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STRUCTURE AND PROPERTIES OF LYMPHOCYTES' SURFACES IN PATIENTS WITH CHRONIC LYMPHOBLASTIC LEUCOSIS

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Abstract
In the investigation that was performed, the authors studied the structure and properties of lymphocytes' surfaces in patients with chronic lymphoblastic leukemia with application of atomic force microscopy technology. It was stated that development of chronic lymphoblastic leukemia is characterized with circulation of immature forms of lymphocytic series in peripheral bloodstream, for which increase of surface potential by 456% ($p<0.05$) as compared with control group, is typical. Surface relief of abnormal cells is characterized by abundance of globular structures with reduced height, which endows it with relief view. Stiffness of lymphocytes in patients with lymphoblastic leukemia is reduced by 51.4% ($p<0.05$) as compared with control group. Revealed peculiarities of morphological and functional properties of abnormal cell clones lead to microcirculatory embarrassment in vessels, which should be considered at providing standard treatment schemes.
Key words: chronic lymphoblastic leukemia, Young's modulus, surface potential, surface relief.

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СТРУКТУРА И СВОЙСТВА ПОВЕРХНОСТИ ЛИМФОЦИТОВ БОЛЬНЫХ ХРОНИЧЕСКИМ ЛИМФОБЛАСТНЫМ ЛЕЙКОЗОМ

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Аннотация
В выполненном исследовании изучены структура и свойства поверхности лимфоцитов больных хроническим лимфобластным лейкозом с использованием технологий атомно-силовой микроскопии. Установлено, что развитие хронического лимфолейкоза

характеризуется циркуляцией в периферическом русле незрелых форм лимфоцитарного ряда для которых характерно увеличение поверхностного потенциала на 456% ($p<0,05$) по сравнению с контролем. Рельеф поверхности аномальных клеток характеризуется обилием глобулярных структур со сниженной высотой, что придает ей вид рифлености. Жесткость лимфоцитов больных ХЛЛ снижена на 51,4% ($p<0,05$) по сравнению с контролем. Выявленные особенности морфофункциональных свойств аномальных клонов клеток приводят к нарушению микроциркуляции в сосудах, что необходимо учитывать при проведении схем стандартной терапии.
Ключевые слова: хронический лимфолейкоз, модуль Юнга, поверхностный потенциал, рельеф поверхности.

Chronic lymphocytic leukemia (CLL) is a progressive disease, which is characterized by accumulation of monoclonal $CD5^+$, $CD19^+$, $CD23^+$ B-cells in blood, lymphoid tissues and bone marrow. According to experimental data, the main reason of accumulation of incompetent lymphocytes that are present in blood at Go/G1 stage lies in fault of apoptosis mechanisms [10]. Currently scientists are obtaining the evidence that elements of adenylate cyclase pathways are involved in the process of avoidance of apoptosis by lymphoid cells – in particular, β_2-adrenoreceptors [8]. It was shown that in lymphocytes of patients with CLL the number of β_2-adrenoceptors is lowered, which leads to reduction of adenylate cyclase's stimulation [9]. This leads to development of lymphocytes' resistance to apoptosis and advance of the disease [7]. In connection with the above mentioned, a special topicality belongs to study of structure and function of lymphocytes of patients with CLL, as cellular models, which do not have advanced proliferative capacity, which, however, favor development of cytoplasia lymphoid blood lineage at the account of dysfunction of apoptosis mechanisms.

The objective of the work is investigation of peculiarities of structural organization and properties of lymphocytes' surfaces in patients with CLL.

Materials and methods of research

Experimental part of the work has been performed on the base of clinical diagnostic laboratory of regional clinical hospital named after St. Ioasaf of city of Belgorod and Department of Ecology, Physiology and Biological Evolution of Belgorod State National Research University.

In experimental part of the work venous blood of patients with CLL (50 persons), who were undergoing medical treatment in hematological department of regional clinical hospital of Belgorod was used. Control was provided by blood of healthy people (50 persons).

Blood was obtained by means of venous puncture. Analysis was performed immediately within 1 hour after sampling. Samples were collected in vacuum tubes Vacuette K3E. Lymphopoietic cells were being egested from the whole blood by means of centrifuging at 1500 rpm during 5 min, with further three-stage washing in RPMI-1640 medium and re-suspending in the same medium. Before performing experiments, cell vitality was assessed. Samples with cell vitality of not less than 98% were used in the experiment.

General and differential blood counts were performed at direct participation of doctor-laboratory assistants of clinical laboratory at Beckman Coulter LH500 automatic hematology analyzer (France, 2010).

