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Original article

Adhesion Force of Tlymphocytes in the pathogenesis of patients with light and severe bronchial and atopic asthma

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ABSTRACT

The emergence of various diseases specifically disabling diseases such as bronchial and atopic asthma is associated with quantitative and qualitative changes of lymphocytes. Therefore, the detection in the peripheral blood of some diseases, the so-called degenerative lymphocytes might constitute a new diagnostic and prognosis test and might serve as markers, and even constitute innovative therapeutic targets. Atomic force microscope, considered as a new method of investigation, allowed us to visualize the structure of lymphocytes at a degree of molecular resolution, to study the spatial distribution of mechanical properties of cellular structures within a single cell and the adhesive force of cell surface of lymphocytes in light and severe asthmatics. The results showed that the more the inflammatory process is high the more important the cell adhesion. With Atomic Force Microscope method a relationship of interdependency between morphological changes and the degree of severity was established. These morphological changes might lead to the increase in the force of adhesion, the release of lipidic mediators, the lymphokines capable of activating the surrounding cells and to boost the allergic inflammation. The images obtained on the morphology of patient lymphocytes could suggest a variability of morphological parameters of cells according to the severity of the disease. But these parameters vary significantly within each group. This suggests that the emergence and intensification of the inflammatory process are not related to changes in cellular parameters but rather the functioning of lymphocyte involved in the activation of immuno-inflammatory system. Therefore, using the Atomic Force Microscope method in the field of biomedical science can lead significantly to go beyond the study of morphological parameters of human blood cells and especially apoptotic lymphocytes.

1.Introduction:

The lymphocytes are fundamentally important cells of the immune system which determine the specificity of the immune response to non-indigenous such as infectious organisms and other foreign substances as allergens [1]. The emergence of various diseases specifically severe diseases such as bronchial asthma is associated with quantitative and qualitative changes of lymphocytes [2]. Therefore, the detection in the peripheral blood of some diseases, the so-called degenerative lymphocytes (having undergone a modification) might constitute a new diagnostic and prognosis test and might serve as markers [3], and even constitute innovative therapeutic targets in the healing of some diseases especially bronchial and atopic asthma.

Asthma is a chronic inflammatory disease of the respiratory tracts [4], which is characterized by widespread but miscellaneous bronchial obstruction and by hyper-responsiveness to several triggers in which T lymphocytes play a central role [5-6-7-8]. The inflammatory process in asthmatics is characterized by the accumulation of eosinophilia, T lymphocytes of CD4+ or CD8+ phenotype in the bronchial wall [9], γα T lymphocytes and B cells, macrophages, dendritic cells and mast cells [10-11-12]. Most of these cells show markers of activation on their surface [10]. The inflammatory process may depend in part on the activation of T-helper lymphocytes which induce pro-inflammatory cytokines. One of the key questions in the inflammatory process is the ability of T lymphocytes to induce immune response, on the basis of specific recognition between T-cell receptors and MHC complexes expressed at the cell surface show antigens. An increase in the expression of activation of CD4-positive lymphocytes in peripheral blood from patients with severe asthma was demonstrated as compared with those from relatively healthy patients. These

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changes might be associated with improvement of lung function [13]. The activation of T cells in the peripheral blood also occurs in asthmatic patients with light asthma [14]. On electron microscopy, bronchial-biopsy specimens from patients with asthma revealed morphological characteristics of atypical lymphocytes indicating the activation [15], the increase in these cells in the ?uide of broncho-alveolar washing of atopic asthmatics [16-17-18] and increase further following the introduction of an allergen [19-20-21-22], the immunocytochemical analysis showed an increase in the number of stained cells which activate CD25 marker (the interleukin-2 receptor) [16]. Similarly, with electron microscopy, Vodounon et al (2011) showed that the morphological characteristics of lymphocytes in the peripheral blood of patients with light and severe bronchial and atopic asthma [23]. For more information on the implication of lymphocytes in the pathogenesis of bronchial and atopic asthma, atomic force microscopy was used and considered as the new method of investigation of various micro-and nanostructures on the surface of a material [24-25]. Nowadays, Atomic Force Microscope (AFM) has the unique capability of visualizing biological samples with molecular resolution in buffer solution (this allows keeping the biological samples alive). In addition to providing topographical images of surfaces with nanometer- to angstrom-scale resolution, AFM allows to study biophysical characterizations of cell surface such as force of morphological adhesion, which gives fundamental information on cell structures and their biological functions. Various diseases, including cancer, originate from abnormalities in the adhesion process. The establishment of a pathogen in a specific tissue and / or infection of target cells also require adhesive interactions. T lymphocytes closely regulate the adhesive interactions at each stage of the immune response and inflammation in asthmatics. Currently, data associated with changes in the properties of cells of connective and epithelial tissue were obtained, and also the study of the morphology of leukocytes of human blood [26]. However, these data are still fragmentary and insufficient. Therefore, the aim of this study was to identify the morphological changes of lymphocytes, to study the spatial distribution of mechanical properties of cellular structures within a single cell by the atomic force microscopy (AFM) technique and the adhesive force involved in the initiation of inflammatory immune responses in patients with light and severe bronchial and atopic asthma.

