Morphological and biochemical characteristics of apoptosis lymphocytes of peripheral blood in the pathogenesis of atopic bronchial asthma of light and serious severity

Vodounon Alode Cyrille*1,2, Skibo Yulia1, Aikou Nicolas2, Akpona Simon2, Abramova Zinaida Ivanovna1, Kotchoni Oloni Simeon3, Baba-Moussa Lamine2

1Laboratory of Acid Nucleic. Department of Biochemistry. Faculty of Biology. Kazan Federal University (KFU-Russian), Kremlyovskaya str. 18. Kazan 420008. Republic of Tatarstan, (RUSSIAN FÉDÉRATION)
3Department of Biology and Center for Computational & Integrative Biology, Rutgers University, Camden, NJ 08102, (USA)

E-mail: sweetiebj@yahoo.fr; yuliya_ksu@mail.ru; aikounicolas@yahoo.fr; akponasimon@yahoo.fr; ziabramova@mail.ru; sok8@camden.rutgers.edu; laminesaid@yahoo.fr

Received: 17th May, 2012 ; Accepted: 14th September, 2012

ABSTRACT

Background: The deregulation of the apoptosis of immune-competent cell has been suggested to play a key role in the development of the inflammatory reactions in allergic diseases like asthma. This study aimed to investigate the particularity of apoptosis of peripheral blood lymphocytes in allergic asthmatic patients (light and severe persistent asthma). The study of apoptosis peripheral blood lymphocytes were assessed by electron and optic microscopes and flow cytometry.

Result: The apoptosis of the lymphocytes of asthmatics shows structural and biochemical particularities. The initial phase of the apoptosis is expressed through the correlation between the number of cells with a decrease in the mitochondrial potential and the translocation of the phosphatidylerine on the surface of lymphocytes of patients. The extension of the survival of lymphocytes in asthmatics of light severity is associated with the increase of the proliferating activity on the slowdown of the apoptosis of cells. On the other hand, the increase of the rate of self-antibodies directed against DNA in asthma of serious severity is associated with the death of lymphocytes by means of programmed necrosis, followed by the development of the alteration of the nearby cells and the inflammatory reactions, but also the phagocytosis of the remaining cellular fragments and the development of the immune response, if these cellular fragments contain chromatin associated with antigens.

Conclusion: The results obtained (especially with the patients of serious severity) allowed to understand the impact of the apoptosis on the immunity of the patients and to forecast the degree of serious asthma. Further study in immunology will benefit from these findings in order to better understand the process of apoptosis in atopic bronchial asthma patients. It appears to be obvious that diseases such as cancer, autoimmune disorders may derive benefit from such studies.

© 2012 Trade Science Inc. - INDIA
INTRODUCTION

At the cellular level, the biological support of the integrity of human body is performed by a regulating influence of assignable signals which support balance conditions between 3 physiological and integrative processes namely: the proliferation, the differentiation, and the cellular-programmed death (apoptosis, the autophagy and the programmed death by necrosis)\(^\text{[1]}\). During the last few years, the study of the apoptosis of isolated cells mainly the lymphocytes have become one of the most complicated and serious problem in the field of biomedical science. Until now, the mechanism of regulation of the apoptosis of an isolated cell has not been clarified in detail. This problem is of paramount importance and is seen by the fact that, any abnormality of the apoptosis can be responsible for triggering and developing numerous pathologies\(^\text{[2,3]}\). What is very important is that the development of the acute and serious diseases is associated with the abnormality of apoptotic process that sometimes when the cell escapes the process and in that case it leads to cancer development\(^\text{[4,6]}\) or otherwise apoptosis “embraces” a number of cells, which is a pathogenic factor for the development of other forms of diseases among which may include the self-immune and immune-deficiency diseases\(^\text{[4,5]}\). Note that the apoptosis may be the cause of allergy. The role of the mechanism of the apoptosis in the pathogenic field of allergic diseases was actively studied. The particular interest is conditioned by the fact that, on the basis of allergic asthma, excessive activation of the immuno-competent cells, leading to the accumulation of auto-reacting clones (the positive activation resulting in the absence of apoptosis) were observed\(^\text{[7-9]}\). The specificity of the inflammatory process at respiratory level in the case of asthma is characterized by an increase in reactive cells such as: eosinophiles, macrophage, lymphocyte-T\(^\text{[10,11]}\) and the loss of the epithelial cells in bronchitis\(^\text{[12]}\). The apoptosis of lymphocytes-T is considered by some authors as a mechanism of selection of lymphocytes directed by antigens\(^\text{[13,14]}\). Considering that the change occurs more quickly at the cells level, it is interesting and of paramount importance to carry out the comparative study of the morphological and the biochemical parameters of lymphocytes between relatively healthy patients (control) and asthmatics according to degree of severity.

