The Use of Nanomechanic Sensor for Studies of Morphofunctional Properties of Lymphocytes from Healthy Donors and Patients with Chronic Lymphoblastic Leukemia

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Tapping mode-atomic force microscopy and force spectroscopy were used for studies of the topography of the cell surface and elastic properties of lymphocytes from healthy donors and patients with chronic lymphoblastic leukemia. It was demonstrated that the decrease in lymphocytes stiffness in patients with chronic lymphoblastic leukemia by 51.4% ($p<0.05$) was accompanied by spatial modification of the cell surface, in particular, increase in the number of globular protrusions and depressions by 247 and 122%, respectively ($p<0.05$), in comparison with normal lymphocytes.

Key Words: *lymphocytes; surface topography; Young's modulus; atomic force microscopy*

Introduction of technologies of atomic force microscopy (AFM)-scanning as the nanomechanic sensor into biological studies opens new vistas in evaluation of morphofunctional properties of blood cells without violation of their morphological integrity [10,14]. Evaluation of structural peculiarities of the cell surface and local modulus of elasticity is interesting for the studies of mechanical properties of cells during cell migration [8], differentiation, and proliferation accompanying tumor metastasizing [9]. The advantages of AFM over traditional methods are 3D visualization of topography [13], integration of morphological images of the cell surface with its mechanical properties, *e.g.* elasticity in 2D resolution and cell-surface and cell-cell interaction forces [18], and evaluation of viscoelastic [4] and adhesion properties of cells [1].

Here we studied topography of the cell surface and viscoelastic properties of lymphocytes from healthy donors and patients with chronic lymphoblastic leukemia (CLL) using the AFM-scanning technique.

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MATERIALS AND METHODS

Lymphocyte isolation. Experiments were carried out on peripheral blood samples from 100 healthy donors (age 25-45 years) and patients (N=50) with CLL (age 17-32 years). Leukocytes were isolated by centrifugation of the EDTA-stabilized whole blood at 1500 rpm for 10 min. The upper layer of the plasma was removed and the lower leukocyte-rich portion of the plasma and the leukocyte ring were collected. Erythrocyte admixtures were lyzed with 0.83% ammonium chloride. The cells were washed twice with isotonic buffer solution (Dulbecco saline, pH=7.4).

Studies of topography. Surface topography of blood cells was studied using an INTEGRA Vita atomic force microscope (configuration on the basis of an Olympus IX-71 inverted microscope). Lymphocyte suspension was applied onto clean degreased glass plates and transferred into a humid chamber [3] for maintaining their viability. Scanning of 25 cells from each experimental and control sample was performed in a tapping mode (scan frequency 0.6-0.8 Hz) using a NSG03 cantilever with tip radius of 10 nm and spring constant of 1.1 N/m. The obtained scans were used for construction of 3.5×3.5-μ surface profiles using

Nova software (NT-MDT, Zelenograd, 2009) and morphological structures were measured on these profiles (the number and height of globular prominences were determined).

Elastography of lymphocytes. Young modulus quantitatively characterizing stiffness of the surface was measured using the method of elastography. This method is based on measurement of sample surface deformation during its interaction with the tip of the AFM probe. For calculation of the Young modulus, the experimental force-distance curves were transformed from the D-z coordinates to the F-Δh system, where D is photodiode mismatch current and z is cantilever deflection during AFM piezoscanner displacement. The attraction force was calculated by Hooke's law:

$$F=k\times x \quad \textbf{(1)},$$

where k is probe stiffness and x is the depth of probe indentation into the sample.

The Young modulus for the sample–tip system was calculated by the formula:

$$F=\frac{4\sqrt{R}}{3}E\Delta h\frac{3}{2} \quad \textbf{(2)},$$

where F is the force acting on the sample, R is tip radius, Δh is depth of probe indentation into the sample, and E is Young modulus.

For experiments with blood cells, modified AFM probes shaped as hemispheres with a radius of 2.5 μ were used.