2. Materials and methods:

2.1.Patients and blood sampling:

The study was carried out on the lymphocytes isolated from peripheral blood of relatively healthy and asthmatic individuals. The group of patient consisted of people of different severity: 15 light persistent asthma with mean age 45 ± 14 years (group A), a group of 15 patients with severe persistent asthma (mean age 43 ± 18 years) (group B) and a group of relatively healthy donors of 15 people (Group C) (mean age 40 ± 5 years). All the donors were non-smokers, and did not receive corticosteroids within 2 weeks before the study and were selected after written consent. These patients were hospitalized in the department of pneumology in the hospital of Kazan city (RKB), Republic of Tatarstan, Russian Federation. The work was done jointly with medical doctor who received approval from the Local Ethics Committee of the Medical University of Kazan for the conduct of biomedical research. The work was performed in accordance with the rules of the Ethics Committee in the laboratory of Clinical Immunology and Allergy of RKB in compliance with the Helsinki Declaration. All patients (groups A and B) had difficulty in breathing or shortness of breath, at least 5 years prior to participation, in compliance with the data from Global Initiative for Asthma [27-28]. None of the patients (groups A, B and C) had any respiratory infections in the month prior to their inclusion in the study. No other clinically relevant diseases were reported.

2.2.T-lymphocyte isolation:

Eight milliliters of blood from the veins of donors (A, B, C) were collected in special tubes containing heparin-Na (Eurotube, Spain). The lymphocytes were isolated according to the standard method of zonal centrifugation suggested by Patel et al [29] with a mixture of Ficoll-verographfin (ρ = 1077 g.cm-1) [30-31]. This method involves isolating 95% of T lymphocytes and their viability was determined by the method of trypan blue exclusion [32-33]. To obtain a pure population of T lymphocytes, we used the negative isolation method with the use of super paramagnetic beads CD3 (Dynabeads Untouched Human T cells, Dynal, Invitrogen).

2.3.Study of T-lymphocytes with the atomic force microscope:

The AFM method allows obtaining images of biological materials under native conditions, without distorting the fine structure of molecules [34]. The lymphocytes were placed on a skimmed glass microscope and incubated at room temperature for one hour. The study of cell surface was performed by a scanner device (Integra or PRIMA) in tapping mode (NSGO1 was used as a probe with an atomic radius of about 10 nm and a coefficient of elasticity of 2.5 to 10N / m with a resonant frequency of 150-170 kHz) ("NT-MDT", Russia). Image contrast is generated by monitoring the forces of interaction between the tip and the surface. A small laser, which is focused on the cantilever, detects any bending or twisting of the cantilever. The reflection of the laser beam is focused on a photodiode detector. The interaction of the sample with the tip is measured by the variation in the reflected beam's point of incidence on the photodiode. Deflection of the cantilever by interaction with features on the sample surface is monitored during scanning and is translated into a three-dimensional image of the surface [35].

2.4.Statistics:

The image processing and the determination of the size of the scanned objects i.e the variations in heights were recorded as images using the software Nova RC 1.0.26.1348 for scanning probe microscopes of "NT-MDT." The distribution model and the analysis of the data obtained (mean and standard deviation) in individual samples were analyzed using the Excel package.

3.Results:

The images of lymphocytes of relatively healthy donors and patients with light and severe asthma were obtained with the atomic force microscope (fig.1,2,3,4). The lymphocytes of patients with bronchial asthma show morphological changes that are related to the severity of the disease (fig.3, 4).

In our study, 2.5% glutaraldehyde was used for fixing the lymphocytes of relatively healthy donors (fig.1). A classic fixing allows keeping the shape of the lymphocytes, but most of cellular components outside the nucleus are not distinctive with a significant reduction in lymphocyte parameters. Therefore, we decided to study the lymphocytes in their native form without the use of fixer (fig.1). The lymphocytes from healthy donors have a relatively typical rounded shape. On the cell surface, cellular microvilli and pseudopodia were visible (fig.1). The height was measured in nanometers (10-9 м). Generally, the height of the cells

determines the intracellular components (such as the nucleus, mitochondria and granules). With the atomic force microscope method the image of the topography of lymphocytes were recorded. On the surface of the cytoplasmic membrane the corpuscles were clearly identified, without doubt being a part of the integrative protein of membrane or fragments of glycocalyx. The size of corpuscle was 50 ± 7.5 нм, p <0.05.

