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Features of immunoregulation in patients with pulmonary tuberculosis with blood eosinophilia

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ABSTRACT

Aim of the research – to investigate the features of the immune response regulation in pulmonary tuberculosis (TB) and analyze the role of regulatory T cells in the immunopathogenesis of TB with blood eosinophilia in different clinical forms of the diseases and with regard to the sensitivity of Micobacterium tuberculosis to antituberculosis drugs.

Materials and methods. 157 patients with newly diagnosed infiltrative and disseminated TB were examined. The material of the study was venous blood and the culture of mononuclear leukocytes isolated from venous blood. The level of interleukin (IL) 4, IL-10 and transforming growth factor beta (TGF□) in supernatants of culture suspensions of mononuclear leukocytes in vitro and the level of IL-5 in the blood were determined with the enzyme-linked immunosorbent assay. Evaluation of the expression of surface molecules CD4, CD20, CD25 and intracellular transcription factor Foxp3 in blood lymphocytes was performed by flow cytometry. The results were analyzed by statistical methods.

Results. It was shown that in patients with TB, excessive generation of regulatory T cells is associated with eosinophilia of the blood and an imbalance in the mechanisms of regulation of the immune response. In TB with eosinophilia, an increase in the number of Foxp3-positive regulatory T cells in the blood is combined with hypersecretion in vitro of anti-inflammatory cytokines TGF□, IL-10, IL-4 and an increase in CD20+ B-lymphocyte and IL-5 in the blood. These changes were most pronounced in the disseminated form of TB in combination with drug resistance of the pathogen.

Conclusion. Features of immunoregulation in TB with eosinophilia of blood are associated with activation of immunosuppression mechanisms and polarization of the immune response in the direction of the Th2-dependent pathway.

Key words: pulmonary tuberculosis, regulation of the immune response, regulatory T cells, eosinophilia.

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INTRODUCTION

Pulmonary tuberculosis (TB) is an immunocompromising disease with intracellular parasitization of the pathogen. Infection with *Mycobacterium tuberculosis* (MTB) leads to the mobilization of various populations of myeloid and lymphoid immune cells which are capable, through intercellular contacts and secretion of immunoregulatory biomolecules, of influencing all links and stages of the immune response, thus determining the direction of its development, activation and suppression. Factors of innate and adaptive immunity in the process of immunological surveillance of antigens of different origin do not function in isolation from one another, but have a mutually directed positive and negative regulation [1–3].

One of the mechanisms of the formation of the suppressor regimen of immunoregulation in TB is an excessive generation and conversion of regulatory T cells, which contribute to the reduction of the number of different subpopulations of T-helpers and weaken the antimycobacterial immunity of the macroorganism as a whole [3]. Studies carried out in the field of immunopathogenesis of TB indicate that in case of tuberculosis infection, an increase in the number of Tregs in the blood is associated with an increase in the number of eosinophilic granulocytes [4].

Eosinophils are the polyfunctional granulocytes possessing a wide spectrum of bactericidal and cytotoxic factors (metabolites of oxygen and nitrogen, lipid mediators, granule proteins), phagocytic, antigen-presenting and immunoregulatory properties [5]. Increasing knowledge about the components of granules, inducible mediators and surface receptors of eosinophils indicates that these cells are the active participants of the reactions that form the basis of the pathogenesis of tuberculosis infection. However, the modulating role of eosinophils in the development of antituberculosis immunity remains to be fully elucidated.

Thus, the purpose of this work was to analyze the role of regulatory T cells in the regulation of the immune response in TB with eosinophilia of the blood.