MATERIALS AND METHODS

Patients and blood sampling

The study was carried out on the apoptosis of lymphocytes isolated from peripheral blood of relatively healthy and asthmatic individuals. Eighty-six (86) patients including 40 men and 46 women with an average age of 31 +/- 8 years and 30 control donors who were not suffering from the atopic bronchial asthma (ABA) with an average age of 28 +/- 5 years were studied. The group of patients consisted of people of different severity: 48 patients were slightly affected, and 38 patients were severely affected. The patients were hospitalized in the detachment of pneumology in the hospital of Kazan city (RKB), Republic of Tatarstan, Russian Federation.

The severity of asthma in these patients was assessed according to the Global Initiation for Asthma guideline\(^\text{[15,16]}\). The diagnosis of the ABA was established on the basis of the data of allergic anamnesis, results of cutaneous experiments of skin with allergens and dust. All patients and control subjects had not received oral steroids in the 2 weeks prior to their recruitment for the study and were selected after written consent was obtained. All patients were informed and they gave their written consent. 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. All subjects were free from upper respiratory tract infections. The blood was taken from the veins of donors in the morning before their breakfast. Nine (9) ml of blood was taken in a specialized tube containing heparin-Na (EUROTUBO, Spain) from each patient and control subject. Leucocytes cells were counted using Garaeva camera and optical microscope (ocular 10x, objective 8x).

Isolation of lymphocytes

Lymphocytes were isolated according to the standard method of zonal centrifugation proposed by Patel\(^\text{[17]}\) with the mixture of ficoll-verograffin (r=1.077 g / cm)\(^\text{[18,19]}\). Patel method consists in isolating 95 % lymphocytes-T. The viability of lymphocytes was determined by the trypan blue exclusion method\(^\text{[20-22]}\).
The culture of lymphocytes

The cells obtained (2×10^6) were diluted in 1 ml solution of RPMI-1640 medium in a flat-bottomed plastic plank (Nung), then adding 10% serum of foetal calf as well as 10 µl of L-glutamine (200 µg/ml) (Flow)[23]. The cells were cultured and incubated in CO₂-incubator (5 % CO₂) for 1-6 days[24,25].

Morphological study of lymphocytes

The cells obtained after zonal centrifugation were successively precipitated in 2.5 % glutaraldehyde and 1 % OsO₄ for 1 hour. The sample was then plunged into the “epon 810” after dehydrating it with ethanol (30°C, 40°C, 60°C, 70°C, 96°C) in acetone and propylene oxide. Cuts were realized with ultra microtome LKB-3 and observed with electron microscope (Hitachi 125-Japon)[26], after putting the material in uranyl acetate[27] and lead citrate[28] for the contraction.

Flow cytometric analysis

The quantification of the apoptosis of lymphocytes was performed with cytometer in flux on FacsSalibur device (“Becton Dickinson”) by using some parameters such as the determination of DNA fragmentation by means of iodide propidium (IP) (“Sigma”)[29], the translocation of membrane phosphatidylserines at the surface of lymphocytes with the aid of merocyanine (MC540), the measurement of the variation of potential of mitochondrial membrane of lymphocytes according to the intensity of florescence CMX-Ros (“Molecular Probes”)[30]. On every variant of the experiment we counted not less than 10000 cells (units).