The lymphocytes from healthy donors had a rounded shape (Fig. 2). The elevated portion of the cell, corresponding to the brightest area on the color scale represents the nucleus (Fig. 2, a). In addition, the entire cytoplasm of lymphocytes was spread over the cover glass during the preparation of the sample, allowing to see the nucleus (fig.2, a, b), its shape, and other components of the cell. The scanning of lymphocytes and their three-dimensional structure (Fig. 2, a, b) showed that cells have a large nucleus occupying the entire volume of cytoplasmic volume of the cell and the presence of some smaller roughness comparable to the granules (fig. 2 a , b).

Fig. 1: Three-dimensional structure of lymphocytes from relatively healthy donor.

a- fixed with 2,5% glutaraldehyde;

b- b- without fixing.

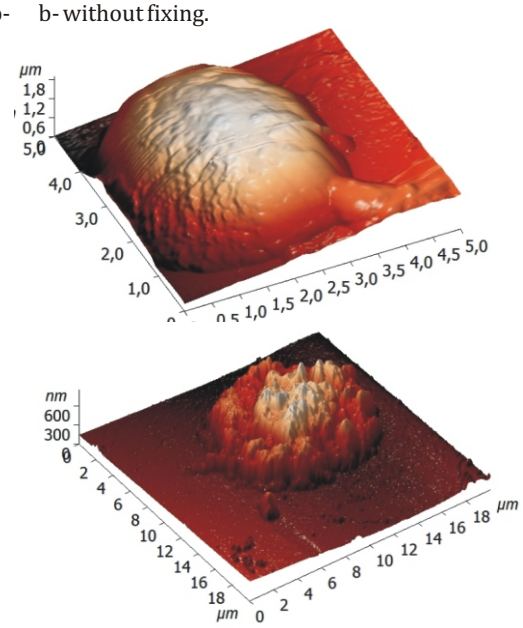
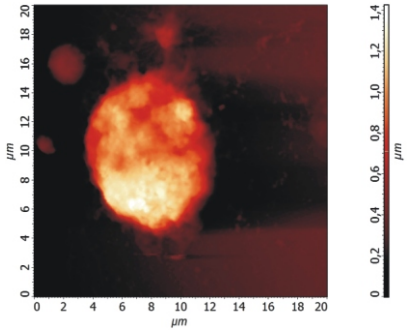
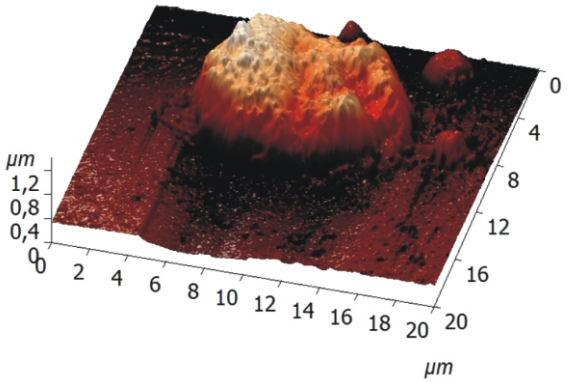


Fig 2: Schematic diagram showing the structure of lymphocytes from relatively healthy donor.

(a) - Scanning of the morphology of lymphocytes from relatively healthy donor : a typical two-dimensional image of T-lymphocytes. Arrow showed microvilli and pseudopods.



(b)- The image corresponding of the three-dimensional structure of lymphocytes from relatively healthy donor.



C- The topography of a typical surface of T-lymphocytes from relatively healthy donor, obtained by atomic force microscope.