Significance of differences was evaluated using Student t test.

RESULTS

On the surface profile curves of lymphocytes from healthy donors and CLL patients, morphological structures presented by globular prominences and depressions were found (Fig. 1). The configuration of lymphocyte surface in CLL patients had a marked corrugated pattern in comparison with lymphocytes from healthy donors: the number of globular structures increased by 247% ($p<0.05$) and their height decreased by 42.6% ($p<0.05$; Table 1). The number of depressions in lymphocyte membranes in CLL patients increased by 122% ($p<0.05$) and their diameter and depth decreased by 67.4 and 46.3%, respectively ($p<0.05$) in comparison with the corresponding parameters in healthy donors.

During elastography of lymphocyte surface, force curves were recorded and used for calculation of the probe–sample interaction force. The obtained curves were transformed from the "mismatch current (DFL, nA)–height (μ)" coordinates (Fig. 2, *a*) to "force (DFL, nN)–height (μ)" coordinates (Fig. 2, *b*). The transformation was performed using DFL_to_Force script for Nova software (NT-MDT, Zelenograd, 2009). On the transformed force curves (Fig. 2, *b*), the values of DX (nm), DY (nN), and Y (nN) were found and used for calculation of probe indentation into the sample [12]:

$$x=\frac{\Delta Z(\text{SetPoint-DFL0})}{\Delta \text{DFL}} \quad \textbf{(3)},$$

where ΔZ is the converted value of the force curve by DX (nm), SetPoint is a functional parameter determining the value of the input signal of the feedback circuit during scanning (nA), DFL0 is converted value of the force curve by Y (nA), and ΔDFL is converted value of force curve calibration by DY (nN),

It was found that the Young modulus in lymphocytes from CLL patients decreased by 51.4% ($p<0.05$) and the depth of cantilever indentation increased by 199% ($p<0.05$) in comparison with lymphocytes from healthy donors. These changes attest to increased resistance of the cell surface against elastic deformations and improvement of its viscoelastic properties.

TABLE 1. Structural Peculiarities and Mechanical Properties of Lymphocytes from Healthy Donors and CLL Patients

Parameters of structures and viscoelastic properties of the surface		Healthy donors	CLL patients
Globular prominences	height, nm	41.3±3.7	17.6±0.9*
	number	36.0±0.9	125.0±1.1*
Depression in the membrane	diameter, nm	221.8±24.0	149.4±12.9*
	depth, nm	17.3±0.6	8.01±0.90*
	number	18.0±1.1	40.0±2.3*
Stiffness	Young modulus, μPa	3.5±0.2	1.80±0.01*
	cantilever indentation depth, nm	345.20±3.74	1035.20±7.32*

Note. *$p<0.005$ in comparison with healthy donors (Student's test).