MATERIALS AND METHODS

A total of 157 patients with TB (108 men and 49 women) aged 18 to 55 years (4.94±10.63 years old in average) were enrolled in the study. TB was diagnosed on the basis of a clinical picture of the disease, lung X-ray, and microscopic and bacteriological sputum examination data. All patients with TB were examined prior to administration of etiotropic antituberculsis chemotherapy. Based on the number of eosinophils in the blood of TB patients, two main study groups were formed. The first group (n=76) consisted of patients with eosinophilia-associated TB who had absolute and relative number of eosinophils in the blood of (0.966±0.110)Ч10^9/l and (8.790±0.250)%, respectively. The second group (n=81) included TB patients without blood eosinophilia (number of eosinophils in the blood of (0.249±0.010)Ч10^9/l and 2.572±1.190%). Out of all examined patients, 94 people had infiltrative TB, and 63 people – disseminated TB. In all enrolled TB patients, the sensitivity of the MBT to the main antituberculosis drugs was investigated. As a result, in 99 patients MTB was found to be sensitive to the main antituberculosis drugs (isoniazid, rifampicin, streptomycin, ethambutol), while in 58 patients MTB was drug-resistant.

The comparison group consisted of 78 healthy donors (52 men and 26 women) aged 23 to 50 (41.31±7.47 years old in average).

The criteria for excluding TB patients from the study were the treatment with anti-tuberculosis, nonsteroidal anti-inflammatory drugs, and glucocorticosteroids; immunotherapy; concomitant oncological, endocrine, autoimmune and allergic diseases; co-inflection with hepatitis, HIV, and other viruses.

In all patients with TB and in healthy donors, parasitic infestation was ruled out (based on the results of anamnesis, coroscopy and antibody titres to helminth antigens assessed by the enzyme immunoassay).

The material of the study was blood from the ulnar vein, taken in the morning on an empty stomach in an amount of 20 ml, and supernatants of a suspension culture of mononuclear leukocytes. Blood sampling in healthy volunteers and patients with TB (before the appointment of specific chemotherapy) was performed only once.

Isolation of mononuclear leukocytes from venous blood was performed on a ficoll-urographin density gradient (1.077 g/ml) (Medbiospectr, Russia).

Measurement of the concentration of interleukin (IL) 4, IL-10, and transforming growth factor beta (TGFβ) in supernatants of culture suspensions of mononuclear leukocytes and IL-5 in the blood was performed by solid-phase enzyme-linked immunosorbent assay (ELISA), according to manufacturer's instructions (Protein Contour, Russia; Biosource, USA). The optical density of the plate-well contents was evaluated using a photometer-analyzer Multiscan EX (Finland) at a wavelength of 450 nm.

To determine the surface molecules CD4, CD20, and CD25, and intracellular transcription factor Foxp3 in the blood lymphocytes, flow cytometry was performed using fluorochrome-labeled monoclonal antibodies (PerCP, FITC, PE), according to the manufacturer's protocol (Becton Dickinson (BD), USA). Samples were analyzed on the «FACS Calibur Flow cytometer BD» (BD, USA). Data analysis was performed using «BD CellQuest for Mac OS® X» software (BD, USA).

For statistical analysis, an application package «Statistica for Windows» Version 8.0 («StatSoft Inc.», USA) was used. The compliance of the sample data with the normal distribution law was verified by the Shapiro-Wilk test. Since all quantitative parameters in the comparison groups did not have a normal distribution, the results were presented as a median (Me), upper (75%) and lower (25%) quartiles (Me (Q1-Q3)). The reliability of the differences in the samples that did not follow a normal distribution was estimated using the nonparametric U Mann-Whitney criterion for independent samples. Differences were considered reliable at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Number of Tregs in blood in patients with pulmonary tuberculosis with and without eosinophilia

Various subpopulations of regulatory T cells and eosinophilic granulocytes are involved in the reactions of both the innate and acquired immune response. Imbalance of cytokines produced by Treg and eosinophils programs the directivity of the immune response along the path of dominance of individual subpopulations of T-lymphocyte helper cells – Th (Th1, Th2 and/or Treg), which can lead to inefficient implementation of protective antituberculous immunity.