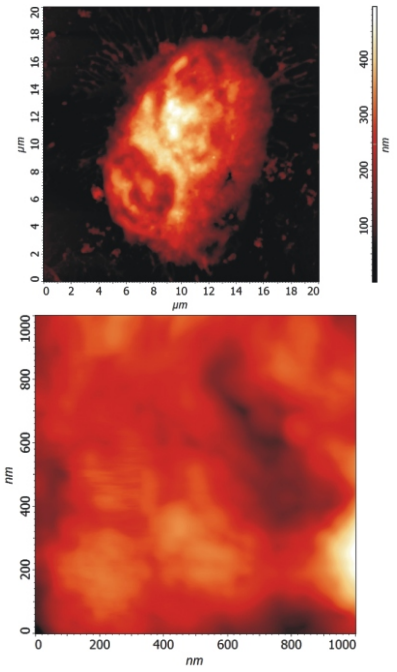
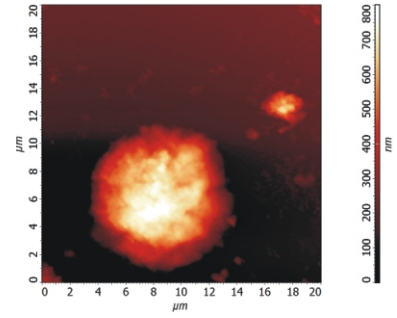
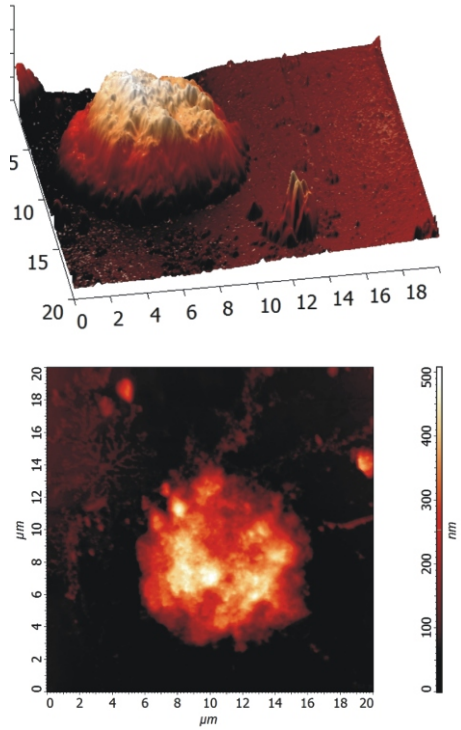


Fig 3: the structure of T-lymphocytes of asthmatics with light severity

A Scanning of the morphology of T-lymphocytes. Two-dimensional image of lymphocytes with light severity.



b- Three-dimensional structure (3D) of lymphocytes from asthmatics with light severity.



c- The topography of the surface of T-lymphocytes from asthmatics of light severity.

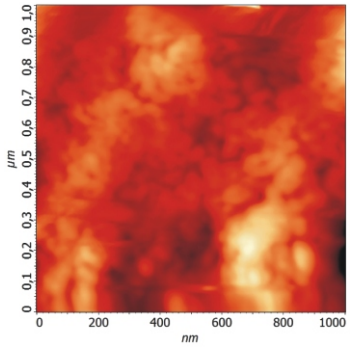
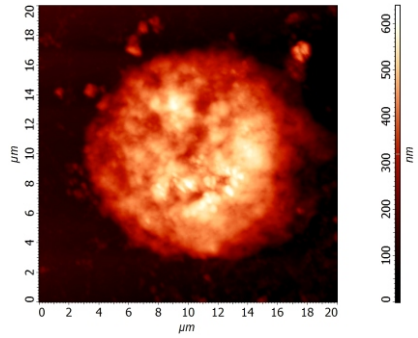
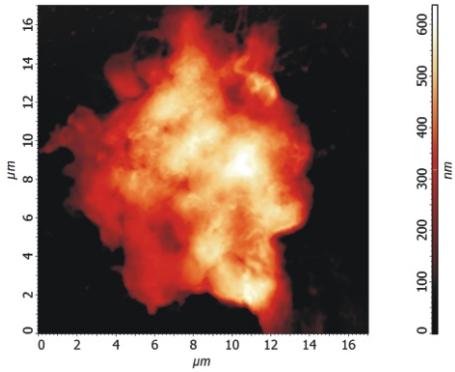


Fig 4: Scan of the morphology of lymphocytes of asthmatics patients with serious severity.

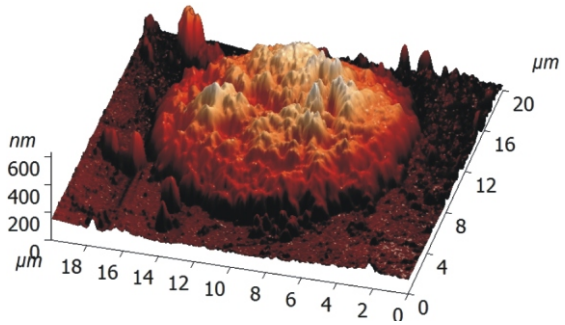
a-1. Two-dimensional representation of T-lymphocytes with a normal morphology.



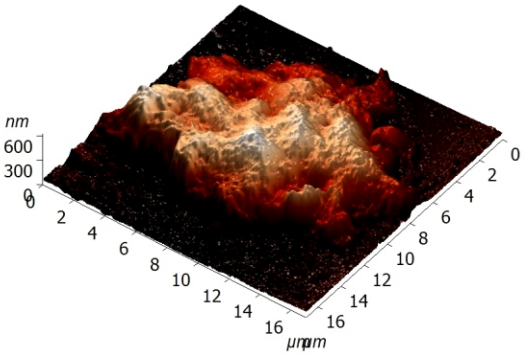
a-2. Two-dimensional representation of T-lymphocytes with a change in morphology



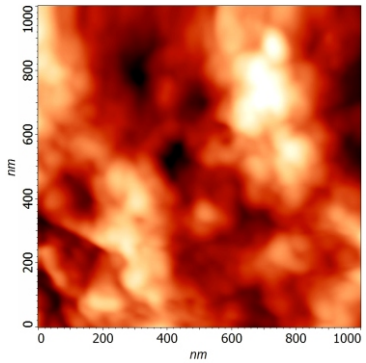
b-1. Three-dimensional structure (3D) of lymphocytes with a normal morphology.



b-2 . Three-dimensional structure (3D) of T- lymphocytes with a change in morphology.



c-1. Topology of the surface of T-lymphocytes with a normal morphology.



c-2. Topology of the surface of T- lymphocytes with a change in morphology.