Measurement of the potential of mitochondrial membrane of the lymphocytes

The difference of potential of mitochondrial membrane of lymphocytes was measured with the aid of fluorochrome CMX-Ros[31] by adding 5 µl of Fluorochrome CMX-Ros[32] in 1 ml of cellular suspension containing 10⁶ cells/ml. The mixture was incubated in darkness for 30 min at 37ºc in a cytometric tube[33]. The dead cells were not counted.

Expression of the phosphatidylserine on the cellular membrane

5 µl of MC-540 was added to 1 ml of cellular medium and then incubated in darkness for 5 min at the ambient temperature[33]. The dead cells were not counted.

Determination of DNA fragmentation of lymphocytes

The number of cells being in a stage of apoptotic process was calculated by the rate of cells recorded in the diploidic zone after colouring the cells with the iodide propidium (IP)[29,34]. The cells were put in 70°C of ethanol for an hour, then rinsed in a buffer solution and coloured with IP containing 0.1 % of triton x-100 and 0.1 % of sodium in darkness for 15 min at ambient temperature. The percentage of cells in the diploidic zone was determined by histogram 2 located at the left of the basic peak which corresponds to diploidic cells.

Statistical analysis

All analyses were performed using Excel program and statistics software 5. 0. The Mann-Whitney rank sun test, a non-parametric method and the Fisher exact test were used to assess the differences between the asthmatic patients of light and severe disease. A p value of ≤ 0.05 was considered to be significantly different.

RESULTS

Patients with allergic asthma

The severity of asthma in this group of patients according to the Global Initiative for Asthma guideline, which was based on daytime symptoms, nocturnal symptoms, and lung function, was as follows light persistent asthma (56%) and severe persistent asthma (44%).

Variation of the number of lymphocytes in vivo

The total number of leukocytes in the blood of atopic asthmatics (6.18 × 10⁹/L) did not significantly differ from the number of leukocytes in the blood of the relatively healthy donors (6.3 × 10⁹/L)[35]. But when studying in detail the different types of leukocytes in the blood, there was a significant increase in the number of neutrophiles (δ=0.04) and eosinophiles (δ=0.26) compared to the normal (TABLE 1).

The increase in the number of neutrophiles and eosinophiles was counterbalanced with a decrease in the number of lymphocytes (0.05) and monocytes (0.03) (TABLE 1). The decrease in the number of lymphocytes was proportional to the degree of severity of the disease (TABLE 2). The use of Mann-Whitney Criterion, a non-parametric method, showed a difference of
95% (δ=0.05) between the number of lymphocytes of the patients of light and severe disease (TABLE 2). The co-relational method of analysis showed a reverse relation of dependence between the number of lymphocytes and the degree of severity of the disease (r = -0.3; δ =0.04; n=49). Some biochemical and morphological parameters were studied in vitro to explain the decrease in the number of lymphocytes observed.

TABLE 1 : The quantitative analysis of the maintenance of leukocytes in peripheral blood of control (N) and the patient of ABA.

<table>
<thead>
<tr>
<th>Leukocytes:</th>
<th>Leukocytes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>N 6.3×10^9/L</td>
<td>BAA 6.18×10^9/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leukocytes:</th>
<th>maintenance of colored elements, in % from Σ quantities of leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>In norm (N), n=20</td>
<td>BAA, n=70</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>30%  27.3%   ↓</td>
</tr>
<tr>
<td>Neutrophiles</td>
<td>59%  63.5%   ↑</td>
</tr>
<tr>
<td>Monocytes</td>
<td>6.6%  5.7%   ↓</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>2.5%  4.6%   ↑</td>
</tr>
</tbody>
</table>

TABLE 2 : Variation of leucocytes formula of venal blood in bronchial and atopic asthmatic patient with different severity (light and grave severities).