Among Treg cells, two main subpopulations are currently identified: natural thymic (Tnr) and induced on periphery Tregs (Tir) [6–8]. It is shown that Tregs express a variety of surface and intracellular molecules which contribute to their immunosuppressive effect. For example, using the membrane molecule CD25 (α-chain of the receptor for IL-2), Tregs competitively bind IL-2, affecting the process of cytokine-induced activation of other T-cells that are sensitive to it. The maximum suppressor activity is characteristic to Foxp3-positive Tregs [9]. Expression of the intracellular transcription factor Foxp3 leads to the induction of the Treg differentiation genes and of the inhibitory cytokines production (IL-10 and TGFβ), which suppress the functional activity of effector T cells [10–12]. According to a number of researchers, excessive activation of Treg mediates the decrease in the effectiveness of antituberculosis effector immune response [2].

It is known that due to the expression of the membrane TLR2 receptor (Toll-like receptor) and γδTCR (T-cell receptor), eosinophils are able to recognize MTB antigens with subsequent secretion of suppressive cytokines [13, 14]. Thus, TGFβ secreted by eosinophilic granulocytes induces the expansion and increased functional activity of Tregs [15].

Table 1

Number of $CD4^+CD25^+Foxp3^+$ regulatory T cells in the blood of pulmonary tuberculosis patients, *Me* (Q_1–Q_3)

Groups of enrolled patients		$CD4^+CD25^+Foxp3^+$ Treg (%)	
Healthy donors		2.63 (2.00–3.29)	
Pulmonary tuberculosis patients (TB)		With drug-sensitive TB	With drug-resistant TB
With blood eosinophilia	Infiltrative	3,93 (3,11–6,00) $p_1 < 0,05$	4,83 (5,21–8,24) $p_1 < 0,05$
	Disseminated	6,67 (4,56–8,20) $p_1 < 0,05$	7,04 (6,78–10,48) $p_1 < 0,05$
Without blood eosinophilia	Infiltrative	3,26 (2,00–7,15) $p_1 < 0,05$	3,98 (1,95–6,85) $p_{1,2} < 0,05$
	Disseminated	4,66 (4,02–5,48) $p_{1,2,3} < 0,05$	5,25 (2,78–6,00) $p_{1,2,3} < 0,05$

N o t e. Here and in Tables 2-7: p_1 – the level of statistical significance of the differences in comparison with parameters of healthy donors; p_2 – in patients with pulmonary tuberculosis with eosinophilia; p_3 – in patients with infiltrative pulmonary tuberculosis; p_4 – in patients with drug-sensitive pulmonary tuberculosis.

Table 2

Number of $CD4^+CD25^-Foxp3^+$ regulatory T cells in the blood of pulmonary tuberculosis patients, *Me* (Q_1–Q_3)

Groups of enrolled patients			
Healthy donors		5,12 (4,76–9,75)	
Patients with pulmonary tuberculosis (PT)		Drug-sensitive TB	Drug-resistant TB
With blood eosinophilia	infiltrative	6,06 (4,70–8,34)	5,65 (4,94–13,02)
	disseminated	5,76 (3,00–5,99)	6,36 (3,29–7,04) $p_1 < 0,05$
Without blood eosinophilia	infiltrative	5,00 (3,04–6,95) $p_2 < 0,05$	4,62 (3,54–7,00) $p_2 < 0,05$
	disseminated	6,00 (5,79–7,14) $p_3 < 0,05$	6,75 (5,95–7,43) $p_1 < 0,05$

In the course of the study, a significant increase (in comparison with a group of healthy individuals) in the number of $CD4^+CD25^{+-}$ $Foxp3^+$ Tregs in the blood of TB patients was found, and was especially pronounced, in patients with blood eosinophilia (compared to patients without eosinophilia), with disseminated form of TB (compared to infiltrative TB) and with drug-resistant TB (compared to drug-sensitive TB). At the same time, the number of CD25-negative Treg cells expressing FoxP3 was increased in patients with drug-resistant disseminated TB alone, regardless of the presence of blood eosinophilia (Table 1, 2).