Geometric parameters and micro relief of lymphocytes' surface were studied in tapping mode at atomic-force microscope INTEGRA Vita, produced by NT-MDT (Zelenorgad, 2009). For scanning cantilevers of NSG03 series were used, which had rounded radius of 10 nanometers. Preparation of blood samples for AFM was performed according to method [2]. Fifteen lymphocytes from each sample were scanned. At obtained scans, cells' morphometric parameters were measured and micro relief of cellular surface was analyzed at membrane units with the area of 3.5 x 3.5 μm. Cells' morphometry was performed with the application of program products of Nova (NT-MDT, Russia, 2009).

Elastic properties of lymphocytes were studied in the mode of force spectroscopy. Young's modulus was measured with the usage of modified AFM probe, produced on the base of polymer micro spheres, attached to tipless one of CSG11 series, according to method [1]. Calculations of probe's depth of immersion into the sample, force of pressing probe to the sample and Young's modulus were performed according to commonly known formulas [5, 6].

Calculation of surface potential (SP) of lymphocytes was performed in Kelvin probe mode.

Suspended mixture of lymphocytes for measuring surface potential was prepared according to method [3]. Measuring of SP was performed with the usage of cantilever with titanium current conducting coating of NSG03/TiN series (Nanoworld, USA). Fifteen cells were scanned from each sample; processing of obtained scans was performed in Nova program (NT-MDT, Russia).

Obtained results were processed by means of variation statistics. Statistical significance was defined with application of t of Student's tests at $p<0.05$.

Results of the research and their discussion

As a result of performed researches, leukocytosis was determined in patients with chronic leukemia. Thus, the number of patients' leukocytes six fold exceeded indices of healthy persons. In differential blood cell count, shifts towards mononuclear forms were observed. Against the background of sharp increase of lymphocytes' number by 124% ($p<0.05$), the ratio of granular forms decreased, thus, neutrophils – by 67% ($p<0.05$), eosinophils – by 61% ($p<0.05$), basophiles – by 61% ($p<0.05$), as compared to control (Table 1).

Table 1

Hematological indices of blood system of patients with CLL

Indices	Control	CLL
WBC, 10^9 л$^{-1}$	9.5±0.3	60.2 ±0.2*
Neutrophils, %	55.5±1.5	18.5±4.3*
Eosinophils, %	1.8±0.4	0.7 ±0.1*
Basophils, %	0.8±0.01	0.4 ±0.3*
Lymphocytes, %	34.4±1.8	77.2 ±5.7*
Monocytes, %	4.5±1.2	3.2 ±1.3
RBC, 10^{12} l^{-1}	3.7±0.08	3.3±0.2
Hb, g/l	121.3±3.2	102.4±1.5*
Ht, %	35.8±0.9	30.0 ±1.6*
MCV,fL	95.7±0.9	91.6±2.6
MCH, pg	32.4±0.3	31.6±0.9
MCHC, g/l	339.9±1.7	346.2±7.8
PLT 10^9 l^{-1}	320.2±1.5	185.1±0.6*
MPV, fL	9.2±0.3	7.1±0.5

*Note: * statistically significant differences of values in comparison with control at p < 0.05.* RBC stands for the number of red blood cells, Hb stands for hemoglobin, Ht stands for hematocrit, MCV stands for mean red cell volume, MCH stands for mean corpuscular hemoglobin concentration, PLT means the number of thrombocytes, MPV stands for mean platelet volume. CLL stands for chronical lymphoblast leucosis, WBC is the number of leukocytes. The number of leukocytes in blood of patients with CLL increased correspondingly by 534% ($p<0.05$) in comparison with control.

Lymphocytes of healthy persons (Figure 1*a*) and persons with CLL (Figure 1*b*) are characterized with round shape; the main part of the cell is taken by nucleus, which projects above the surface.

