Morphological features of histogenic differon cells in connective tissue of guinea pigs' lungs after sensitization with ovalbumin

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

An urgent issue of modern morphology is establishing a number of patterns of morphological changes and reactivity of connective tissue components of lungs in case of experimental sensitization with allergens.

The aim is to estimate morphological features of histogenic differon cells in connective tissue of guinea pigs' lungs after sensitization with ovalbumin.

Materials and methods. Using morphometric and histological method, we have estimated the lung connective tissue of 48 male guinea pigs with experimental ovalbumin-induced allergic inflammation, simulated by subcutaneous sensitization and aeroallergization with ovalbumin. The number of fibrocytes, fibroblasts per 5000 μm^2 and their ratio – fibroblast/fibrocyte coefficient were determined.

Results. We have established the regularity of morphological changes dynamics in the cellular elements of pulmonary connective tissue. Experimental sensitization and inhalated allergization with ovalbumin leads to a statistically significant increase in the average number of fibroblasts and fibrocytes throughout the observation period in all experimental groups. It has been proved that the dynamics of cells has a multidirectional character, demonstrated by indicators of the fibroblast/fibrocyte coefficient, which shows the disproportion in the fibroblast/fibrocyte ratio and proves the tendency to the development of fibrosis in guinea pigs' pulmonary connective tissue in case of experimental sensitization with ovalbumin.

Conclusions. A gradual increase in the number of fibrocytes, against the background of a decrease in the number of fibroblasts is observed from the 23rd day to the completion of experimental sensitization with ovalbumin in the lungs of guinea pigs, compared with control group. A decrease of fibroblast/fibrocyte coefficient from 1.37 ± 0.03 in the early period to 0.82 ± 0.03 in the late period of the allergic inflammation demonstrates multidirectional nature of the dynamics in the number of connective tissue cells and indicates a tendency towards the development of fibrosis in pulmonary connective tissue.

Key words: guinea pigs, fibrocyte, fibroblasts, pulmonary connective tissue, experimental allergic inflammation, ovalbumin.

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Морфологічні особливості клітин гістогенного диферону сполучної тканини легень після сенсибілізації овальбуміном

С. С. Попко, В. М. Євтушенко

Актуальною проблемою сучасної морфології є питання щодо встановлення низки закономірностей морфологічних змін і реактивності компонентів сполучної тканини легень при експериментальній сенсибілізації алергенами.

Мета роботи – з'ясувати морфологічні особливості клітин гістогенного диферону сполучної тканини легень морської свинки після сенсибілізації овальбуміном.

Матеріали та методи. Використовуючи морфометричний і гістологічний методи, дослідили сполучну тканину легень 48 самців морської свинки з експериментальним алергічним запаленням, змодельованим сенсибілізацією та аероалергізацією овальбуміном. Визначали середню кількість фіброцитів, фібробластів на умовну одиницю площі 5000 мкм^2 та їхнє співвідношення – фібробластно-фіброцитарний коефіцієнт.

Результати. Встановили закономірність динаміки морфологічних змін клітинних елементів сполучної тканини легень. Експериментальна сенсибілізація та інгаляційна алергізація овальбуміном призводять до статистично значущого збільшення середньої кількості фібробластів і фіброцитів протягом усіх термінів спостереження в усіх експериментальних групах. Динаміка клітин має різноспрямований характер, що підтверджено показниками фібробластно-фіброцитарного коефіцієнта, які свідчать про диспропорційність у співвідношенні фібробласти/фіброцити, показують тенденцію до розвитку фіброзу та склеротичних процесів у сполучній тканині легень морських свинок під час експериментальної сенсибілізації овальбуміном.

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Key words: guinea pigs, fibrocyte, fibroblasts, pulmonary connective tissue, experimental allergic inflammation, ovalbumin.

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Висновки. Від 23 до 44 доби після початку експериментальної сенсибілізації овальбуміном у легенях морських свинок порівняно з контролем спостерігали поступове збільшення середньої кількості фіброцитів, але зменшення середньої кількості фібробластів. Різноспрямований характер у динаміці кількості клітин сполучної тканини показує зменшення показника фібробластно-фіброцитарного коефіцієнта від 1,37 ± 0,03 в ранньому періоді до 0,82 ± 0,03 в пізньому періоді розвитку алергічного запалення, що свідчить про тенденцію до розвитку фіброзу в сполучній тканині легень морської свинки.