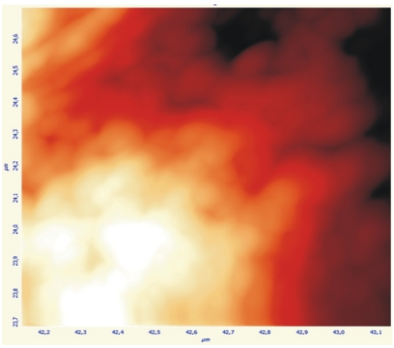


Fig. 5: the change of morphological parameters of lymphocytes (length-width-height) of relatively healthy donors and asthmatic patients with light and serious severity.

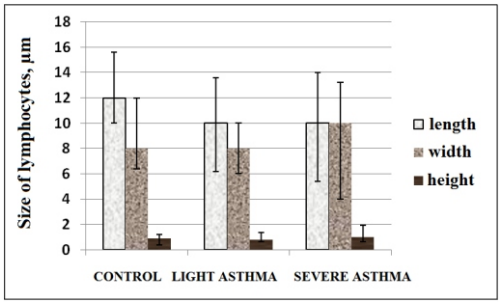


Fig. 6: the spectroscopic curve of the derivation of the cantilever showing the fragmentation, the length of the projection on the x-axis, which determines the magnitude of curvature.

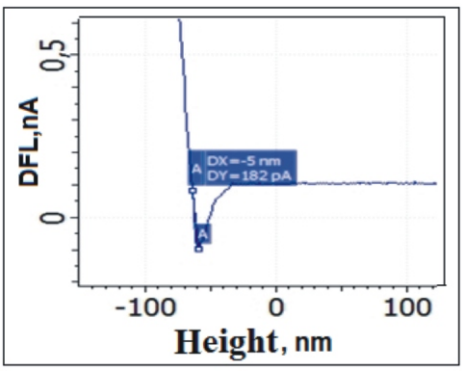


Fig. 7: Diagram of cell adhesion force from relatively healthy donors and patients with light and severe asthma - obtained by atomic force microscopy.

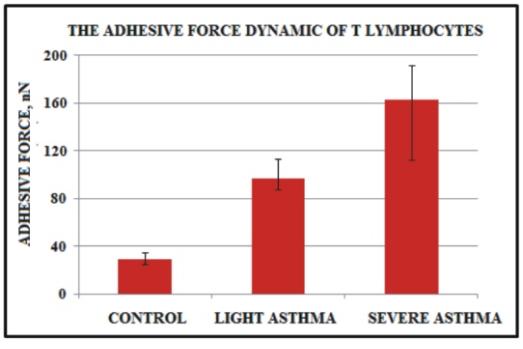
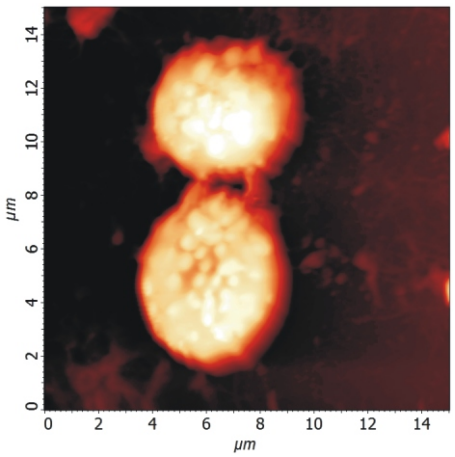
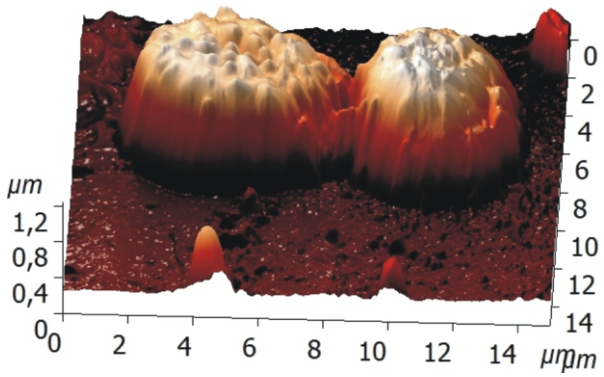


Fig. 8: cell adhesion with the formation of cytoplasmic bridges



(a) - cytoplasmic bridge between the cytotoxic T lymphocytes and target cells.