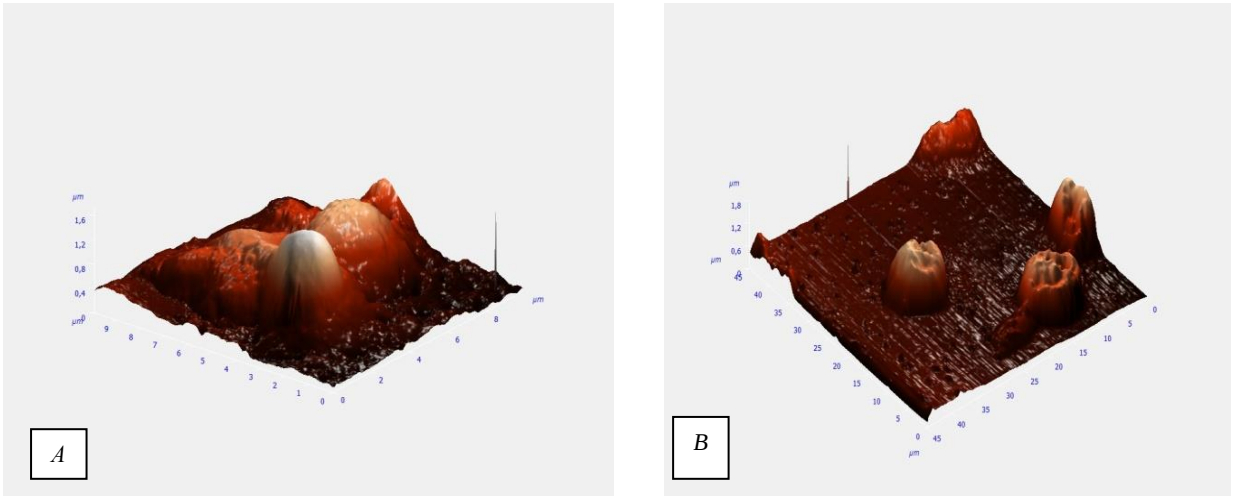

Fig. 1. 3D image of lymphocytes of healthy persons *(a)* and patients with CLL *(b)*
(atomic force microscopy, tapping mode of scanning)

In peripheral blood of patients with CLL, blastic forms of cells were found (Figure 2).

Morphometric parameters of lymphocytes of patients with CLL and healthy people are presented in Table 2.

As it can be seen from Table 2, diameter, volume and square of the surface of lymphocytes of persons with CLL are larger by 30%, 17% and 50% ($p<0.05$), respectively, in comparison with sizes of cells in control. Similar tendency was observed for nuclei. In group of patients with CLL a difference in morphometric parameters between lymphocytes and blast-like forms was stated. For the latter ones typical is reduction of height of cells and nuclei by 40% and 60% ($p<0.05$), respectively, in comparison with cytes, while their volume and square of the surface increased.

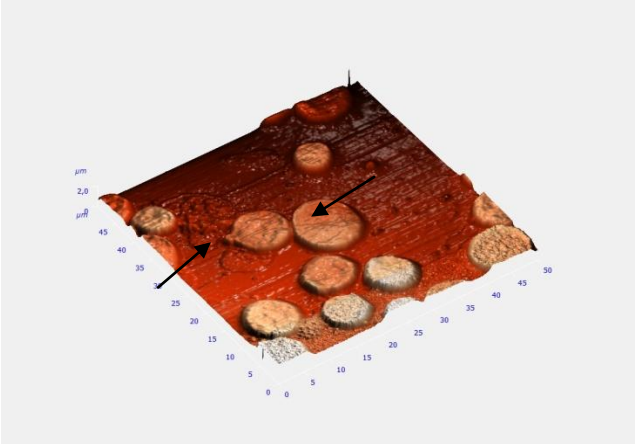

Fig. 2. 3D image of blast forms in blood of patient with CLL
(various forms of blasts are identified with arrows; atomic force microscopy, tapping mode of scanning)

Table 2

Morphometric parameters of lymphocytes

Parameter	Control		Patients with CLL			
			Lymphocytes		Blast-like forms	
	cell	nucleus	cell	nucleus	cell	nucleus
D, μm	6.54±0.26	4.28±0.13	8.54±0.50*	7.26±0.90*	8.92±0.4	7.56±1.12
H, μm	1.97±0.52	1.27±0.18	1.00±0.05	0.95±0.03	0.60±0.01**	0.38±0.04**
V, μm^3	68.24±0.35	54.13±0.86	79.08±1.60*	64.21±1.20*	92.80±1.20**	86.28±1.36**
S, μm^2	39.21±2.45	29.10±1.18	58.80±1.60*	49.18±0.96*	63.50±1.39**	53.80±1.26**

* Statistical significance of parameters of cells and nuclei in patients with leucosis in comparison with control at $p<0.05$.
** Statistical significance of parameters between blasts and cytes in patients with leucosis in comparison with control at $p<0.05$. D stands for diameter, H – height, V – volume, S – square of the surface.

Morphological features, identified by method of AFM at lymphocytes' surfaces of healthy donors (Figure 3*a*) and persons with CLL (Figure 3*b*) are represented by globular projections and cavities.