Ключові слова: морська свинка, фіброцит, фібробласт, сполучна тканина легень, експериментальне алергічне запалення, овальбумін.

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Морфологические особенности клеток гистогенного дифферона соединительной ткани лёгких после сенсибилизации овальбумином

С. С. Попко, В. М. Евтушенко

Актуальная проблема современной морфологии – вопрос об установлении ряда закономерностей морфологических изменений и реактивности компонентов соединительной ткани лёгких при экспериментальной сенсибилизации аллергенами.

Цель работы – установить морфологические особенности клеток гистогенного дифферона соединительной ткани лёгких морских свинок после сенсибилизации овальбумином.

Материалы и методы. Используя морфометрический и гистологический методы, исследовали соединительную ткань лёгких 48 самцов морской свинки с экспериментальным аллергическим воспалением, смоделированным сенсибилизацией и аэроаллергизацией овальбумином. Определяли среднее количество фиброцитов, фибробластов на условную единицу площади 5000 мкм^2 и их соотношение – фибробластно-фиброцитарный коэффициент.

Результаты. Установлена закономерность динамики морфологических изменений клеточных элементов соединительной ткани лёгких. Экспериментальная сенсибилизация и ингаляционная аллергизация овальбумином приводят к статистически значимому увеличению среднего количества фибробластов и фиброцитов на протяжении всех сроков наблюдения во всех экспериментальных группах. Динамика клеток имеет разнонаправленный характер, который отражают показатели фибробластно-фиброцитарного коэффициента, свидетельствующие о диспропорции в соотношении фибробласты/фиброциты и являющиеся подтверждением тенденции к развитию фиброза и склеротических процессов в соединительной ткани лёгких морских свинок при экспериментальной сенсибилизации овальбумином.

Выводы. С 23 по 44 сутки после начала экспериментальной сенсибилизации овальбумином в лёгких морских свинок по сравнению с контролем отмечено постепенное увеличение среднего количества фиброцитов, но уменьшение среднего количества фибробластов. Уменьшение показателя фибробластно-фиброцитарного коэффициента с 1,37 ± 0,03 в раннем периоде до 0,82 ± 0,03 отражает разнонаправленный характер в динамике количества клеток соединительной ткани в позднем периоде развития аллергического воспаления, свидетельствует о тенденции к развитию фиброза в соединительной ткани лёгких морских свинок.

Ключевые слова: морская свинка, фиброцит, фибробласт, соединительная ткань лёгких, экспериментальное аллергическое воспаление, овальбумин.

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The variety of connective tissue functions, such as morphogenetic, reparative, biomechanical, metabolic reflects a wide range of its morphological adaptive changes in response to various factors, including allergenic ones [1,2]. Airway hyperreactivity develops as a result of allergic airway inflammation in case of experimental sensitization with ovalbumin [3]. Asthma pathogenesis is represented as a multiple process in which nonspecific and specific immune and neuroendocrine points are involved. Interactions between them lead to the development of asthma hallmarks – inflammatory process and as its result – airway remodeling [4–6]. A specific sign of connective tissue reaction in response to the action of an allergen as a result of the development of allergic inflammation is subepithelial fibrosis in airways [7]. The thickened basal lamina of the airway epithelium and the connective tissue of their respiratory mucosa contain an increased number of collagens types I, III and V, fibronectin and tenascin, which is a pathognomonic point of allergic inflammation and is not observed in case of other inflammatory diseases of the respiratory system [7,8]. The appearance and accumulation of deposits from glycosaminoglycans and collagen in the ground substance of extracellular matrix of connective tissue is associated with fibroblasts' or myofibroblasts' secretory function. The latter originate from resident cells of pulmonary connective tissue, such as fibroblasts and myocytes [8,9]. The mechanisms of differentiation of fibroblasts and myocytes into myofibroblasts, actively synthesized collagen types I, III, V in case of allergic inflammation, are currently being actively investigated by scientists [10,11]. However, morphological changes in the cellular component of the connective tissue of airways and lungs, experimentally sensitized, have not been sufficiently studied in the chronobiological aspect.

Aim

To estimate morphological features of histogenic differon cells in connective tissue of guinea pigs' lungs after sensitization with ovalbumin.