(b) - three-dimensional structure showing communication between a cytotoxic cell and a target cell.

The lymphocytes from patients with light severity were characterized by an absence of microvilli, the formation of numerous intussusceptions on the surface of the cell membrane when compared with the lymphocytes controls. There was also a globular structure on the cell surface. As the number of corpuscles increased their size decreased (20.5 ± 5.5 нм; $p < 0.05$). The nucleus of lymphocytes of asthmatics with light severity (as well as the cell itself) was rounded, occupying most of the cytoplasmic volume (Fig. 3. a, b). There is a clear area in the nucleus recalling the structure of a condensed chromatin (Fig. 3, a). In most cases, the plasmatic membrane of lymphocytes presents invaginations. At the same time, lymphocytes were detected with unmodified surfaces. The cytoplasmic organelles were clearly visible and close to the nucleus (fig.3.b) with occasional eccentric nuclei compared with the cell membrane (fig.3.b).

This group of donors (asthmatic patients with serious severity) was characterized by significant morphological differences between lymphocytes when compared to two other groups mentioned above (fig.4.ab). The membrane was characterized by a loss of microvilli and desmosomes. However, the integrity of the membrane structure was retained (Fig. 4). The granules were very poorly differentiated. The nucleus had a variety of intussusceptions with subdivisions (Fig. 4). Inside the nucleus was observed the spreading of chromatin occupying the

entire nuclear volume. The topography of the surface of normal lymphocytes of asthmatics with serious severity show like lymphocytes from light asthmatics recorded a large number of globular structure with a big size (47.1 ± 11.3 нм; p <0.05) (fig. 4, c-1). At the same time in the modified lymphocytes, the corpuscles were hardly discernible, diffuse with a decrease in their number.
One might assume that the similar structure is related to the formation of different proteins at the cell surface such as markers of CD4 activation of lymphocytes, or adhesion proteins (L-celectine) whose expression increased during bronchial asthma.
Based on the results obtained, one might assume that the size of lymphocytes varies from one group of patients to another depending on the severity of asthma. However, the changes observed in the size of lymphocytes in the experimental conditions do not show with certainty a dependent relationship between cell size and the severity of disease.

The change of morphological parameters (length, width and height) was studied according to the severity of asthma (Fig. 5). These parameters varied within each group (Fig. 5). According to the literature, the size of lymphocytes can vary from 5 to 20 microns and this according to the stage of differentiation in which cells are found. We can conclude that the increase in the degree of severity of disease does not affect the parameters studied or at least these changes were not identified in our study. The lymphocytes of relatively healthy donors were longer (12 microns) and had a width and a height substantially equal to those of lymphocytes of asthmatics with light severity (8 microns) (Fig. 5). On the other hand the lymphocytes of asthmatic patients with serious severity dominate in width (10 micron) with a height slightly higher than the other groups studied (Fig. 5).

The atomic force microscope allowed us also to study the adhesive force on the surface of lymphocytes of asthmatics with light and serious severity. The withdrawal of the cantilever from the sample creates a significant impact of a force of adhesion which may cause the flexion before the separation from the surface. The linear dependence of the connection force from the withdrawal of the cantilever compared with the cell surface (adhesion force F) was measured by Hooke's law: where dH - is the value of flexion of the cantilever, and k-constant force of the cantilever.
The discharge curve of the cantilever was set out from the height of the DFL cantilevered with the cell surface (Fig. 6). The signal corresponds to the difference of DFL signal between the upper and lower halves of the photodiode, through which the laser beam from the cantilever went through. The value of flexibility due to adhesion corresponds to the height differences between the smallest amplitude of the DFL signal and the DFL signal in the free state (DX in Fig. 5). According to the tables of data, constant force is k = 5 N / m, which allowed us to compare the adhesive force of different cells.
For the determination of cell adhesion, 3 groups composed of 10 donors each and from each donor 10 cells were studied. A total of 100 cells were studied in each group. The results showed a variation in the force of adhesion on the cell surface. These changes varied depending on the severity of the disease (Fig. 7). The lowest values ??of the force F of adhesion on the surface of lymphocytes were recorded in the cells from relatively healthy donors. The average of the maximum value was roughly 30 nN and that of the minimum value was 19 nN (Fig. 7).