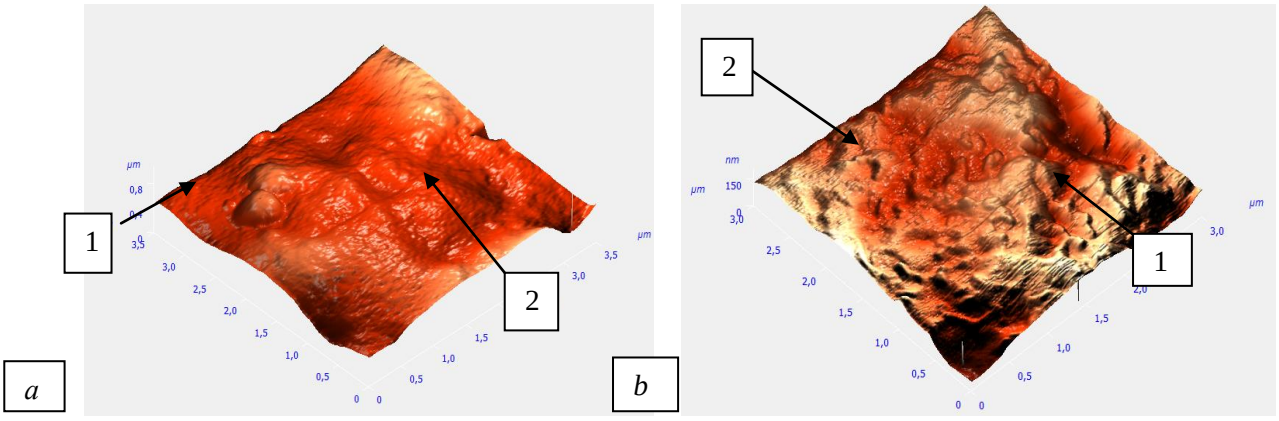

Fig. 3. Relief of lymphocytes' surface:
a – healthy persons, b – patients with CLL: 1 – globular projections, 2 – cavities

(atomic force microscopy, tapping mode)

Configuration of lymphocytes' surface in patients with CLL was corrugated. It was characterized by increase of the number of globular formations by 247% ($p<0.05$) and lowering of their height by 42.6% ($p<0.05$; Table 3).

Table 3

Structural features and mechanical properties of lymphocytes

Parameter		Control	CLL
Globular projections	Height, nm	41.3 ± 3.7	17.6 ±0.9*
	Number	36 ± 0.9	125 ± 1.1*
Cavities in membrane	Diameter, nm	221.8 ± 24.0	149.4 ± 12.9*
	Depth, nm	17.3 ± 0.6	8.01 ± 0.9*
	Number	18 ± 1.1	40 ± 2.3*
Stiffness	Young's modulus, μPa	3.50 ± 0.20	1.80 ± 0.01*
	Depth of immersion of cantilever, nm	345.20 ± 3.74	1035.20 ± 7.32*
Surface potential, mV		- 37.3 ± 0.6	- 6.7 ± 0.2*

*- Statistically significant differences between values of patients with leucosis as compared with control at $p<0.05$.

The number of cavities in lymphocytes' membranes in patients with CLL increased at 122% ($p<0.05$), their diameter and depth decreased at 67.4% and 46.3% ($p<0.05$), respectively, as compared with overall dimensions of cavities at the surface of cells in control.

Young's modulus of lymphocytes of patients with CLL decreased by 51.4% ($p<0.05$), depth of immersion of cantilever increased at 199% ($p<0.05$) in comparison with control. Charge of cells' surface of patients with CLL increased by 456% ($p<0.05$), in comparison with control. Changes in stiffness and charge of the surface point at increased ability of cellular surface to resist elastic deformations and their increased ability to adhere in microvascular flow.

Detected lowering of Young's modulus in tumor cells is consistent with literature data. According to parallel researches, made at atomic-force and confocal laser scanning microscopes, a strong connection was detected between lowering of Young's modulus in blood cells in persons with leucosis and content of F-actin, concentration of which lowers in cells twice in comparison with normal lymphocytes [4]. Reduction of thickness and content of actin-like structures in lymphocytes of patients with CLL is reflected at peculiarities of morphology and charge of transformed cells' surface. Some researches tend to consider the abundance of morphological formation at stage of neo-differentiated cells with reduced level of intracellular cAMP, and, consequently, change of tension in actin-myosin system [8], because of which reorganization of cytoskeletal structures is violated. Lowering of Young's modulus of lymphocytes in patients with CLL points at increase of ability of cellular surface to resist elastic deformations and increase of their elastic features.

Conclusion

Thus, chronic lymphoblastic leucosis is one of the types of differentiated lymphoma, which is characterized by slow accumulation of transformed forms of blast-like lymphocytes with increased sizes, changed properties and surface relief, in flow. Lymphoid cells that obtain the ability to avoid apoptosis are characterized with corrugated surface, increased number of globular structures with reduced height. At the same time, surface's stiffness reduces, and charge of cell's surface increases, which points at increased ability of transformed lymphoid cells to deform in microvascular flow and to adhere to vascular wall.

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