<table>
<thead>
<tr>
<th>Number of leukocytes in %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>patient of ABA, n=49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>Grave</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>30%</td>
<td>37.8%</td>
</tr>
<tr>
<td>Neutrophiles</td>
<td>59%</td>
<td>52.4%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>6.6%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>2.5%</td>
<td>5.4%</td>
</tr>
</tbody>
</table>

Biochemical study of apoptosis of lymphocytes

During the determination of biochemical parameters of the apoptotic process, the percentage of cells (units) observed in the diploidplioic area of the histogram in three days was compared with the number of cells before the culture, and in six days with those obtained for three days of culture (TABLE 3). Referring to TABLE 3 the number of lymphocytes obtained in three days increased approximately by 45% in the culture of the cells of the relatively healthy donors (controls), and by 58% for patients with light asthma. A decrease in the number of lymphocytes with both patients and relatively healthy donors was observed within 6 days (TABLE 3). After 6 days of culture, the number of cells for the relatively healthy donors decreased by 65%. For the asthmatic patients with light severity, the cells continued to grow, but their power of proliferation decreased (38%) (TABLE 3).

It is advisable to pay a particular attention to the change in the number of lymphocytes in the case of serious asthma. If the number of cells increased by 15% within 3 days (TABLE 3), after 6 days of cell culture, the number of lymphocytes decreased by 65% compared to the number of cells before the culture of cells (TABLE 3).

The quantification of the apoptosis of lymphocytes was performed with cytometer in flux by using some parameters such as the determination of DNA fragmentation by means of iodide propidium (IP) (figure 1), the translocation of membrane phosphatidylserines at the surface of lymphocytes with the aid of merocyanine (MC540) (figure 1), the measurement of the variation of potential of mitochondrial membrane of lymphocytes according to the intensity of fluorescence CMX-Ros (figure 1).

Within 3 days of culture, the number of lymphocytes with the decrease of mitochondrial potential was 21%, 20%, and 27% (TABLE 4) in relatively healthy donors, asthmatic patients with slight severity and high severity, respectively. In this present study of cellular growth, there was no significant difference in changing the mitochondrial potential (PM) amongst the groups studied for 3 days (TABLE 4). For 6 days of culture, we observed an increase of the number of cells in relatively healthy donors with a decrease of mitochondrial potential (changes > of 70%) (MP) (TABLE 4). The cells of the patients with light severity having an active power of proliferation increased the number of cells with a decrease of the MP (20% à 42%) (TABLE 4). The cells of the patients with serious severity having lost the power to divide their cells did not show an apoptotic change (27% to 30%) (TABLE 4). However, the dynamic between the lymphocytes of the various groups studied differed. The incubation of lymphocytes for 6 days indicates that the number of cells with a decrease of MP for the asthmatics with light severity was high compared to those of the relatively healthy donors and serious severity (TABLE 4). A prolongation of the culture of cells in the medium for 6 days showed the development of apoptosis according to the degree of severity, at the same time the lymphocytes of the donors showed a progressive increase at apoptosis level under these conditions (TABLE 4).
TABLE 3: Variation of the number of lymphocytes (%) in standard conditions (control) and within the pathology (light and grave severity asthma) during 3 and 6 days of culture in vitro (spontaneous apoptosis). And corresponding structure.

<table>
<thead>
<tr>
<th>Beginning cell number-2.10^6 cells/ml</th>
<th>Degree of severity:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n=10</td>
<td>Light, n=10</td>
</tr>
<tr>
<td></td>
<td>Grave, n=10</td>
</tr>
<tr>
<td>2.9± 0.22</td>
<td>3.1± 0.11</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>&gt; 58</td>
</tr>
<tr>
<td>2.3± 0.1</td>
<td>&gt; 15</td>
</tr>
<tr>
<td>1.0± 0.12</td>
<td>1.9± 0.2</td>
</tr>
<tr>
<td>&lt; 65</td>
<td>&lt; 38</td>
</tr>
<tr>
<td>0.8± 0.14</td>
<td>&lt; 65</td>
</tr>
</tbody>
</table>

/>\- increasing and decreasing of cells’ number.