Secretion *in vitro* of immunosuppressive cytokines IL-10 and TGFβ in patients with pulmonary tuberculosis depending on the content of eosinophils in the blood

It is known that IL-10 inhibits the synthesis and secretion of regulatory cytokines by all subpopulations of helper T-lymphocytes (Th1, Th2 and Th17), and also reduces the functional activity of antigen presenting cells (APC). The significant role of IL-10 in limiting the development of reactions of innate and acquired anti-infective immunity is well established [16–18], including the inhibition of the proliferative response of T cells against the MTB [3].

In the course of the study, we found an increase in basal and BCG-induced IL-10 secre-

tion *in vitro* by mononuclear blood leukocytes in drug-sensitive infiltrative TB without eosinophilia (Table 3). In patients with TB with eosinophilia, regardless of the form of the disease and the drug sensitivity of the pathogen to the antituberculosis drugs, the basal secretion of IL-10 was comparable to the norm, while the BCG-induced level of the cytokine secretion significantly exceeded that of healthy volunteers (Table 3).

It should be noted that with TB with eosinophilia, the normal basal secretion level of IL-10 *in vitro* was combined with an increased content of $CD4^+CD25^+Foxp3^+$ T-lymphocytes in the blood.

Table 3

IL-10 secretion in vitro in the culture of mononuclear leukocytes in patients with pulmonary tuberculosis (in the numerator – intact, in the denominator – BCG-induced) (pg/ml) (*Me* (Q_1–Q_3))

Groups of enrolled patients			
Healthy donors		25,29 (13,50–33,56) 26,21 (22,74–60,22)	
Patients with pulmonary tuberculosis (PT)		Drug-sensitive TB	Drug-resistant TB
With blood eosinophilia	infiltrative	20,91 (14,07–29,24) 43,17 (32,73–61,28) $p_{1,5} < 0,05$	22,56 (13,70–29,89) 42,98 (31,63–63,15) $p_1 < 0,05$
	disseminated	19,00 (16,740–24,71) 46,81 (23,90–70,52) $p_{1,5} < 0,05$	20,37 (17,29–25,12) 44,93 (25,18–69,30) $p_1 < 0,05$
Without blood eosinophilia	infiltrative	44,92 (18,75–57,64) $p_{1,2} < 0,05$ 55,49 (32,22–65,28) $p_{1,2,5} < 0,05$	27,51 (24,18–42,71) $p_4 < 0,05$ 24,51 (25,62–53,21) $p_{2,4} < 0,05$
	disseminated	24,11 (9,54–50,72) $p_{2,3}<0,05$ 33,52 (20,64–66,17) $p_{3,5}<0,05$	20,07 (18,22–21,13) 26,52 (23,57–35,24) $p_2<0,05$

Note. Here and in Tables 4–5: p_5 – compared with intact cell culture.

$CD4^+CD25^+Foxp3^+$ regulatory T lymphocytes are considered to be the main TGFβ-producing cell type, though other cells are also able to secrete this cytokine, including activated T-lymphocytes, monocytes/macrophages, eosinophils, platelets, chondrocytes, osteoblasts and osteoclasts [16]. The main property of TGFβ is an ability to suppress all types of immune responses, primarily those mediated by helper T-lymphocyte type 1 [16]. Under the action of TGFβ, with the participation of costimulatory molecules, T-helpers are converted into regulatory T cells on the periphery of the immune system (from $CD4^+CD25^-$ T-cells into $CD4^+CD25^+Foxp3^+$ Tregs) [7, 19]. The manifestation of this kind of transformation is the expression of the transcription factor Foxp3 (scurphin) directly inside the cell, as well as the CD25 and CTLA-4 molecules (cytotoxic T-lymphocyte-associated protein 4) on its surface. It is known that TGFβ can alter the functional activity of Treg and their sensitivity to apoptosis by increasing the expression of the FOXP3 gene that is localized in the X chromosome [8, 9].