Materials and research methods

The experiment was performed on 48 male guinea pigs weighing 450–600 g, which were housed under standard environmental conditions in the animal facility of Zaporizhzhia State Medical University. All experimental procedures and the animal care were carried out according to ethical guidelines (Strasbourg, 1986; Kyiv, 2001).

Experimental model of allergic airway disease. Allergic airway inflammation was induced as previously described [12]. Guinea pigs received three subcutaneous injections and eight inhalations with ovalbumin (OVA) (Sigma Aldrich, USA). We use alum (10 mg/mL in saline) as an adjuvant (AlumVax Hydroxide vaccine adjuvant, OZ Biosciences France) with OVA injections to counteract tolerance.

Experimental design. We use six groups of guinea pigs in the experimental protocol (8 guinea pigs each). Animals of the first four groups with asthma model were euthanized with an overdose of thiopental (50 mg/kg) on the 23rd, 30th, 36th and 44th days of the experiment respectively; control guinea pigs of group 5 were administered with equivalent amount of saline; group 6 was intact. We subdivided the experiment to the early (23rd, 30th days of the experiment) and late (36th and 44th days of the experiment) periods of the pulmonary allergic inflammatory process. Paraffin sections of guinea pigs' lungs were stained by the method of Mason to estimate the number and distribution of collagen fibers, alcyan blue with a critical concentration of $MgCl_2$ 0.2 M to determine the dynamic of glycosaminoglycans distribution and morphometric evaluation of histogenic differon cells of connective tissue [13]. The sections were examined by light microscope (Primo Star, Zeiss, Germany). We counted the number of fibrocytes and fibroblasts per 5000 μm^2 in 10 fields of lung strip in each animal and their fibroblast/fibrocyte ratio coefficient. Fibroblasts and fibrocytes were identified and differentiated by their morphological points at the light-optical level. Fibroblasts are large polygonal cells up to 50 μm in size with rounded or oval euchromatic nucleus, have processes. Fibrocytes are spindle-shaped cells with the large, identical-shaped, dense nucleus, little volume of cytoplasm and long processes.

Statistical analysis was performed using Microsoft Excel and Statistica for Windows 13 (StatSoft Inc., № JPZ804I382130ARCN10-J). Data were evaluated by Shapiro–Wilk test and the Kolmogorov–Smirnov test. Values are expressed as means M ± m. Multiple comparisons were made using the parametric Student's t-test (P*) and the nonparametric U-Whitney–Mann test (P**). The obtained data were compared between the median and interquartile range Me (Q_1; Q_3). P value of <0.05 was considered significant.

Results

Morphological evaluation of guinea pigs' lungs after experimental sensitization with ovalbumin reveals reactive morphological changes in the connective tissue of airways and pulmonary interstitium. There is edema and disorganization of collagen fibers and changes in the tinctorial properties of the ground substance, such as more intensive metachromatic staining, compared to the control group. Along with this, we observe a reaction from the fibroblastic elements of the connective tissue of lung. Some of the fibroblasts increase in size, the cytoplasm acquires a more pronounced basophilia. Some cells divide mitotically. In addition, in case of ovalbumin sensitization, intense diffuse and focal perivascular and peribronchial infiltration by lymphocytes is observed *(Fig 1)*.

Morphometric assessment of diffusely located histogenic differon cells of connective tissue in lungs of intact group guinea pigs has shown that their average number was as follows: fibrocytes 6.50 ± 0.24, fibroblasts 7.50 ± 0.16, fibroblast/fibrocyte coefficient 1.46 ± 0.06. We have not found statistically significant difference in the number of cells between control and intact groups (P*/** > 0.05), indicated that the procedure has not influenced changes in data content from connective tissue cells. Therefore, we compare results of the experimental and control groups in further observation.

