УДК 616.988-002.954-02:616.155.32
https://doi.org/10.20538/1682-0363-2024-3-25-33

Features of subset composition and functional activity of blood lymphocytes in tick-borne infections of different etiologies

Voronkova O.V., Ilyinskikh E.N., Hasanova R.R., Esimova I.E., Nevskaya K.V., Karpova M.R., Chernyshov N.A., Yampolskaya A.V., Yampolskaya O.V.

Siberian State Medical University
2, Moscow Trakt, 2, Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To perform a comparative assessment of subset composition and functional activity of peripheral blood lymphocytes in patients with tick-borne encephalitis (TBE) and ixodid tick-borne borreliosis (ITBB) in the acute phase of the disease.

Materials and methods. The study involved 22 patients with febrile and meningeal TBE, 15 patients with ITBB with and without erythema, and 11 healthy controls. Subset composition of blood lymphocytes was determined by flow cytometry. The blast transformation assay was applied to assess lymphocyte proliferation. Cytokine-producing activity of cells was studied in 24-hour incubated mononuclear cell cultures. Cytokine concentrations (interleukin (IL)-2, IL-4, IL-10, interferon (IFN)γ) were determined in the supernatants by the enzyme-linked immunosorbent assay (ELISA).

Results. Patients with TBE demonstrated an increase in the proportion of helper – inducer T-cells, a pronounced decrease in the proportion and absolute count of cytotoxic T cells, and low T lymphocyte count compared to the control values. The study in ITBB patients revealed an increase in the helper – inducer T-cell count and the proportion of NK-cells, a decrease in the cytotoxic T cell count, and the T lymphocyte count comparable to normal values. The most significant decrease in the levels of phytohemagglutinin-induced lymphocyte proliferation was found in patients with TBE. Patients of both groups showed a decrease in IL-2 secretion in the mononuclear cell culture, a rise in IL-4 and IL-10 production, and IFNγ production levels comparable to control values.

Conclusion. The study of TBE patients revealed relative lymphocytopenia with changes in the subset composition of lymphocytes characterized by an increase in the proportion of helper – inducer T-cells and a decrease in the absolute cytotoxic T lymphocyte count. Patients with ITBB demonstrated an increase in the proportion of NK-cells and a more pronounced imbalance in the T-helper / cytotoxic T lymphocyte ratio. Changes in the functional phenotype of lymphocytes, regardless of the etiology of tick-borne infection, were characterized by reduced proliferative reserve, low IL-2 secretion, increased IL-4 and IL-10 production, and depressed reactivity of lymphocytes with respect to IFNγ secretion.

Keywords: tick-borne encephalitis, ixodid tick-borne borreliosis, lymphocytes, cytokines

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The study was supported by the Russian Science Foundation grant (No. 22-15-20010, https://rscf.ru/project/22-15-20010/) and the funds of the Tomsk Regional Administration.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the Ethics Committee at Siberian State Medical University (Protocol No. 9119/1 of 30.05.2022).

For citation: Voronkova O.V., Ilyinskikh E.N., Hasanova R.R., Esimova I.E., Nevskaya K.V., Karpova M.R., Chernyshov N.A., Yampolskaya A.V., Yampolskaya O.V. Features of subset composition and functional activity of blood lymphocytes in tick-borne infections of different etiologies. *Bulletin of Siberian Medicine.* 2024;23(3):25–33. https://doi.org/10.20538/1682-0363-2024-3-25-33.

✉ *Voronkova Olga V.,* voronkova-ov@yandex.ru

Особенности субпопуляционного состава и функциональной активности лимфоцитов крови при клещевых инфекциях разной этиологии

Воронкова О.В., Ильинских Е.Н., Хасанова Р.Р., Есимова И.Е., Невская К.В., Карпова М.Р., Чернышов Н.А., Ямпольская А.В., Ямпольская О.В.

Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

РЕЗЮМЕ

Цель – провести сравнительную оценку субпопуляционного состава и функциональной активности лимфоцитов периферической крови у больных клещевым энцефалитом (КЭ) и иксодовым клещевым боррелиозом (ИКБ) в остром периоде заболевания.

Материалы и методы. В исследовании приняли участие 22 больных с лихорадочной и менингеальной формами КЭ, 15 пациентов с безэритемной и эритемной формами ИКБ и 11 здоровых лиц. Определение субпопуляционного состава лимфоцитов в крови проводили методом проточной цитофлуориметрии. Пролиферативную активность лимфоцитов исследовали в реакции бластной трансформации. Цитокинпродуцирующую активность клеток исследовали в 24-часовых культурах мононуклеарных лейкоцитов; концентрацию цитокинов (интерлейкина (IL) 2, IL-4, IL-10, фактора интерферона гамма (IFNγ)) определяли в культуральной жидкости методом иммуноферментного анализа.