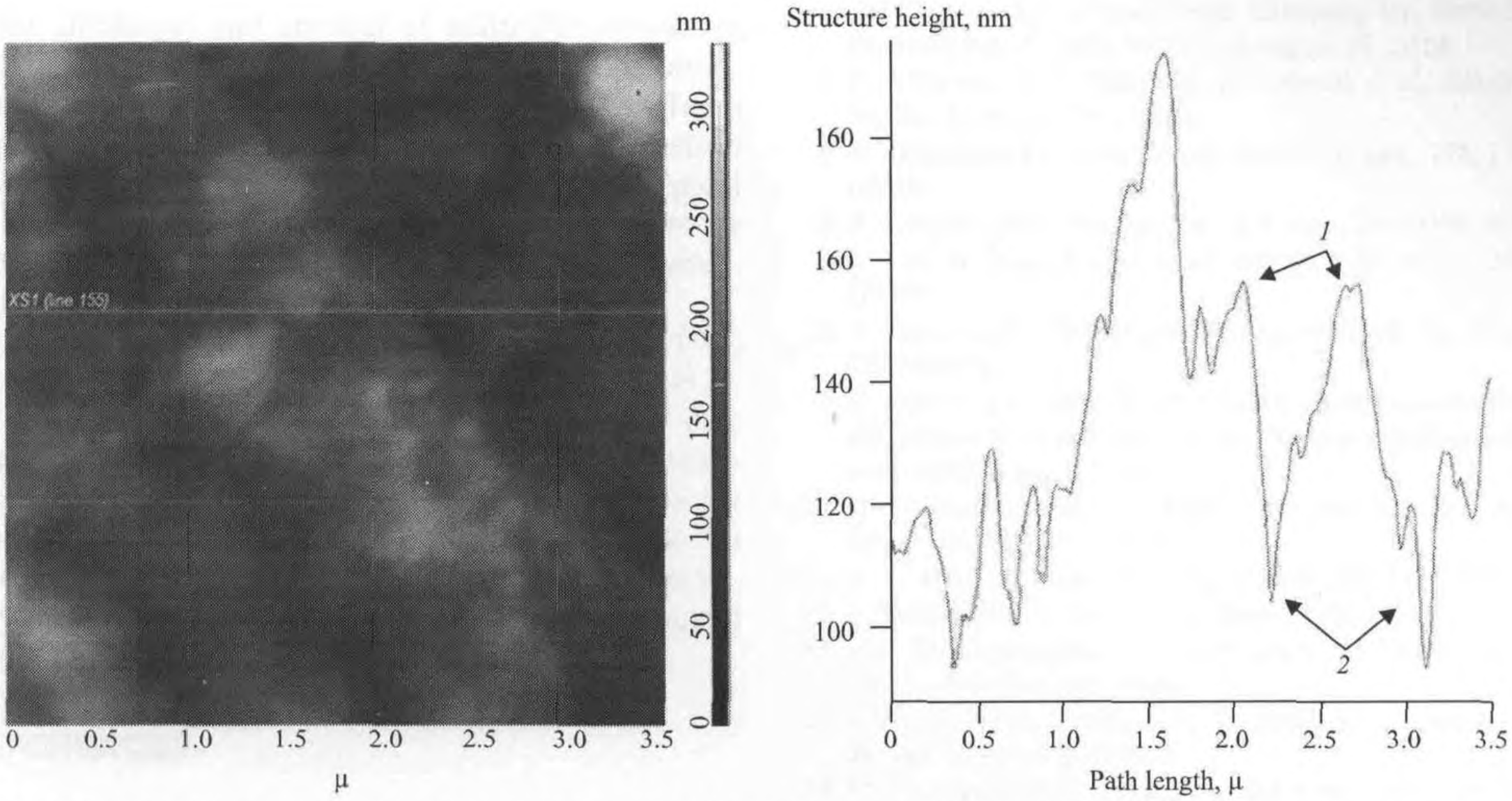

Fig. 1. AFM image (*a*) and profile of lymphocyte surface along the scanning path (*b*). *1*) globular prominences; *2*) depressions

According to scanning electron microscopy data, the lymphocytes from healthy donors and CLL patients have villous morphology [2]. The unique advantage of AFM over scanning electron microscopy is the possibility of studying micro- and nanomechanic properties of the cell structures, modifications of surface relief, and spatial distribution of local elasticity modulus within a single cell [11]. It should be noted that AFM visualization of the surface of blood cells allows identification and analysis of the finest structures with high spatial resolution due to monitoring of attraction forces between the probe and the scanned surface [15]. The use of scanning electron microscopy for studies of biological object morphology has a number of limitations related to changes in cell morphology and appearance of artifacts caused by surface probing with a sharply focused electron beam (electron probe) and the use of fixatives and low or high vacuum conditions [16].