The number of fibrocytes and fibroblasts in the connective tissue of guinea pigs' lungs changes after sensitization and inhalation with ovalbumin. Analysis of the average number of fibrocytes in the early period of allergic inflammatory process in the lungs reveals that their number increases statistically significantly compared to the control group since the 23rd day after the experiment and is 9.5 ± 0.09 in the field of view, which is 1.7 times more than in the control group *(Fig. 2)*. We have declared a tendency to an increase in the number of fibrocytes on the 30th day of the experiment in the 2nd experimental group. There is also a statistically significant difference between the average number of fibrocytes in animals of the control group and after sensitization with ovalbumin, observed in the late period of the experiment (36th and 44th days) in animals of 3rd and 4th experimental groups. Thus, on the 36th day after the start of the experiment, the average number of fibrocytes is 12.88 ± 0.15 in the field of view, the coefficient of increase is 2, compared to the control group. On the 44th day of observation, the maximum value of the average number of fibrocytes reaches 13.38 ± 0.14 in the field of view. The maximum coefficient of increase (2.3) is observed in the 4th experimental group, compared to the control group *(Fig. 4)*.

Analyzing the number of connective tissue fibroblasts in guinea pigs' lungs in the early period of allergic inflammation, we have found that their number increases statistically significantly (P*/** < 0.05) from the 23rd day of the experiment (12.75 ± 0.21 in the field of view), compared to the control group *(Fig. 3)*. The maximum coefficient of increase in the average number of fibroblasts compared to the control group is 1.8 and is observed on the 23rd day of the experiment in the 1st experimental group. Fibroblast/fibrocyte coefficient is 1.37 ± 0.03 in the 1st experimental group, which is slightly less than the same indicator in the control group. We have reported the tendency to increase of the number of fibroblasts on the 30th day of the experiment in the 2nd experimental group. The fibroblast-fibrocyte