Результаты. У пациентов с КЭ на фоне низкого по сравнению с контрольными значениями числа Т-лимфоцитов зарегистрировано повышение доли Т-хелперов/индукторов и выраженное снижение относительного и абсолютного количества Т-цитотоксических лимфоцитов. У больных ИКБ выявлено повышение числа Т-хелперов/индукторов и доли NK-клеток, а также сопоставимое с нормой количество Т-лимфоцитов и низкое содержание Т-цитотоксических клеток. Наиболее значимое снижение уровня ФГА-индуцированной лимфопролиферации зарегистрировано у пациентов с КЭ. У пациентов обеих групп выявлено снижение секреции IL-2 в культуре мононуклеарных лейкоцитов, повышение наработки IL-4 и IL-10 и сопоставимый с нормой уровень продукции IFNγ.

Заключение. У больных КЭ на фоне относительной лимфоцитопении регистрируются изменения субпопуляционного состава лимфоцитов, характеризующиеся повышением доли Т-хелперов/индукторов при абсолютной недостаточности Т-цитотоксических лимфоцитов. При ИКБ наблюдается повышение доли NK-клеток, а дисбаланс соотношения Т-хелперы/Т-цитотоксические лимфоциты выражен сильнее, чем при КЭ. Изменения функционального фенотипа лимфоцитов вне зависимости от этиологического варианта инфекции характеризуются снижением резерва пролиферативной активности на фоне низкой секреции IL-2, повышением наработки IL-4 и IL-10 и недостаточной реактивностью лимфоцитов в отношении секреции IFNγ.

Ключевые слова: клещевой энцефалит, иксодовый клещевой боррелиоз, лимфоциты, цитокины

Конфликт интересов. Авторы гарантируют отсутствие потенциальных и явных конфликтов интересов, связанных с публикацией данной статьи.

Источник финансирования. Исследование выполнено за счет гранта Российского научного фонда (№ 22-15-20010, https://rscf.ru/project/22-15-20010/) и средств Администрации Томской области.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом СибГМУ (протокол № 9119/1 от 30.05.2022).

Для цитирования: Воронкова О.В., Ильинских Е.Н., Хасанова Р.Р., Есимова И.Е., Невская К.В., Карпова М.Р., Чернышов Н.А., Ямпольская А.В., Ямпольская О.В. Особенности субпопуляционного состава и функциональной активности лимфоцитов крови при клещевых инфекциях разной этиологии. *Бюллетень сибирской медицины.* 2024;23(3):25–33. https://doi.org/10.20538/1682-0363-2024-3-25-33.

INTRODUCTION

Despite advances in the fields of prevention, diagnosis, and treatment of most infectious diseases, the problem of tick-borne infections is far from being completely resolved. High incidence of chronic ixodid tick-borne borreliosis (ITBB), pronounced polymorphism of clinical manifestations and features of the course of tick-borne encephalitis (TBE) (from suppressed forms to severe and chronic ones) with the development of long-term complications indicate a lack of scientific knowledge about the relationship between the pathogen and the host body [1, 2].

It is known that the pathogenesis of any infectious disease is a complex dynamic process during which the pathogenic potential of the microbe is realized through interaction with factors of innate and adaptive immunity of the host. At the same time, the functional viability and productive cooperative interactions of antigen-presenting, regulatory and effector immune cells during the immune response largely determine not only features of clinical manifestations, but also options for the outcome of the infectious process [3, 4]. In some cases, the absence of specific clinical and laboratory manifestations in some forms of vector-borne infections transmitted by ixodid ticks causes difficulties in differential diagnosis at the early stage of the disease [5]. In this regard, the study of the characteristics and mechanisms of development of various etiological variants of tick-borne infections has both theoretical and practical significance, in particular, to identify new biomarkers of disorders of the structural and functional phenotype of immune cells which are significant for the diagnosis and prognosis of diseases.

The aim of the study was to perform a comparative assessment of subset composition and functional activity of peripheral blood lymphocytes in patients with TBE and ITBB in the acute phase of the disease.

MATERIALS AND METHODS

The study involved 37 patients with acute tick-borne infections, of which 22 patients had acute febrile and neuroinvasive forms of TBE (mean age of the patients was 49.88 ± 2.81 years), and 15 individuals were ITBB patients with and without erythema migrans (mean age of the patients was 46.00 ± 2.79 years). The diagnosis was verified based on the medical history and the results of an objective examination, which included laboratory tests and the enzyme-linked immunosorbent assay (ELISA) with the determination of IgM and IgG to *Borrelia burgdorferi* s.l. and to TBE in the blood, as well as the TBE virus antigen. Additionally, relapsing tick-borne fever caused by *Borrelia miyamotoi*, ehrlichiosis, and human granulocytic anaplasmosis were excluded in all patients by polymerase chain reaction (PCR) (RealBest kits, Vector-Best, Russia). The control group involved 11 healthy individuals (mean age was 48.13 ± 2.76 years). The material for the study was venous peripheral blood collected from the patients upon admission to the Infectious Disease Clinic of Siberian State Medical University.