According to the results obtained, the level of secretion of TGFβ in an *in vitro* culture of mononuclear leukocytes in TB changed in different ways (Table 4). Thus, in patients with TB in combination with eosinophilia of blood, a significant increase in basal secretion of TGFβ was identified. At the same time, BCG-induced *in vitro* TGFβ secretion by mononuclear leukocytes in patients with drug-sensitive and drug-resistant infiltrative TB with eosinophilia was decreased, while in patients with dissem-

inated TB with eosinophilia (regardless of the sensitivity of the pathogen to the antituberculosis drugs), on the contrary, it was higher than in group of healthy donors. Similarly, an elevated level of BCG-stimulated *in vitro* secretion of TGFβ was detected in drug-resistant disseminated TB without eosinophilia; in patients with drug-sensitive infiltrative TB without eosinophilia, basal and induced secretion of TGFβ varied within normal limits, while in the remaining groups of patients without eosinophilia, its deficiency was revealed. In general, the maximum increase in basal and BCG-induced secretion of this mediator *in vitro* was detected in drug-resistant disseminated TB with eosinophilia (Table 4).

Table 4

TGFβ secretion *in vitro* in the culture of mononuclear leukocytes in patients with pulmonary tuberculosis (in the numerator – intact, in the denominator – BCG-induced) (pg/ml), *Me* (Q_1–Q_3)

Groups of enrolled patients			
Healthy donors		1108,75 (929,80–1487,20) 1087,80 (500,00–1412,60)	
Pulmonary tuberculosis patients (TB)		With drug-sensitive TB	With drug-resistant TB
With blood eosinophilia	infiltrative	1299,03 (840,59–1327,78) $p_1 < 0,05$ 627,50 (553,50–731,71) $p_{1,5} < 0,05$	1001,60 (630,74–1103,00) $p_4 < 0,05$ 652,10 (545,91–743,06) $p_{1,5} < 0,05$
	disseminated	1421,31 (769,45–2140,73) $p_{1,3} < 0,05$ 1267,83 (771,45–1663,00) $p_{1,5} < 0,05$	1632,12 (774,90–2005,78) $p_{1,3,4} < 0,05$ 1365,48 (829,40–1748,22) $p_{1,3,5} < 0,05$
Without blood eosinophilia	infiltrative	1062,91 (792,24–1613,57) $p_2 < 0,05$ 1125,92 (875,16–1215,07) $p_2 < 0,05$	578,02 (315,29–781,46) $p_{1,2,4} < 0,05$ 752,17 (495,32–991,45) $p_{1,4,5} < 0,05$
	disseminated	923,62 (728,24–1427,19) $p_{1,2,3} < 0,05$ 873,18 (571,11–031,92) $p_{1,2,3} < 0,05$	1072,33 (915,61–2452,27) $p_{1,2,3} < 0,05$ 1986,58 (792,53–3009,68) $p_{1,2,3,4,5} < 0,05$

Hypersecretion of TGFβ in TB accompanied by eosinophilia indicates an increase in the reactivity of the main producing cells of this cytokine, the Tregs. Given that TGFβ mediates proliferation, differentiation, and activation of immunosuppressive properties of Tregs, its excessive secretion may be the cause of an increase in the total number of Treg and their individual subpopulations in the blood in patients with TB with eosinophilia. At the same time, it is shown that in diseases accompanied by eosinophilia, eosinophilic granulocytes may also represent a source of TGFβ [13, 15, 20].