Mitochondrial Potential MP

Phosphatidylserine PS

Fragmentation of DNA

Control

Light

Grave

Figure 1: Cytofluorogramme showing the quantification of the apoptosis of lymphocytes with cytometer in flux by using some parameters such as the determination of DNA fragmentation (with iodide propidium (IP)), the translocation of membrane phosphatidylserines at the surface of lymphocytes with the aid of merocyanine (MC540), the measurement of the variation of potential of mitochondrial membrane of lymphocytes according to the intensity of florescence CMX-Ros.

In the beginning of the apoptotic stage with membrane asymmetry dysfunction the translocation of the phosphatidilserine (PC) on the surface of cell were observed. The incubation of the lymphocytes led to an increase in the expression rate of PC on the cells wall for 3 days of incubation (control=18%, light=21%, serious=26%) (TABLE 4). The prolongation of the cells culture within 6 days also showed the development of the apoptosis (TABLE 4) as in the case of mitochondrial potential (MP).
The use of the iodide propidium as marker revealed the presence of cells with a DNA fragmentation. The number of cells with a decrease of the membrane mitochondrial potential and the number of cells with a decrease of the expression of phosphatidylserine).

### TABLE 4: Variation of the membrane mitochondrial potential (MP) of the lymphocytes of control (C) and lightly (L) and severely (S) patients corresponding to the initial stages of apoptosis (A) and the variation of the translocation of phosphatidylserine (PS) of the lymphocytes of control (C) and lightly (L) and severely (S) patients (B). (% corresponds to the number of cells with a decrease of the membrane mitochondrial potential and the number of cells with a decrease of the expression of phosphatidylserine).

<table>
<thead>
<tr>
<th>A</th>
<th>Spontaneous apoptosis (Comparison between 3 and 6 days.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>Control, n=12</td>
<td>21%</td>
</tr>
<tr>
<td>Light, n=12</td>
<td>20%</td>
</tr>
<tr>
<td>Grave, n=12</td>
<td>27%</td>
</tr>
</tbody>
</table>

### TABLE 5: Cytofluorogramme showing the average percentage of degradation of DNA of lymphocytes during the final phase of the apoptosis in % at the control (C) and at the diseases of asthma of light severity (L) and serious (S) severity (% counts of cells with a fragmentation of DNA after colouring the cells with the iodide propidium (IP)).

<table>
<thead>
<tr>
<th></th>
<th>3 days</th>
<th>6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatively healthy donors (C), (n=12)</td>
<td>9%</td>
<td>18%</td>
</tr>
<tr>
<td>Asthmatics with light severity (L),(n=12)</td>
<td>8%</td>
<td>15%</td>
</tr>
<tr>
<td>Asthmatics with grave severity, (G) (n=12)</td>
<td>14%</td>
<td>21%</td>
</tr>
</tbody>
</table>

The method of electronic microscopy by transmission make it possible to identify the morphological differences in lymphocytes of healthy people (control) and asthmatic patients in vivo for the majority of the parameters. As shown in figure 2, the lymphocytes of relatively healthy donors had a normal shape. The cellular membrane was well drawn. The nucleus generally occupies a large portion of the cell: in the nucleus there are nucleolus, the peripheral chromatin, and clear karyoplasm (figure 2). The ultra structure of lymphocytes of the patients differs from those of healthy ones by voluminous nucleus, and a segregation of the nucleolus. Lymphocytes with intussusceptions at membrane surface and formation of blebbing were observed on the cellular

---

**Figure 2:** Ultrastructure of lymphocytes in vivo of controls, of light and grave severity asthma patients.
membrane. The nucleus takes a “lopaste” shape with segregation of the chromatin which concentrates in the form of “glibok” at the periphery of the nucleus (figure 2). A difference occurs with severe asthma where fragmentation of the nucleus and invaginations of the inner cell membrane was observed (figure 2).