The force of adhesion of lymphocytes from asthmatics with light severity was higher than that obtained from relatively healthy donors. The maximum was 100 nN and the minimum 30 nN (Fig. 7). The highest values ??were obtained in asthmatic patients with serious severity where the maximum value was roughly 180 nN that is six times larger than that obtained in controls, and the minimum was 100 Nn (Fig. 7). These differences in the values ??of adhesive force can be attributed to the increase in the inflammation associated with increasing severity of the disease. Besides the above results on the force of adhesion of lymphocytes, we found that cells were linked amongst themselves by "bridges" (Fig. 8). We assumed that the figure obtained was nothing other than lymphocytes and cytotoxic lymphocytes and T-lymphocytes carrying antigens. In this case, the formation of this bridge comes from the cytoplasm and may serve as a springboard for the transmission of lympho-toxins for eventual lysis of the target cells. This bridge expresses the communication between cells involved in inflammatory immune responses in asthma.

4.Discussion:

Asthma is a chronic disease that is characterized by inflammation of respiratory tracts [36-4]. The lymphocytes play a central role in the pathogenesis of asthma. Although they are not the most numerous inflammatory cells in either the alveoli or the respiratory tracts of relatively healthy individuals, T lymphocytes are in important quantities in the compartments of the lungs, and their numbers are growing very rapidly in response to inflammatory signals. T lymphocytes are a key component of the immune response in asthma [37-38], as well as in relatively healthy individuals, because they are able to activate other inflammatory cells through cytokines. Active T lymphocytes, spontaneously release cytokines that stimulate eosinophilia and mast cells [39-13-40-41]. The population of lymphocytes increases in the lungs of asthmatic donors in particular during the period of the worsening of the disease [2]. This provides evidence that activated CD4+ T lymphocytes can be identified in the peripheral blood of patients with acute severe asthma [42]. The percentage of activated CD4+ T lymphocytes decreased with treatment and clinical improvement [42]. Robinson et al. (1993) showed that CD4+ T lymphocytes in broncho-alveolar washing fluid and blood from asthmatic patients were activated, in comparison to controls.
With the atomic force microscopy, a relationship of interdependency between morphological changes and the degree of severity was established (Fig. 3, 4). The light form of asthma is characterized by a rare allergic reaction, in which cells are directly involved. There is a clear zone at the nucleus level, sometimes eccentric to the nuclear membrane, recalling the structure of condensed chromatin (Fig. 3, a). In most cases, the plasmatic membrane of lymphocytes show excrescence. The cellular components are well differentiated with lymphocytes that are more or less correct and oval (Fig. 3). The severe form is characterized by pronounced morphological differences (Fig. 4) compared to the two previous groups (fig. 2, 3). However, the membrane integrity of lymphocytes is not affected, which is very important for the organism. However, this morphological change could result in the increase in the expression of adhesion molecules, the release of lipid mediators, cytokines, chemokines and growth factors that can, in turn, activate the surrounding cells and amplify tissue inflammation [43]. The prolongation of allergic inflammation in asthma is associated with the increased survival of T lymphocytes [9-44-45] and the loss of their ability to apoptosis [7-46]. The lack of lymphocyte apoptosis could account for their uncontrolled accumulation and persistence in the respiratory tracts and thus maintain the chronic inflammation and tissue damage.

It was found that lymphocytes from patients with bronchial asthma are characterized by inhibition of apoptosis [47-48-49]. The study of molecular mechanisms of cell apoptosis in asthma, in recent years, has become one of the most difficult and pressing problems of biomedical sciences and the role of apoptotic mechanism of immunological suppression in the pathogenesis of allergic diseases have been extensively studied. The apoptotic cells are characterized by the formation of deep invaginations on the cell surface with the generation of cavities [50]. We could, therefore, assume that the morphological changes of cells are associated with apoptosis. The images obtained on the morphology of patient lymphocytes (fig.3,4) could suggest a variability of morphological parameters of cells according to the severity of the disease. The results on cell size (length, width, height) were obtained. However, under the experimental conditions, the parameters of lymphocytes of different groups studied were not statistically different (Fig. 5). It was established that the size of lymphocytes in the experimental conditions did not vary with the severity of asthma. But these parameters vary significantly within each group. As the size of lymphocytes in the process of differentiation and their vital activity may vary within the range of 5 to 20 microns, one might assume that all cells studied were found in different stages of their functioning. We can, therefore, say that the emergence and intensification of the inflammatory process is not associated with the changes in cellular parameters. Among the most important applications of atomic force microscopy in biological studies include the use of AFM as a nano-mechanical sensor, which allows to study the mechanical properties (elasticity) and the adhesion of biomaterials[51-52]. The main advantage of AFM is the ability to record the force of adhesion to the cell surface or other objects.