Absolute and relative lymphocyte counts, including T lymphocytes (CD3+CD19–), helper – inducer T-cells (CD3+CD4+CD45+), cytotoxic T lymphocytes (CD3+CD8+CD45+), natural killer cells (NK-cells, CD3–CD56+CD45+), and B lymphocytes (CD19+CD3–), were assessed by immunophenotyping using fluorescently labeled monoclonal antibodies (Elabscience, China) and subsequent multicolor flow cytometry on the Accuri C6 flow cytometer (BD Biosciences, USA). To correctly exclude all particles from the assay regions that did not correspond in size and granularity to living lymphocytes, some logical constraints were included into the particle distribution histograms according to small-angle and side-scatter characteristics (Fig.). The proportion of positive cells in the total cell count was measured by applying logical constraints to individual markers. At least 10^4 lymphocytes were analyzed in each sample. Absolute subset counts were determined based on their proportion (percentage) and absolute lymphocyte count in the peripheral blood using the Sysmex XN1000 hematology analyzer (Sysmex, Japan).

Spontaneous and mitogen-stimulated proliferative activity of peripheral blood mononuclear cells (PBMC) was studied in the blast transformation assay (BTA). Suspension culture was prepared by mixing heparinized venous blood with the RPMI-1640 medium in a 1:4 ratio supplemented with L-glutamine and fetal bovine serum (BioloT, Russia). Phytohemagglutinin (PHA) (Sigma, USA) (0.01 mg per 2.0×10^6 / ml) was added to one of the two samples and incubated at 37 °C and 5% CO_2 for 72 hours. After incubation, the contents of the vials were resuspended and centrifuged. Smears were prepared from the sediment, fixed and stained with Azur II Eosin. BTA intensity was determined

by standard cell morphology using light microscopy to analyze the count of non-blast and blast forms (in %). The stimulation index was calculated as the ratio of the PHA-induced blast transformation to spontaneous blast transformation.

Cytokine-producing activity of cells was studied in 24-hour incubated mononuclear cell cultures isolated from venous blood by the Ficoll density gradient centrifugation at a density of 1.077 g / cm^3 (BioloT, Russia). Cells were incubated at a concentration of 2×10^6 / ml in the complete growth medium with or without 50 μg / ml PHA. The concentrations of cytokines, including interleukins (IL)-2, IL -4, IL-10, and interferon (IFN)-γ, were assessed in the culture supernatants by ELISA using Vector-Best kits (Russia). The stimulation index was calculated as the ratio of PHA-induced cytokine secretion to spontaneous secretion.

