroff P., Caviezel F., Mueller D., Thoms F., Bachmann M.F., Vogel M. CD23 provides a noninflammatory pathway for IgE-allergen complexes. *J. Allergy Clin. Immunol., 2020, Vol. 145, no. 1, pp. 301-311.*

7. Feng C., Li L., Zhou L., Li D., Liu M., Han S., Zheng B. Critical roles of the E3 ubiquitin ligase FBW7 in B-cell response and the pathogenesis of experimental autoimmune arthritis. *Immunology, 2021, Vol. 164, no. 3, pp. 617-636.*

8. Gallo D., Piantanida E., Gallazzi M., Bartalena L., Tanda M.L., Bruno A., Mortara L. Immunological drivers in Graves' Disease: NK Cells as a Master Switcher. *Front. Endocrinol. (Lausanne), 2020, Vol. 11, 406.* doi: 10.3389/ fendo.2020.00406.

9. Giuliani C., Saji M., Bucci I., Napolitano G. Bioassays for TSH Receptor Autoantibodies, from FRTL-5 Cells to TSH Receptor-LH/CG Receptor Chimeras: The Contribution of Leonard D. Kohn. *Front. Endocrinol. (Lausanne), 2016, Vol. 7, 103.* doi: 10.3389/fendo.2016.00103.

10. Ji X., Wan J., Chen R., Wang H., Huang L., Wang S., Su Z., Xu H. Low frequency of IL-10-producing B cells and high density of ILC2s contribute to the pathological process in Graves' disease, which may be related to elevated-TRAb levels. *Autoimmunity, 2020, Vol. 53, no. 2, pp. 78-85.*

11. Kageyama Y., Katayama N. Ontogeny of human B1 cells. *Int. J. Hematol., 2020, Vol. 111, no. 5, pp. 628-633.*

12. Klotz L., Burgdorf S., Dani I., Saijo K., Flossdorf J., Hucke S., Alferink J., Nowak N., Beyer M., Mayer G., Langhans B., Klockgether T., Waisman A., Eberl G., Schultze J., Famulok M., Kolanus W., Glass C., Kurts C., Knolle P.A. The nuclear receptor PPAR gamma selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS autoimmunity. *J. Exp. Med., 2009, Vol. 206, no. 10, pp. 2079-2089.*

13. Kudryavtsev I.V., Subbotovskaya A.I. Application of six-color flow cytometric analysis for immune profile monitoring. *Medical Immunology (Russia), 2015, Vol. 17, no. 1, pp. 19-26.* (In Russ.). doi: 10.15789/1563-0625-2015-1-19-26.

14. Li J.F., Xie L.J., Qin L.P., Liu Y.F., Zhang T.J., Huang Y., Cheng M.H. Apoptosis gene reprograming of human peripheral blood mononuclear cells induced by radioiodine-131 ((131)I) irradiation. *Indian J. Med. Res., 2019, Vol. 149, no. 5, pp. 627-632.*

15. Lindgren O., Asp P., Sundlöva., Tennvall J., Shahida B., Planck T., Åsman P., Lantz M. The effect of radioiodine treatment on trab, Anti-TPO, and Anti-TG in graves' disease. *Eur. Thyroid J., 2019, Vol. 8, no. 2, pp. 64-69.*

16. Lushova A.A., Zheremyan E.A., Astakhova E.A., Spiridonova A.B., Byazrova M.G., Filatov A.V. B-lymphocyte subsets: functions and molecular markers. *Immunologiya, 2019, Vol. 40, no. 6, pp. 63-76.* (In Russ.). doi: 10.24411/0206-4952-2019-16009.

17. Mahmoodpoor A., Paknezhad S., Shadvar K., Hamishehkar H., Movassaghpour A.A., Sanaie S., Ghamari A.A., Soleimanpour H. Flow Cytometry of CD64, HLA-DR, CD25, and TLRs for Diagnosis and Prognosis of Sepsis in Critically Ill Patients Admitted to the Intensive Care Unit: A Review Article. *Anesth. Pain Med., 2018, Vol. 8, no. 6, e83128.* doi: 10.5812/aapm.83128.

18. Riley A.S., McKenzie G.A.G., Green V., Schettino G., England R.J.A., Greenman J. The effect of radioiodine treatment on the diseased thyroid gland. *Int. J. Radiat. Biol., 2019, Vol. 95, no. 12, pp. 1718-1727.*

19. Sánchez Á., Contreras-Jurado C., Rodríguez D., Regadera J., Alemany S., Aranda A. Hematopoiesis in aged female mice devoid of thyroid hormone receptors. *J. Endocrinol., 2020, Vol. 244, no. 1, pp. 83-94.*

20. Savchenko A.A., Dudina M.A., Borisov A.G., Dogadin S.A., Kudryavtsev I.V., Moshev A.V., Mankovskiy V.A. Effects of helper and regulatory T cells upon phenotypic composition of blood B lymphocytes and thyroid gland in Graves' disease. *Medical Immunology (Russia), 2018, Vol. 20, no. 3, pp. 431-438.* (In Russ.). doi: 10.15789/1563-0625-2018-3-431-438.

21. Struja T., Kutz A., Fischli S., Meier C., Mueller B., Recher M., Schuetz P. Is Graves' disease a primary immunodeficiency? New immunological perspectives on an endocrine disease. *BMC Med., 2017, Vol. 15, no. 1, 174.* doi: 10.1186/s12916-017-0939-9.

22. Sutherland D.R., Ortiz F., Quest G., Illingworth A., Benko M., Nayyar R., Marinov I. High-sensitivity 5-, 6-, and 7-color PNH WBC assays for both Canto II and Navios platforms. *Cytometry B Clin. Cytom., 2018, Vol. 94, no. 4, pp. 637-651.*

23. Sutton B.J., Davies A.M. Structure and dynamics of IgE-receptor interactions: FcεRI and CD23/FcεRII. *Immunol. Rev., 2015, Vol. 268, no. 1, pp. 222-235.*

24. Troshina E.A., Sviridenko N.Yu., Vanushko V.E., Rumyantsev P.O., Fadeev V.V., Petunina N.A. Federal clinical recommendations of the Russian Association of Endocrinologists for the diagnosis and treatment of toxic goiter. *Clinical and Experimental Thyroidology, 2014, Vol. 10, no. 3, pp. 8-19.* (In Russ.).

25. Wiersinga W.M. Graves' Disease: Can It Be Cured? *Endocrinol. Metab. (Seoul), 2019, Vol. 34, no. 1, pp. 29-38.*

26. Zhang D., Qiu X., Li J., Zheng S., Li L., Zhao H. MiR-23a-3p-regulated abnormal acetylation of FOXP3 induces regulatory T cell function defect in Graves' disease. *Biol. Chem., 2019, Vol. 400, no. 5, pp. 639-650.*

27. Zohouri M., Mehdipour F., Razmkhah M., Faghih Z., Ghaderi A. $CD4^{+}CD25^{-}FoxP3^{+}$ T cells: a distinct subset or a heterogeneous population? *Int. Rev. Immunol., 2021, Vol. 40, no. 4, pp. 307-316.*

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