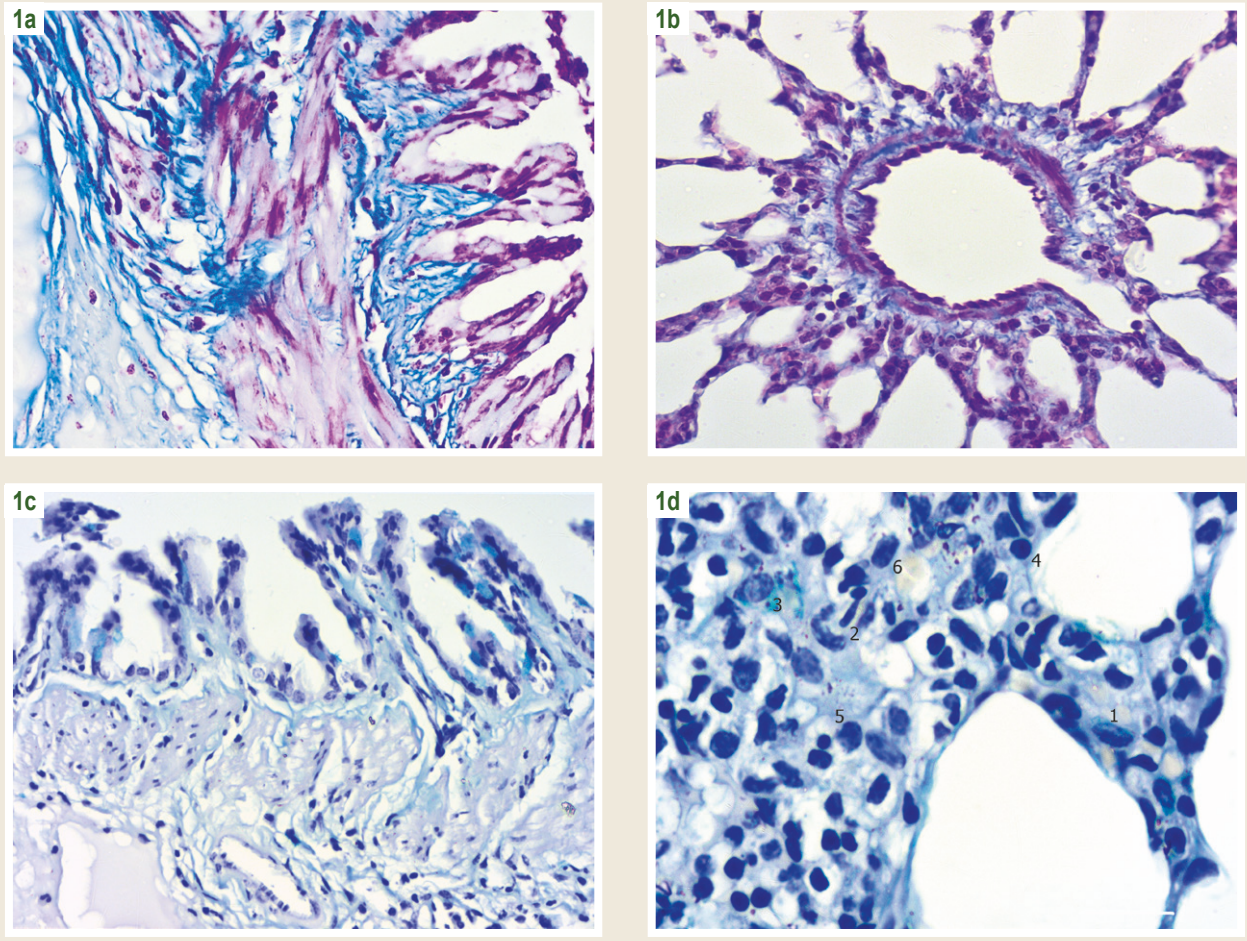

Fig. 1. Microscopic changes of connective tissue elements of the airways and pulmonary interstitium of guinea pigs after sensitization and challenging with ovalbumin on the 30th (1a, 1b), 36th (1c), 44th (1d) days after the start of experiment. **1a, 1b:** edema and disorganization of collagen fibers of connective tissue in the bronchial wall (**1a**), uneven thickening of the subepithelial layer in terminal bronchiole (**1b**). **1c:** more intensive metachromatic staining of the ground substance of connective tissue in the bronchial wall; **1d:** connective tissue cells in lung: **1** – fibroblast; **2** – fibrocyte; **3** – mast cell; **4** – lymphocyte; **5** – macrophage; **6** – plasma cell. Staining: **1a, 1b** – Mason staining; **1c, 1d** – alcian blue. **1a, 1b, 1c** × 400; **1d** ×1000.

coefficient in the 2nd experimental group is 1.30 ± 0.04 *(Fig. 4)*.

The statistically significant difference in the average number of fibroblasts compared to similar indicators in animals of the control group is also observed in the late period of allergic inflammatory process in lung (36th and 44th day after the start of the experiment) in animals of the 3rd and 4th experimental groups. On the 36th day after the start of the experiment, the average number of fibroblasts ss 10.38 ± 0.27 in the field of view, the magnification factor is 1.5, compared to the control group. The tendency to gradually significant decrease of the number of fibroblasts is shown with increasing experiment time. Fibroblast/fibrocyte coefficient decreases statistically significantly in 1.8 times in the late period of development of allergic inflammatory process in lung, compared to the similar indicator in the control group.

Discussion

Thus, our study determines the pattern of dynamics of morphological changes in the cellular elements of the respiratory connective tissue. Experimental sensitization and challenging with ovalbumin leads to a statistically significant increase in the average number of fibroblasts and fibrocytes during all observation periods in all experimental groups. The multidirectional nature of their dynamics attracts attention. The maximum number of fibroblasts occurs during the first period of pulmonary allergic inflammation on the 23rd day of observation, after which there is a tendency to gradually reduce their content to the 44th day of the experiment. In contrast to the dynamics of fibroblasts, the average content of fibrocytes gradually increases during the experiment, with the maximum coefficient of increase on the 44th day. This multidirectional nature of cell dynamics reflects the fibroblast/fibrocyte coefficient, during the experiment, indicated a disproportion in the fibroblast – fibrocyte ratio, especially in the late period of allergic inflammation. The latter fact is a confirmation of the tendency to the development of fibrosis and sclerotic processes in connective tissue of guinea pigs' lungs during experimental sensitization with ovalbumin.