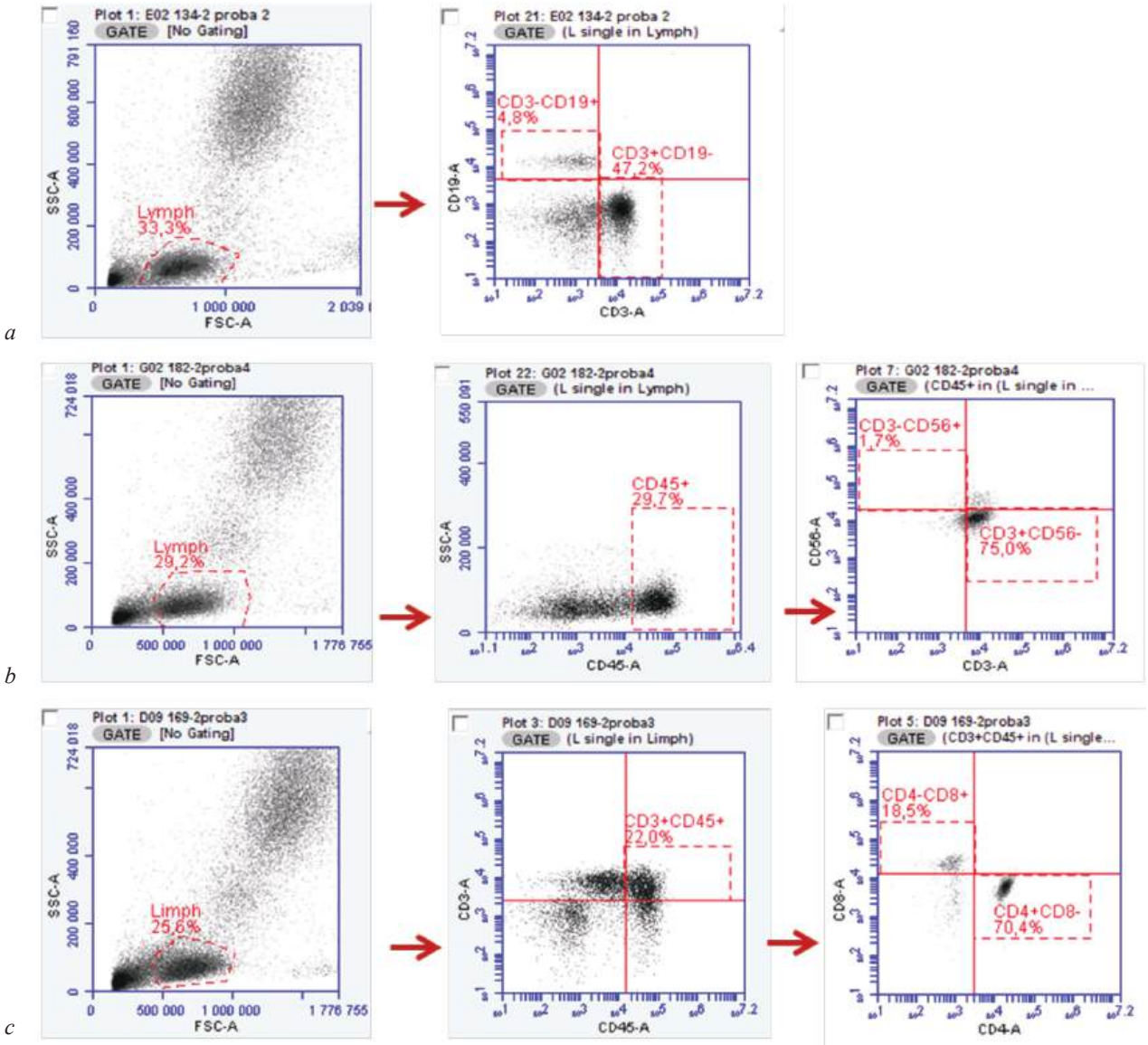

Figure. Distribution of lymphocyte subsets depending on the expression of surface CD-markers: *a* – distribution histogram of T lymphocytes (CD3+) and B lymphocytes (CD19+); *b* – isolation of NK-cells (CD3–CD56+) from the general subset of CD45+ lymphocytes; *c* – distribution histogram of helper – inducer T-cells (CD4+) and cytotoxic T lymphocytes (CD8+) after applying the logical constraint to CD45+ and CD3+

The results were processed using the Statistica 12.0 software (StatSoft, USA). Normally distributed data (Shapiro – Wilk test) were presented as the mean and the standard deviation ($M \pm SD$). Non-normally distributed data were presented as the median and the interquartile range Me (Q_1; Q_3). The Student's t-test or the Mann – Whitney U-test with the Bonferroni correction were used to compare differences between the groups. The differences between the two compared variables were considered statistically significant at $p < 0.05$.

RESULTS

When analyzing the hemogram parameters in the group of patients with TBE compared to the controls, we revealed a statistically significant decrease in the proportion of lymphocytes along with an increase in the total leukocyte count in the peripheral blood (Table 1). Nevertheless, the absolute lymphocyte counts in the peripheral blood in both groups of patients with TBE and ITBB did not differ significantly from the control values. According to the results of immunophenotyping of peripheral blood lymphocytes, patients with TBE with a decrease in the total T lymphocyte count showed an increase in the proportion of helper – inducer T-cells as well as a significant decrease in the relative and absolute cytotoxic T lymphocyte count (on average by 1.8 and 2.0 times, respectively) compared to the control values (Table 1).

In patients with ITBB, the total T lymphocyte count did not differ significantly from the values in the control group. However, they had a decrease in the cytotoxic T lymphocyte count and an increase in the absolute helper – inducer T-cell count.

Quantitative changes in these lymphocyte subsets affected the helper T cell / cytotoxic T cell ratio, which was on average 2 times higher than the control values in both groups (Table 1).

Table 1

Total leukocyte count and lymphocyte subset composition in the peripheral blood of patients with tick-borne infections, *Me* (Q_1; Q_3)

Group of the examined individuals	Total leukocyte count, $\times 10^9$ / l	Total lymphocyte count in the hemogram (in % in the numerator, $\times 10^9$ / l in the denominator)	Lymphocyte subset counts (in % in the numerator, $\times 10^9$/l in the denominator)					Helper T cell / cytotoxic T cell ratio
			NK-cells	Helper – inducer T-cells	Cytotoxic T lymphocytes	T lymphocytes	B lymphocytes	
Control group, $n = 11$	5.76 (4.32; 5.90)	36.00 (32.00; 37.01) 2.08 (1.45; 2.18)	11.02 (8.98; 12.97) 0.21 (0.19; 0.26)	49.32 (47.30; 51.12) 1.01 (0.66; 1.08)	31.00 (29.9; 32.10) 0.62 (0.47; 0.70)	80.32 (78.2; 82.03) 1.70 (1.09; 1.71)	9.07 (8.97; 9.8) 0.16 (0.14; 0.19)	1.59 (1.54; 1.70)
Patients with TBE, $n = 22$	8.36 (5.82; 10.99) $p_1 = 0.01$	20.65 (13.2; 28.9) $p_1 = 0.04$ 1.43 (1.00; 2.38)	11.80 (8.01; 13.52) 0.21 (0.12; 0.28)	58.22 (53.12; 69.1) $p_1 = 0.04$ 0.76 (0.56; 1.10)	17.37 (11.48; 22.45) $p_1 = 0.01$ 0.29 (0.17; 0.41) $p_1 = 0.01$	73.76 (66.81; 86.12) $p_1 = 0.04$ 1.12 (0.77; 1.84) $p_1 = 0.04$	9.31 (4.52; 12.66) 0.17 (0.06; 0.29)	2.85 (2.10; 5.68) $p_1 = 0.03$
Patients with ITBB, $n = 15$	7.23 (5.49; 8.57) $p_1 = 0.03$	34.40 (19.85; 43.7) $p_2 = 0.04$ 2.09 (1.72; 2.79)	14.92 (11.13; 21.53) $p_1 = 0.04$ $p_2 = 0.04$ 0.26 (0.13; 0.46)	60.99 (52.93; 64.26) $p_1 = 0.04$ 1.19 (1.14; 1.72) $p_1 = 0.04$ $p_2 = 0.03$	16.81 (14.84; 18.29) $p_1 = 0.01$ 0.34 (0.27; 0.40) $p_1 = 0.01$	77.14 (71.22; 81.87) 1.54 (1.46; 2.08)	7.83 (4.92; 10.27) 0.16 (0.09; 0.27)	3.69 (3.40; 4.20) $p_1 < 0.001$

Here and in Table 2 and 3: p_1 is the level of significance of differences when compared with parameters in the control group; p_2 is the level of significance of differences when compared with parameters in TBE patients.