DISCUSSION

The atopic bronchial asthma (ABA) refers to the number of the most wide-spread chronic and inflammatory diseases of the respiratory system. In spite of advanced studies in the field of immunology, the functional diagnosis of the bronchial asthma and the problem of the definition of the criteria to assess the degree of severity of the disease are of current interest. That is why one of the most important tasks of modern pneumology is the guarantee of the control over ABA and particularly the severe form of the disease. Thus, the research for factors leading to the development and the progress of the disease is necessary. Recent studies underline the role of the programmed cellular death and the self-immunity as potentially important factors in the pathogenesis of the chronic and obstructive diseases of the respiratory tracts especially asthma. According to some authors, the prolongation of the inflammation generally in the case of the asthma was related to an increase of life expectancy of lymphocytes and to their ability to escape the cellular death. Therefore, we studied the dynamism of the variation of the number of lymphocytes in vivo. The co-relational method of analysis showed a reverse relation of dependence between the number of lymphocytes and the degree of severity of the disease (TABLE 2). The decrease in number of lymphocytes could be related to the disturbance of the proliferating activity of the lymphocytes or their migration from the peripheral blood towards lungs under the influence of allergens. On the other hand, the prolongation of the influence of allergens may lead to the loss of apoptotic power of lymphocytes and resulting in the increase of their lifetime. Some morphological and biochemical parameters were studied in vitro to explain the decrease in the number of lymphocytes observed. Considering that change (morphological and biochemical) occurs faster in the cells than in the organism, it is therefore interesting to update the comparative analysis of the morphological structure of lymphocytes in healthy people and asthmatic patients according to the degree of severity. The method of electronic microscopy by transmission make it possible to identify the morphological differences in lymphocytes of healthy people (control) and asthmatic patients in vivo for the majority of the parameters reported that, Light and electron microscopy are two of the classical techniques for the study of this process. Because of the lack of cellular synchronisation in apoptosis and for the fact that the apoptotic cell is rapidly disposed through phagocytosis, study methods based on morphological criteria are adequate for the demonstration of the process, but are not useful for quantifying it. As shown in figure 1, the lymphocytes of relatively healthy donors had a normal and rounded shape. The ultra structure of the lymphocytes of the patients differs from those of healthy ones by voluminous nucleus, and a segregation of the nucleolus. Lymphocytes with intussusceptions at membrane surface and formation of blebbing were observed on the cellular membrane. The nucleus takes a “lopaste” shape with segregation of the chromatin which concentrates in the form of “glibok” at the periphery of the nucleus. A difference occurs with severe asthma where fragmentation of the nucleus and invaginations of the inner cell membrane was observed. Referring to the entire observation, the immediate question is that: how do cells with such a structure behave during the cellular growth in vitro? The study of the apoptotic process in vitro with the use of the cellular medium unravels today the mechanism of molecular dysfunction and the kinetics of this process during the development of asthma. In most part of the systems of culture, the lymphocytes of relatively healthy donors die with apoptosis within few days. On the other hand, we notice an opposite phenomenon with asthmatic patients. That could be the cause of the change observed at morphology level of cells in vivo; that is why the time of appearance lymphocytes in the asthma which reached an apoptotic state presents a practical and particular interest in the characterization of the degree of severity of the asthma. In vitro the number of lymphocytes varies according to the time of culture with both asthmatic patients and relatively healthy donors, and a difference in cells survival due to the degree of severity. The results of our studies highlight a reverse relation between the number of lymphocyte of the peripheral blood and the degree of se-
verity of the asthma in vivo; On the other hand, a slow development of cells in the medium for patients with serious asthma was observed, but the lymphocytes of the asthmatic patients with light severity show a proliferating activity (TABLE 3). Under which shape and in which stage of the development of the programmed death of lymphocytes can the dysfunction of the apoptotic process happen at asthmatic patients? To answer this question we studied the basis of the development of the apoptosis at the biochemical level, which takes place in the mitochondrion and in the nucleus. During the study of the apoptotic mechanism a close attention must be given to mitochondria because during the process of the cellular programmed death the mitochondrion gets rid of a good quantity of biologically active substance, capable of making the apoptosis reach the terminal and irreversible phase\[^{46}\]. Some authors reported that the properties of mitochondria show a correlation between the ultra structure and the state of functioning of these structures. A disappearance of mitochondrial filaments, and the mitochondrial crests change their configuration was observed\[^{47}\]. The cells of the patients with light severity having an active power of proliferation increased the number of cells with a decrease of MP. The cells of the patients with serious severity having lost the power to divide their cells did not show an apoptotic change (TABLE 4). A prolongation of the culture of cells in the medium for 6 days showed a resistance to the development of apoptosis according to the degree of severity, at the same time the lymphocytes of the donors showed a progressive increase at apoptosis level under these conditions (TABLE 4). In the beginning of the apoptotic stage with membrane asymmetry dysfunction the translocation of the phosphatidylserine (PC) on the surface of cell were observed\[^{48-52}\]. According to some authors, the atopic asthma is accompanied by an infiltration of the cells from blood track towards the ramifications of the bronchial track. With time, they have to be eliminated by the apoptotic process. A dysfunction of the elimination of the cells from targeted organs may be related to some deregulation of the expression on their surface, the phosphatidylserine molecule indicating the apoptosis which leads to a recognition followed by the digestion of the cells. There are hypotheses of which the cause of the prolonged inflammation in the lungs of asthmatic patients is due not to the dysfunction of the regulation of the apoptotic process of the lymphocytes but to the dysfunction of their recognition\[^{53}\]. A poor expression of the apoptotic markers materializing the early stage of the apoptosis of the cells allows us to assume that the deregulation of this process takes place in the final phase that is in the nucleus (TABLE 5). The use of the iodide propidium as marker revealed the presence of cells with a DNA fragmentation. The exterior morphological behaviour of DNA fragmentation was characterized by the intussusceptions of the nuclear membrane and the condensation of the chromatin at the periphery of the nucleus\[^{54}\]. The study of the apoptotic process of the lymphocytes in the asthmatic patients shows the resistance of these cells in the apoptosis. However, the incubation of the lymphocytes of the donors and the patients leads to a decrease in MP and the expression of PC in a certain number of cells, at the same time in the other cells left the parameters did not change. All the cells did not undergo the apoptotic stage after coloration of cells with the iodide propidium. In the similar way with the cells from the donors only a part of the lymphocytes from asthmatic patient in vitro was exposed to spontaneous apoptosis and were capable of disappearing in vivo. Patient with serious severity who found their cells exposed to the terminal phase of the apoptosis were between 14–21 % (TABLE 5).