One of the mechanisms of mutually directed regulation of the functional activity of Tregs and eosinophils in TB can be the formation of an «immunosuppressive» microenvironment in the focus of granulomatous inflammation through the secretion of a specific enzyme indolyl-2,3-dioxygenase (IDO) by these cell populations. Information on the role of the enzyme in the formation of the suppressor regimen of immune regulation appeared in the literature relatively recently and was referred to as the «tryptophan degradation mechanism» [6,21]. Simultaneous induction of IDO in tolerogenic dendritic cells (TDC) and Tregs is considered as a possible mechanism of an inhibitory effect of $CD4^+CD25^+Foxp3^+$ natural regulatory T cells [6, 9]. Eosinophilic granulocytes can also synthesize IDO, catalyzing the conversion of tryptophan to kynurenin, which in turn regulates the balance of Th1/Th2 lymphocytes by inducing apoptosis of Th1 cells [22].

Parameters of Th2-immune response in patients with pulmonary tuberculosis with eosinophilia

The key cytokine of the Th2 immune response is IL-4, which stimulates the clonal proliferation of B-lymphocytes and their maturation into plasma cells secreting antibodies. In addition, IL-4, along with IL-2, regulates the balance between suppression and activation of immune responses [23]. Some authors suggest the ability of IL-4 to inhibit apoptosis of Treg and increase their suppressor activity [24].

According to the results of this study, the level of basal secretion of IL-4 by mononuclear leukocytes *in vitro* was significantly higher than normal in all TB patients with eosinophilia, regardless of the clinical form of the disease and the sensitivity of the pathogen to the antituberculosis drugs, while in patients without eosinophilia, the increase in basal cytokine secretion was recorded only with a drug-resistant variant of disseminated TB (Table 5). BCG-induced *in vitro* secretion of IL-4 was increased only in disseminated TB, both with and without eosinophilia. In general, the most pronounced changes in the secretion of IL-4 *in vitro* were established in disseminated TB in combination with drug resistance of the pathogen (Table 5), that fits into the existing ideas about the immunopathogenesis of this form of TB. It is known that in disseminated TB, a predominant Th2 type of immune response is realized with activation of B-lymphocytes and immunoglobulin-secreting function of plasma cells formed from them [3].

Table 5

IL-4 secretion *in vitro* in culture of mononuclear leukocytes in patients with pulmonary tuberculosis (in the numerator - intact, in the denominator – BCG-induced), *Me* (Q_1–Q_3)

Groups of enrolled patients			
Healthy donors		39,98 (21,14–55,04) 43,69 (26,46–68,55)	
Pulmonary tuberculosis patients (TB)		With drug-sensitive TB	With drug-resistant TB
With blood eosinophilia	infiltrative	57,13 (35,72–78,41) $p_1 < 0,05$ 45,64 (32,04–60,00)	62,36 (37,11–79,45) $p_1 < 0,05$ 39,23 (35,65–56,32)
	disseminated	56,39 (24,14–70,63) $p_{1,3} < 0,05$ 60,47 (40,11–78,49) $p_1 < 0,05$	52,24 (23,55–66,23) $p_{1,3} < 0,05$ 59,92 (41,34–77,39) $p_{1,3} < 0,05$
Without blood eosinophilia	infiltrative	30,54 (16,30–51,42) $p_2 < 0,05$ 29,15 (19,55–52,42) $p_2 < 0,05$	36,29 (17,18–47,53) $p_2 < 0,05$ 35,82 (20,22–54,61) $p_2 < 0,05$
	disseminated	38,32 (39,94–74,81) $p_2 < 0,05$ 59,13 (44,37–75,12) $p_{1,3,5} < 0,05$	60,73 (41,15–72,39) $p_{1,3,4} < 0,05$ 51,74 (44,79–59,72) $p_{1,3} < 0,05$

Another mediator of the Th2-mediated (humoral) immune response is IL-5 [25]. IL-5 possesses not only eosinophil-activating properties but, in cooperation with IL-4 and IL-13, also induces the proliferation and differentiation of B-lymphocytes ($CD20^+$), secretion of immunoglobulins of different classes by plasma cells and anti-inflammatory cytokines by Th2-lymphocytes [25].