It should be noted that the number of B lymphocytes in both groups of patients was comparable with the control values. At the same time, a statistically significant increase in the relative number of NK-cells was recorded in patients with ITBB compared to the control group.

Assessing the results of lymphocyte proliferative activity in the PBMC cultures *in vitro*, we found an increase in the level of spontaneous blast transformation only in the patients with ITBB compared to the healthy controls (Table 2).

The levels of PHA-induced blast transformation were significantly lower in both groups of patients than in the control group, while the most significant decrease in this parameter was found in patients with TBE, which was reflected in similar changes in the calculated PHA stimulation index (Table 2).

Table 2

The results of the blast transformation assay of the peripheral blood lymphocytes in patients with tick-borne infections, *M ± SD*

Groups of the examined individuals	Blast transformation assay, %		Stimulation index
	without PHA stimulation	with PHA stimulation	
Control group, $n = 11$	6.09 ± 0.97	61.82 ± 7.87	10.41 ± 2.41
Patients with TBE, $n = 22$	7.55 ± 3.31	26.78 ± 11.52 $p_1 < 0.001$	3.79 ± 1.19 $p_1 < 0.001$
Patients with ITBB, $n = 15$	10.40 ± 2.48 $p_1 < 0.001$ $p_2 = 0.003$	49.63 ± 7.12 $p_1 < 0.001$ $p_2 = 0.002$	4.98 ± 1.18 $p_1 < 0.001$ $p_2 = 0.03$

The capability of secreting immunoregulatory cytokines is one of the parameters determining the functional phenotype along with the proliferative activity and expression profile of peripheral blood lymphocytes. In this study, we focused on IL-2, IL-4, IL-10, and IFNγ that have para- and autocrine effects on immune cells by controlling all stages of antigen-specific proliferation, differentiation, and functional activity of T and B lymphocytes [6–8]. When analyzing the cytokine-producing activity of PBMC cultures *in vitro* obtained from both groups of patients with tick-borne infections, regardless of their etiology, we detected a decrease in the spontaneous and mitogen-induced production of IL-2, which was most pronounced in patients with ITBB (Table 3).

Basal IL-4 secretion and the levels of spontaneous and PHA-induced IL-10 secretion in the cell culture supernatants in both groups of patients were significantly higher than those in the controls. We detected that on average the levels of PHA-stimulated IL-4 production in the patients with ITBB were two times higher than those in the control group (Table 3). It should be mentioned that the concentration of spontaneous and mitogen-induced IFNγ secretion in the primary PBMC cultures in both groups of patients with tick-borne infections did not differ from that in the healthy donors (Table 3). The analysis demonstrated a statistically significant decrease in the stimulation indices of IL-2, IL-4, and IL-10 in both groups of patients compared to the control values, which indicates that the PBMC secretory activity in relation to these cytokines decreases.

Table 3

Cytokine concentrations in the supernatants of 24-hour cultures of peripheral blood mononuclear cells in the patients with tick-borne infections, *Me* (Q_1; Q_3)

Parameter		Control group, $n = 11$	Patients with TBE, $n = 22$	Patients with ITBB, $n = 15$
Concentration of IL-2, pg /ml	without PHA stimulation	44.52 (32.74; 112.13)	32.11 (11.56; 48.06) $p_1 = 0.01$	10.78 (9.78; 13.19) $p_1 < 0.001$; $p_2 = 0.03$
	with PHA stimulation	93.50 (66.96; 275.88)	27.21 (24.28; 68.74) $p_1 = 0.005$	24.27 (21.01; 27.57) $p_1 < 0.001$; $p_2 = 0.03$
	Stimulation index	2.85 (1.57; 5.52)	2.20 (1.64; 2.60); $p_1 = 0.04$	2.11 (1.68; 2.62); $p_1 = 0.04$
Concentration of IL-4, pg / ml	without PHA stimulation	2.92 (2.51; 4.93)	10.51 (6.50; 23.88) $p_1 = 0.01$	26.18 (22.13; 30.35) $p_1 < 0.001$; $p_2 < 0.001$
	with PHA stimulation	19.72 (10.20; 22.32)	19.90 (10.38; 46.97)	44.43 (41.42; 66.06) $p_1 < 0.001$; $p_2 = 0.01$
	Stimulation index	4.00 (3.49; 8.83)	2.11 (1.89; 2.19); $p_1 < 0.001$	1.99 (1.41; 2.48); $p_1 = 0.001$
Concentration of IL-10, pg / ml	without PHA stimulation	9.36 (2.45; 12.65)	37.28 (12.89; 53.71) $p_1 = 0.02$	28.77 (20.01; 90.87) $p_1 = 0.001$
	with PHA stimulation	27.39 (12.87; 59.75)	88.66 (57.09; 141.46) $p_1 = 0.04$	112.39 (48.07; 129.70) $p_1 = 0.004$
	Stimulation index	4.72 (1.38; 11.09)	2.22 (2.13; 2.73); $p_1 = 0.02$	2.20 (1.02; 5.20); $p_1 = 0.01$
IFNy concentration, pg/ml	without PHA stimulation	26.96 (9.47; 43.61)	21.27 (14.82; 25.01)	16.50 (8.81; 41.81)
	with PHA stimulation	25.59 (22.45; 49.30)	24.39 (11.49; 41.25)	21.77 (19.11; 44.34)
	Stimulation index	0.98 (0.51; 4.85)	1.02 (0.50; 2.79)	1.81 (0.43; 3.95)