One of the recent successes in the study of the programmed death of cells was the biochemical changes leading to necrosis. In connection with these data in the current classification of programmed death of the cells (PDC), we call an apoptosis PDC of type 1, a necrosis PDC of type 3 and autophagy PDC of type 2\[^{11}\]. Currently in the foreground there is a preventive difference between apoptosis and necrosis of living cells in the body\[^{55,56}\]. Apoptosis ends with a phagocytosis of dead cells without alteration, or inflammation and development of an immune response\[^{57}\]. The death of cells by necrosis is often associated with a development of the alteration of the nearby cells\[^{58,59}\]. The inflammation and a phagocytosis of the dying cells leading to the development of an immune response, if they contain chromatin with antigens. This difference is very important in pathology. The idea of programmed necrosis is formulated according to the existence of the way of signal announcer of necrosis because of the connection of the receiver of the molecule TNF during the slowing down of the apoptosis.
Edinger and Thompson\cite{60} reported that the mechanism of the apoptosis was designed so as to slow down the activity of an enzyme whose efficiency can lead to the development of necrosis. A negative link between the apoptosis and the Programmed necrosis was established during the degradation of the DNA. Under these conditions, no cancerous cells fit into the control centers at inter-phase level of the cellular cycle and by stopping the entry of cells in mitosis with a dysfunction at the genome level. In the case of a dysfunction of the mechanism of repair, cells die with apoptosis. But if one notices a deformation of DNA, a dysfunction of the apoptotic mechanism, then the cells die with programmed necrosis, and programmed necrosis in this kind of situation has 2 meanings. At first the programmed death of cells by necrosis in the absence of apoptosis diminishes the risk of transmission of mutations to the next generation of cells\cite{60}. On the other hand the destruction of cells by necrosis can lead to an activation of the immune response of the multi-cellular organisms which could explain a secretion of self-antibodies against DNA\cite{61}. They had already demonstrated the existence of a direct correlation of the rate of self-antibodies against the native DNA and the degree of severity of asthma.