In patients with TB with eosinophilia, regardless of the clinical form of the disease and the sensitivity of the pathogen to the antituberculosis drugs, an increase in the content of IL-5 in the blood was detected. Among patients with disseminated TB with eosinophilia, the maximum serum concentration of IL-5 was determined in the case of drug resistance TB. An increase in the concentration

of IL-5 in the blood correlated with basal hypersecretion of IL-4 mononuclear leukocytes *in vitro* ($r = 0.88$, $p < 0.05$ and $r = 0.74$, $p < 0.05$ in infiltrative and disseminated TB, respectively) (Table 6).

In all patients with TB, a statistically significant increase in the relative number of $CD20^+$ B-lymphocytes in the blood was identified. At the same time, their absolute content was increased only in patients with infiltrative and disseminated TB accompanied by eosinophilia (Table 7).

Table 6

IL-5 concentration in the serum of pulmonary tuberculosis patients (pg/ml), *Me* (Q_1–Q_3)

Healthy donors		7,99 (7,56–19,44)	
Pulmonary tuberculosis patients (TB)		With drug-sensitive TB	With drug-resistant TB
With blood eosinophilia	infiltrative	65,66 (51,43–72,28) $p_1 < 0,05$	64,49 (48,12–69,41) $p_1 < 0,05$
	disseminated	54,02 (37,93–62,06) $p_1 < 0,05$	71,52 (56,73–77,32) $p_{1,4} < 0,05$
Without blood eosinophilia	infiltrative	11,32 (9,40–13,54) $p_2 < 0,05$	9,15 (7,11–10,05) $p_2 < 0,05$
	disseminated	10,94 (7,76–11,49) $p_2 < 0,05$	11,46 (10,06–21,30) $p_2 < 0,05$

Таблица 7
Table 7

Number of CD20+ B lymphocytes in the blood in pulmonary tunerculosis patients, *Me* (Q_1–Q_3)

Groups of enrolled patients				
Healthy donors		%	7,13 ± 3,07	
		$\times 10^9$	0,13 ± 0,01	
Groups of enrolled patients			With drug-sensitive TB	With drug-resistant TB
With blood eosinophilia	infiltrative	%	17,34 ± 3,00 $p_1 < 0,05$	18,01 ± 2,42 $p_1 < 0,05$
		$\times 10^9$	0,61 ± 0,02 $p_1 < 0,05$	0,68 ± 0,01 $p_1 < 0,05$
	disseminated	%	22,85 ± 6,29 $p_{1,3} < 0,05$	23,03 ± 6,54 $p_{1,3} < 0,05$
		$\times 10^9$	0,55 ± 0,07 $p_1 < 0,05$	0,52 ± 0,08 $p_1 < 0,05$
Without blood eosinophilia	infiltrative	%	20,00 ± 1,16 $p_1 < 0,05$	19,84 ± 1,99 $p_1 < 0,05$
		$\times 10^9$	0,20 ± 0,05 $p_2 < 0,05$	0,23 ± 0,06 $p_2 < 0,05$
	disseminated	%	21,03 ± 1,92 $p_1 < 0,05$	19,93 ± 3,17 $p_1 < 0,05$
		$\times 10^9$	0,19 ± 0,11 $p_2 < 0,05$	0,17 ± 0,10 $p_2 < 0,05$

It is believed that B-lymphocytes play an important role in the realization of antituberculous immunity due to the synthesis of antibodies that neutralize toxins, opsonize mycobacteria, participate in the mechanisms of antibody-dependent cellular cytotoxicity, etc. However, the humoral immune response in case of tuberculosis infection has no protective significance, and its activation can contribute to progressive dissemination of the pathogen [25].

The established increase in the absolute number of $CD20^+$ B-lymphocytes in the blood in patients with TB with eosinophilia, on the one hand, may represent a cause of hyperproduction of the mediators of the humoral link of the immune system by eosinophilic granulocytes, and on the other hand – its consequence.