DISCUSSION

It is known that the subset composition of lymphocytes in the peripheral blood in the acute phase of an infectious disease is the result of their dynamic redistribution during active migration of naive lymphocytes to peripheral lymphoid organs (where they interact with antigen-presenting cells), their further proliferation and release as mature clones into the blood, migration into tissues with return to the peripheral lymphatic organs, and apoptosis [9, 10]. The quantitative and qualitative characteristics of the immune response to infections depend on many factors, such as the antigen type, its dose, and a route of entry into the body.

According to some of the earlier studies, changes in the distribution of the peripheral blood lymphocyte subsets included a decrease in the CD3+, CD4+, and CD8+ T lymphocyte counts as well as an increase in the B lymphocyte count, typical of the acute phase of TBE [11–13]. In patients with acute febrile and neuroinvasive forms of TBE and relative lymphocytopenia, we revealed a deficit in the T lymphocyte count (CD8+ lymphocyte count), while the B lymphocyte count and NK-cell count did not differ from the control values. Moreover, we detected significant imbalances in the helper T cell / cytotoxic T cell ratio as well as a significant decrease in the PHA-induced proliferative activity of peripheral blood lymphocytes in TBE patients (Table 2).

It seems that the decrease in the CD8+-subset in TBE patients resulted from the insufficient proliferative response of lymphocytes to viral antigens. It should also be assumed that there was a direct cytotoxic effect of the TBE virus and selective damage to the T cell immune response due to its replication in the thymus, which can control maturation, differentiation, and functional activity of T lymphocytes.

Thus, some researchers point to the development of clonal exhaustion of committed T lymphocytes in viral persistence with the immune imbalance polarized toward Th2 [14, 15]. These results were consistent with our data on assessing the cytokine-producing activity of mononuclear cells in the acute phase of TBE and revealed elevated levels of IL-10 and IL-4 secretion in PBMC cultures with decreased IL-2 secretion and insufficient leukocyte cellular activity to release IFNγ (Table 3). When IL-2 secretion was reduced, lymphocytes did not have sufficient reserve to increase proliferation in response to PHA stimulation, which was probably not compensated by the effects of other proinflammatory cytokines, such as TNFα, IL-1β, IL-12, etc.

Unlike cytotoxic T lymphocytes, NK-cells as components of innate immunity do not have pathogen specific recognition function as well as in the mechanisms of antibody-dependent cellular cytotoxicity [16]. NK-cells play a pivotal role of in the immunopathogenesis of viral infections and are also involved in cytolytic effects in bacterial diseases. In bacterial infections, NK-cells can be activated both via cross-interaction with other leukocytes and via direct recognition of pathogen-associated molecular patterns [17].

To date, there is evidence that NK-cells express pattern recognition molecules, such as Toll- and NOD-like receptors [17, 18]. The capability of Borrelia spp. to induce an increase in the NK-cell count at the onset of the disease was a very important finding [19, 20]. At the same time, the cytolytic activity of NK-cells cannot be an effective mechanism for eliminating extracellular pathogens, including Borrelia spp. However, when there is cytotoxic lymphocyte deficiency and ineffective antibody synthesis, NK-cells are likely to play a pivotal role in limiting pathogen dissemination, which was confirmed by the increase in their count that we detected in the patients with ITBB. Moreover, the role of NK-cells and Th1 lymphocytes in the immunopathogenesis of Lyme disease was predominantly determined by the immune cell capability of stimulating phagocytosis via release of IFNγ and IL-12 that induce macrophage activation [21, 22].

Despite the fact that we detected elevated absolute helper T cell count in the patients with ITBB, the levels of basal and PHA-stimulated IFNγ secretion in the PBMC cultures did not differ from those in the control group. Additionally, low IL-2 production and increased secretion of IL-4 and IL-10 were revealed in both groups of patients with ITBB and TBE. The changes in the cytokine secretion profile of PBMC that we revealed in the patients with ITBB seemed quite logical and reflect the effector phase of the immune response to borrelia antigens. It was a result of cooperative interactions between macrophages and lymphocytes mediated by cytokines that induce the accumulation of mature T helper 2 cells, which

can stimulate formation of sufficient subsets of plasma cells releasing specific antibodies to borrelia antigens in the lymphoid organs.