During inflammation occurs over-expression of adhesion molecules in inflammatory sources (Fig. 7), which leads to the accumulation and retention of leukocytes. At the same time, the lack of expression of molecule of adhesion on the cell surface or on the ligands can result in the development of a chronic inflammatory response. It was shown that there is an imbalance in asthmatic patients with light severity, in cytokine production, and this for several reasons. One of these is the increase in over-expression of specific adhesion molecules. Bronchial hyper-responsiveness associated with reactions of delayed or postponed phases are mainly the characteristic features of bronchial asthma. The development of delayed phase reactions is characterized by the following event: the increase in the permeability of vessels, where histamine, C4 and D4 leukotriene and bradykinine play the primary role, the internalization of adhesion molecules on the endothelium (ICAM-1, VCAM-1, ELAM-1) and their association with the receptor-ligand complex of leukocytes (LFA-1, VLA-4), which leads to the migration of leukocytes to the surface of endothelial cells (rolling effect) and then a strong binding of vascular endothelial cells in the reaction site (the main molecule of initiation: IL4 and TNF), a trans-membrane migration or diapedesis of inflammatory cells [53] to the center of inflammation. One of the characteristics of refractory inflammation in the course of asthma is the infiltration in bronchitis of activated CD4 lymphocytes. The results of many authors confirm the participation of several adhesion molecules (ICAM-1, VCAM-1, L-selectin, E-selectin) in the development of allergic process [54-55-56-57]. We observe an increase in adhesion with increasing severity of the disease which can apparently be related to the intensification of the inflammatory process. Therefore, the results obtained in the present study confirm this theory once again.

Based on the results of this study (Fig. 7), we assume that the stronger the inflammatory process the higher the adhesion of cells. The different groups studied showed a direct relationship of interdependence between the adhesion force F and the severity of asthma. According to literature, the increase in lymphocytes in the fluid of broncho-alveolar washing correlates with the severity of bronchial asthma [58]. Interleukins released in the area of inflammation and particularly Ile-2, link to the surface of cell receptors (IL-2R) and cause the expansion of antigens stimulating T lymphocytes and also play a key role in the activation of the immune system and the conversion of a refractory and acute inflammation to chronic inflammation.

With the escalating severity of the disease, an increase in the adhesiveness of cantilever appears to be associated with increased inflammation process. In the present study the active cytotoxic T lymphocytes were observed. This cell population has a lytic effect on target cells through cytoplasmic bridges (Fig. 8). Cytotoxic T lymphocytes are in direct contact with the target cells, increasing their permeability, causing osmotic swelling, the rupture of the membrane and the release of the content into the surrounding areas. The effect of cytotoxic mechanism is associated with the activation of membrane enzyme systems in the area of cell adhesion with the formation of cytoplasmic bridges between the cells and the effect of the lymphotoxin [59]. Previous work in our laboratory revealed the presence of granular DNases with subpopulation T8 and T4 that is associated with an imbalance of cytotoxicity and the repression in asthmatics [60]. Adhesion is a ubiquitous phenomenon necessary for many biological functions. T lymphocytes closely regulate the adhesive interactions at each stage of the immune response. These steps include the firm attachment to endothelial cells as they travel through the blood vessels, the formation of the immunological synapse (with a cell showing antigens) and contact with other cells when they go through the peripheral tissues in the search for invaders or infected cells. Despite the advances in the treatment of asthma, the overall mortality has not decreased significantly, while the prevalence and the severity of the disease are increasing [61]. Respiratory tract inflammation is present in patients with light, moderate or severe asthma [62]. The mechanisms which underlie this bronchial mucosal inflammation are complex. The absence of effective therapy can be explained by the complexity and multiplicity of cellular and molecular events involved in lymphocyte changes, but also by the variability in the time of the occurrence of these abnormalities during the evolution of asthma. These considerations underline the importance of identifying cellular markers [63] and molecular predictors. Estimation of structural of living cells with AFM can be of great importance in diagnostics of different human diseases. So many questions remain unresolved and their elucidation will undoubtedly lead to interesting perspectives.

5.Conclusion:

The deteriorating ecological conditions contribute to an increase in the number of people with various pathologies. Among the most common include bronchial and atopic asthma which is an acute and chronic disease of respiratory tract, with the participation of many cells including T lymphocytes. Many studies have been carried out on this disease. Therefore, using the AFM method in the field of biomedical science can lead significantly to go beyond the study of morphological parameters of human blood cells and especially apoptotic lymphocytes. The morphological study of the different groups showed a significant difference which

increases with the degree of disease severity. These changes could be the consequence of an immuno-inflammatory response. The changes observed in the values ??of adhesive forces of different groups studied are functions of the severity of asthma and may be associated with the involvement of its cells in the inflammatory process. But the question remains open.

Competing of interest: We have no competing interests

Author's contribution: AZI, VAC, TVN designed the study. CYV, MMB, KN, VAC participed in the technical work and the acquisition and interpretation of data. KBSM, SH, BML, VAC evaluated the literature. CYV, AZI, VAC has carried out the experiments of this study.AZI, TVN, VAC,BML, KSO have given final approval of the version to be published.

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