CONCLUSION

In TB patients with eosinophilia, the number of immunosuppressive $CD4^+CD25^+Foxp3^+$ Tregs in the blood is increased. The high reactivity of blood Treg lymphocytes in patients with TB with eosinophilia, regardless of the drug sensitivity of the causative agent, is indicated by the high level of *in vitro* secretion of inhibitory cytokines IL-10 (BCG-induced in patients with infiltrative and disseminated TB) and TGFβ (basal and BCG-induced in patients with disseminated TB). In patients with TB without eosinophilia, the level of secretion of immunosuppressive cytokines is within or below the norm.

The immunomodulatory effect of eosinophilia of blood in TB is confirmed by the fact that the activation of suppressor cells in patients with eosinophilia is combined with an increase in the production of IL-4 by mononuclear leukocytes *in vitro* and an increase in IL-5 and $CD20^+$ B-lymphocytes in the blood, which represents a reflection of immune deviation towards the immune Th2 response. In general, its manifestations are most pronounced in disseminated TB in combination with the drug resistance of the causative agent and eosinophilia of the blood.

CONFLICT OF INTERESTS

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

CONFORMITY WITH PRINCIPLES OF ETHICS

Studies were conducted with the permission of the local ethics committee (protocol N26, 11.15.2010).

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Особенности иммунорегуляции у больных туберкулезом легких с эозинофилией крови

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РЕЗЮМЕ

Цель исследования – установить особенности регуляции иммунного ответа при туберкулезе легких (ТБ) и проанализировать роль регуляторных Т-клеток в иммунопатогенезе ТБ с эозинофилией крови в зависимости от клинической формы заболевания и чувствительности *Micobacterium tuberculosis* к противотуберкулезным лекарственным средствам.

Материалы и методы. Обследовано 157 больных с впервые выявленным инфильтративным и диссеминированным ТБ. Материалом исследования служили венозная кровь и культура мононуклеарных лейкоцитов, выделенных из венозной крови. Методом иммуноферментного анализа определяли содержание интерлейкина (IL) 4, IL-10 и трансформирующего фактора бета (TGFβ) в супернатантах культуральных суспензий мононуклеарных лейкоцитов *in vitro* и IL-5 в крови. Оценку экспрессии поверхностных молекул CD4, CD20, CD25 и внутриклеточного транскрипционного фактора Foxp3 в лимфоцитах крови проводили методом проточной цитометрии. Полученные результаты анализировали статистическими методами.

Результаты. Показано, что у больных ТБ избыточная генерация регуляторных Т-клеток ассоциирована с эозинофилией крови и дисбалансом механизмов регуляции иммунного ответа. При ТБ с эозинофилией увеличение численности Foxp3-позитивных регуляторных Т-клеток в крови сочетается с гиперсекрецией *in vitro* противовоспалительных цитокинов TGFβ, IL-10, IL-4 и повышением содержания CD20+ В-лимфоцитов и IL-5 в крови. Указанные изменения являются наиболее выраженными при диссеминированной форме ТБ в сочетании с лекарственной устойчивостью возбудителя.

Заключение. Особенности иммунорегуляции при ТБ с эозинофилией крови связаны с активацией механизмов иммуносупрессии и поляризацией иммунного ответа в направлении Th2-зависимого пути.

Ключевые слова: туберкулез легких, регуляция иммунного ответа, регуляторные Т-клетки, эозинофилия.

КОНФЛИКТ ИНТЕРЕСОВ

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Уразова О.И., Чурина Е.Г., Колобовникова Ю.В. – разработка концепции и дизайна исследования, анализ и интерпретация данных, написание статьи. Новицкий В.В., Караулов А.В. – проверка критически важного интеллек-

туального содержания, окончательное утверждение для публикации рукописи. Никулина Е.Л. – проведение лабораторных исследований, анализ данных. Полетика В.С. – оригинальный перевод текста статьи.

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