In general, when analyzing the results obtained and comparing them with the literature data, we noted significant variability in changes in immune status parameters in different groups of patients with tick-borne infections, regarding both quantitative changes in these parameters and data interpretation in the context of complex regulatory mechanisms of the immune response [4, 11–13, 22]. Additionally, many authors note the correlation of suppression or activation levels of specific innate and adaptive immunity components with the severity of the clinical course of the disease, which, in our opinion, emphasizes the relevance of further research on immune response mechanisms in different clinical forms of tick-borne infections, including the study of dynamics of the infectious process.

CONCLUSION

The study revealed changes in the lymphocyte subset composition in the blood of patients with TBE and relative lymphocytopenia, characterized by an increase in the proportion of helper – inducer T cells and a decrease in the absolute cytotoxic T lymphocyte count. Patients with ITBB demonstrated an increase in the proportion of NK-cells and a more pronounced imbalance in the T-helper / cytotoxic T lymphocyte ratio. Changes in the functional phenotype of lymphocytes, regardless of the etiology of tick-borne infection, were characterized by reduced proliferative reserve, low IL-2 secretion, increased IL-4 and IL-10 production, and depressed reactivity of lymphocytes with respect to IFNγ secretion.

REFERENCES

1. Provorova V.V., Krasnova E.I., Khokhlova N.I., Savelyeva M.A., Filimonova E.S., Kuznetsova V.G. Old and new tick-borne infections in Russia. *Infectious Diseases: News, Opinions, Training.* 2019;8(2):102–112 (in Russ.). DOI: 10.24411/2305-3496-2019-12013.
2. Andaev E.I., Nikitin A.Ya., Tolmacheva M.I., Zarva I.D., Yatsmenko E.V., Matveeva V.A., et фд. Epidemiological situation on tick-borne viral encephalitis in the Russian Federation in 2022 and forecast of its development for 2023. *Problems of Particularly Dangerous Infections.* 2023;(1):6–16 (in Russ.). DOI: 10.21055/0370-1069-2023-1-6-16.
3. Pinegin B.V., Khaitov R.M. Modern principles of immunotropic drugs creation. *Immunologiya.* 2019;40(6):57–62 (in Russ.). DOI: 10.24411/0206-4952-2019-16008.
4. Andronova N.V., Minoranskaya N.S., Minoranskaya E.I. The specific immune response and some remote results in the acute course of ixodic tick-borne borreliosis and mixed infections of tick-borne encephalitis and ixodic tick-borne borreliosis. *Siberian Medical Journal (Irkutsk)*. 2011;100(1):54–57 (in Russ.).
5. Filimonova E.S., Bondarenko E.I., Krasnova E.I., Provorova V.V., Krinitsyna E.V., Oleinik A.N., et al. Identification of agents of natural foci infections. *Infectious Diseases: News, Opinions, Training.* 2021;10(4):90–97 (in Russ.). DOI: 10.33029/2305-3496-2021-10-4-90-97.
6. Simbirtsev A.S. Immunopharmacological aspects of the cytokine system. *Bulletin of Siberian Medicine.* 2019;18(1):84–95 (in Russ.). DOI: 10.20538/1682-0363-2019-1-84-95.
7. Ng C.T., Oldstone M.B. IL-10: achieving balance during persistent viral infection. *Curr. Top. Microbiol. Immunol.* 2014;380:129–144. DOI: 10.1007/978-3-662-43492-5_6.
8. Welsh R., Bahl K., Marshall H., Urban S. Type 1 interferons and antiviral CD8 T-cell responses. *PLoS Pathog.* 2012;8(1):e1002352. DOI: 10.1371/journal.ppat.1002352.2012. V.8:e1002352.
9. Marelli-Berg F.M., Fu H., Vianello F., Tokoyoda K., Hamann A. Memory T-cell trafficking: new directions for busy commuters. *Immunology*. 2010;130(2):158–165. DOI:10.1111/j.1365-2567.2010.03278.x.
10. Litvinova L.S., Gutsol A.A., Sokhonevich N.A., Kofanova K.A., Khaziakhmatova O.G., Shupletsova V.V., et al. The main surface markers of functional activity of T-lymphocytes (review). *Medical Immunology.* 2014;16(1):7–26 (in Russ.). DOI: 10.15789/1563-0625-2014-1-7-26.
11. Krylova N.V., Leonova G.N., Pavlenko E.V., Zaporozhets T.S., Smolina T.P., Gazh A.K., et al. Comprehensive assessment of immune system in various forms of tick-borne encephalitis in acute phase. *Medical Immunology.* 2012;14(4-5):313–320 (in Russ.).
12. Krylova N.V., Leonova G.N. Features of the immunopathogenesis of tick-borne encephalitis. *Pacific Medical Journal*. 2007;3(29):21–24 (in Russ.).
13. Blom K., Cuapio A., Sandberg J.T., Varnaite R., Michaëlsson J., Björkström N.K. et al. Cell-mediated immune responses and immunopathogenesis of human tick-borne encephalitis virus-infection. *Front. Immunol.* 2018;9:2174. DOI: 10.3389/fimmu.2018.02174.
14. Stone E.T., Pinto A.K. T Cells in tick-borne flavivirus encephalitis: a review of current paradigms in protection and disease pathology. *Viruses.* 2023;15(4):958. DOI: 10.3390/v15040958.
15. Nasyrova R.F., Ryazantseva N.V., Zhukova N.G., Zima A.P., Zhukova O.B., Chechina O.E., et al. Molecular and cellular basis of the pathogenesis of tick-borne encephalitis. *Bulletin of Siberian Medicine*. 2006;(1):42–51 (in Russ.).
16. Abakushina E.V., Kuzmina E.G., Kovalenko E.I. The main characteristics of human natural killer cells. *Immunologiya*. 2012;33(4):220–224 (in Russ.).
17. Athié-Morales V., O'Connor G.M., Gardiner C.M. Activation of human NK cells by the bacterial pathogen-associated molecular pattern muramyl dipeptide. *J. Immunol.* 2008;180(6):4082–4089. DOI: 10.4049/jimmunol.180.6.4082.
18. Freud A.G., Mundy-Bosse B.L., Yu J., Caligiuri M.A. The