**CONCLUSION**

These results could open the door to new perspective for in depth study of the role of apoptotic markers in the mechanism of asthma regulation, the characterisation of pathogenic factors of the disease and the target for its treatment according to the severity. However, further study in pulmonology will certainly benefit from these finding to better understand the process of apoptosis in ABA patients. It appears to be obvious that diseases such as cancer, autoimmune disorders may derive benefit from such studies.

**ACKNOWLEDGEMENT**

This work was supported by federation of Russia which granted a scholarship to one of the authors (Vodounon A.C).

The work was done jointly with medical doctor.

We thank Prof Silbykina B.N and Prof Mustafin I.G for their technical assistance.

**REFERENCES**

\begin{thebibliography}{99}

\bibitem{1} V.N.Manskikh; Path of cell death and their biological significance, Cytology, \textbf{11}, 909-15 (2007).
\bibitem{2} Belushkina II, S.E.Severin; Molecular basis of pathology of apoptosis, Archives of Pathology, \textbf{63}, 51-60 (2001).
\bibitem{3} E.Moskaleva, S.E.Severin; Possible mechanisms of adaptation of cells damage, inducing programmed death, the relationship with the pathology, Pathological Physiology and Experimental Therapy, \textbf{2}, 2-16 (2006).
\bibitem{7} R.I.Sepiashvili, M.G.Shubich; Apoptosis in immunological process, Alerg Immunol, \textbf{1}, 15-23 (2000).
\bibitem{8} E.F.Chernushenko; Actual problems of immunology, Ukraynsky Pulmonological Journal, \textbf{2}, 94-96 (2003).
\bibitem{10} Michel Aubier; Physiopathologie pulmonaire, de la recherche fondamentale aux avancées thérapeutiques, Médecine/Sciences, \textbf{15}, 775-7 (1999).
\bibitem{11} S.M.Gavalov, J.K.Mamoyan, N.T.Yasakova; Morphocytometer characteristics of the nuclei of lymphocytes and the severity of asthma (comparative study), Allergology, \textbf{4}, 60-65 (2001).
\bibitem{12} A.Trautmann, M.Akdis, K.Blaser; Role of dysregulated apoptosis in atopic dermatitis, Apoptosis, \textbf{5}, 425-429 (2000).
\bibitem{14} D.Urbonien, R.Skalauskas, B.Sitkauskiene, Autoimmunity in pathogenesis of chronic obstructive pulmonary disease, Medicina (Kaunas), \textbf{3}, 190-195 (2005).
\end{thebibliography}
[19] Antoneeva Il; Differentiation and activation-associated markers of peripheral blood lymphocytes in the patients with ovarian in the course of tumor progression, Medical Immunology, 6, 649-652 (2007).