The decrease in lymphocyte stiffness in CLL patients against the background of surface relief modification observed in our study agrees with published data that the decrease in elasticity modulus in leukemic blood cells is closely related to organization of actin filaments. Considerable differences in the organization of F-actin filaments in abnormal lymphocytes are detected: they are 2-fold smaller and less numerous that in lymphocytes from healthy donors [7]. The decrease

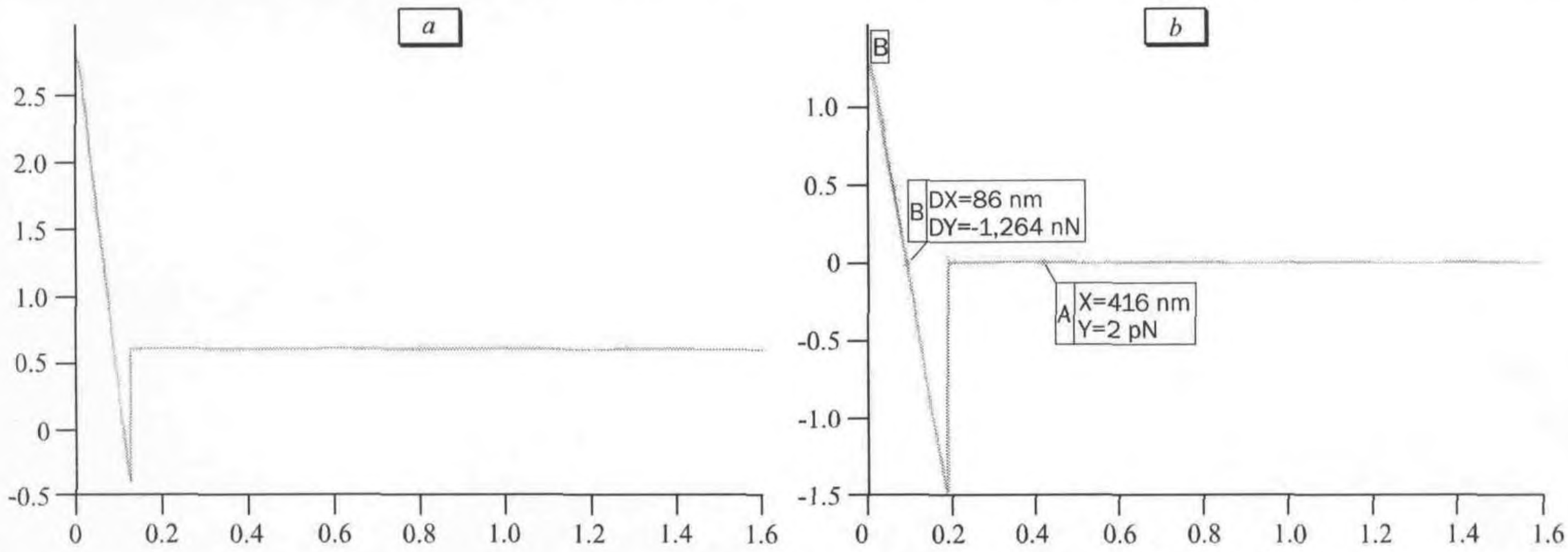

Fig. 2. Force curves of lymphocyte from a CLL patient. *a*) initial force curve in "mismatch current (DFL, nA)–height (μ)" coordinates; *b*) curve in "force (DFL, nN)–height (μ)" coordinates after conversion using DFL_to_Force script for Nova software. DX (nm), DY (nN), and Y (nN) values used for calculation of probe indentation into the sample are shown on the force curve.

in the thickness and content of actin-like structures in lymphocytes of CLL patients leads to transformation of their surface relief. Changes in cell stiffness is an important compensatory and adaptive reaction developing under conditions of lymphocytosis typical of this disease [17]; it ensures deformation of more elastic cells in capillaries and facilitates their movement in blood vessels.

Thus, reduced stiffness of lymphocytes in CLL patients and abundance of morphological structures on the cell surface in comparison with normal cells were demonstrated using AFM tools. Changes in surface topography manifesting in increased number of globular prominences and depressions in the membrane attest to cytoskeleton reorganization and represent a compensatory reaction aimed at maintenance of the rheological properties of the blood.

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