broad spectrum of human natural killer cell diversity. *Immunity.* 2017;47(5):820–833. DOI: 10.1016/j.immuni.2017.10.008.
19. Oosting M., Brouwer M., Vrijmoeth H.D., Pascual Domingo R., Greco A., Ter Hofstede H. et al. *Borrelia burgdorferi* is strong inducer of IFN-γ production by human primary NK cells. *Cytokine.* 2022;155:155895. DOI: 10.1016/j.cyto.2022.155895.
20. Scorza B.M., Mahachi K.G., Cox A.D., Toepp A., Pessoa-Pereira D., Tyrrell P. et al. Role of NK-like CD8+ T cells during asymptomatic *Borrelia burgdorferi* infection. *Infect. Immun.* 2022;90(5):e0055521. DOI: 10.1128/iai.00555-21.
21. Petnicki-Ocwieja T., Kern A. Mechanisms of *Borrelia burgdorferi* internalization and intracellular innate immune signaling. *Front. Cell Infect Microbiol.* 2014;4:175. DOI: 10.3389/fcimb.2014.00175.
22. Tomasiewicz K., Chmielewska-Badora J., Zwolinski J., Murias-Brylowska E. Analysis of main T-cell subsets and activated T suppressor/cytotoxic cells in patients with *Borrelia burgdorferi* s. lato only infection and co-infections with *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti*. *Ann Agric Environ Med.* 2016;23(1):111–115. DOI: 10.5604/12321966.1196864.

Authors' contribution

Voronkova O.V., Ilyinskikh E.N. – conception and design, drafting of the manuscript. Hasanova R.R., Esimova I.E., Nevskaya K.V., Karpova M.R. – analysis and interpretation of the data. Chernyshov N.A., Yampolskaya A.V., Yampolskaya O.V. – implementation of laboratory research methods.

Authors' information

Voronkova Olga V. – Dr. Sci. (Med.), Associate Professor, Head of the Biology and Genetics Division, Siberian State Medical University, Tomsk, voronkova-ov@yandex.ru, https://orcid.org/0000-0001-9478-3429

Ilyinskikh Ekaterina N. – Dr. Sci. (Med.), Associate Professor, Professor of the Infectious Diseases and Epidemiology Division, Siberian State Medical University, Tomsk, infconf2009@mail.ru, https://orcid.org/0000-0001-7646-6905

Hasanova Rezeda R. – Cand. Sci. (Med.), Associate Professor, Biology and Genetics Division, Siberian State Medical University, Tomsk, hasanova_rezeda@mail.ru, https://orcid.org/0000-0002-3250-7688

Esimova Irina E. – Dr. Sci. (Med.), Associate Professor, Biology and Genetics Division, Siberian State Medical University, Tomsk, orevi@mail.ru, https://orcid.org/0000-0002-7508-2878

Nevskaya Ksenia V. – Cand. Sci. (Med.), Researcher, Center for Biological Research and Bioengineering, Siberian State Medical University, Tomsk, nevskaya.kv@ssmu.ru, https://orcid.org / 0000-0003-1659-8812

Karpova Maria R. – Dr. Sci. (Med.), Professor, Head of the Microbiology and Virology Division, Siberian State Medical University, Tomsk, mrkarpova@mail.ru, https://orcid.org/0000-0002-7109-9955

Chernyshov Nikita A. – Post-Graduate Student, Siberian State Medical University, nchernyschov@mail.ru, https://orcid.org/0000-0002-4008-5606

Yampolskaya Olesya V. – Student, Siberian State Medical University, Tomsk, lesyapolskaya.01@mail.ru, https://orcid.org/0000-0003-3362-8307

Yampolskaya Anastasia V. – Student, Siberian State Medical University, Tomsk, nastya.yampolskaya13@mai.ru, https://orcid.org/0009-0002-2003-7225

(✉) **Voronkova Olga V.,** voronkova-ov@yandex.ru

Received 25.01.2024;
approved after peer review 13.03. 2024;
accepted 25.04.2024