Our morphological observation of guinea pigs' lung connective tissue cells in general confirmed with previous studies. Previously reported persistently elevated number of fibrocytes in bronchial mucosa is observed in chronically undertreated or corticosteroid-resistant asthma and are associated with persistent airway inflammation and connective tissue remodeling of the bronchial wall [10]. The asthmatic bronchial epithelium is predominant source of fibrocyte chemoattractants in asthma and contributes with T helper type 2 lymphocytes and eosinophils to promote the proliferation and remodeling function of recruited fibrocytes. The presence of elevated numbers of fibrocytes in the bronchial mucosa of allergic patients may also increase the risk of acute exacerbations because these cells can amplify T helper type 2 inflammation to the clinically relevant allergen and can promote further inflammation [4,14,15]. Several studies have revealed subepithelial fibrosis, primarily mediated by submucosal resident fibroblasts proliferated and differentiated into myofibroblasts [9,10]. However, authors have shown that oncostatin M-induced products of connective tissue cells, such as monocyte chemoattractant protein 1 (MCP-1), IL-6, and PGE2 can modulate macrophage function, including the expression of oncostatin M produced by macrophages and neutrophils, indicating feedback loops characterized macrophage and connective tissue cell interaction in extracellular matrix remodeling and allergic inflammation [16]. Oncostatin M receptor (OSMRβ chains) is expressed by pulmonary connective tissue fibroblasts and bronchial smooth muscle cells. Scientists have associated lung extracellular matrix hyperproduction with defective epithelial – connective tissue crosstalk during allergic inflammation, suggesting the aberrant activation of the asthmatic epithelial-mesenchymal trophic unit (EMTU) may lead to disease pathogenesis [14]. A similar trend has been recently showed in studies of other scientists [9,17].

In conclusion, our results may become a part of the foundation for the further investigation of morphological basis of mechanisms of epithelial neuroendocrine – immune interaction in the animal models of widespread diseases.

Conclusions

1. There is a gradual increase in the average number of fibrocytes (respectively 9.50 ± 0.09 in the 1st experimental group to 13.38 ± 0.14 in the 4th experimental group) and a decrease in the average number of fibroblasts (12.75 ± 0.21 in the 1st experimental group to 10.37 ± 0.27 in the 4th experimental group) from the 23rd to the 44th day after the start of experimental sensitization with ovalbumin in guinea pigs' lungs.

2. A decrease of the fibroblast/fibrocyte coefficient from 1.37 ± 0.03 in the early period to 0.82 ± 0.03 reflects a divergent nature in the dynamics of the number of connective tissue cells in the late period of allergic inflammation, indicated a tendency of development of fibrosis in the connective tissue of guinea pigs' lungs.

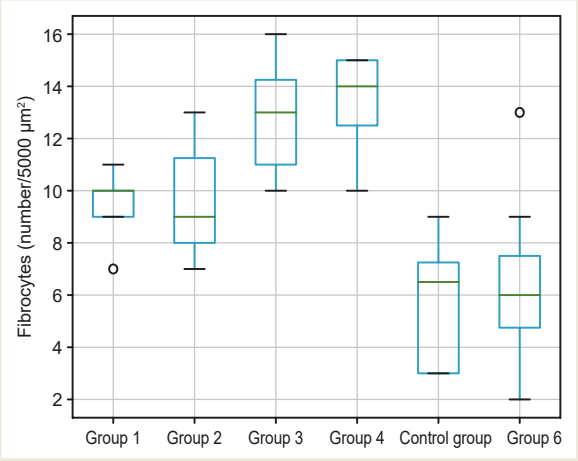

Fig. 2. Morphometric changes in the number of fibrocytes in the connective tissue of guinea pigs' lungs. *: P < 0.05 (Student's t-test); **: P < 0.05 (Whitney–Mann U-test) compared to the control, Me (Q_1; Q_3), M ± m (n = 8).

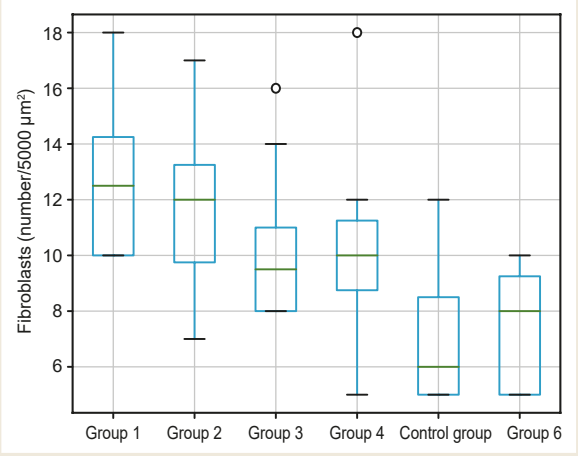

Fig. 3. Morphometric changes in the number of fibroblasts in the connective tissue of guinea pigs' lungs.
*: P < 0.05 (Student's t-test); **: P < 0.05 (Whitney–Mann U-test), compared to the control, Me (Q_1; Q_3), M ± m (n = 8).

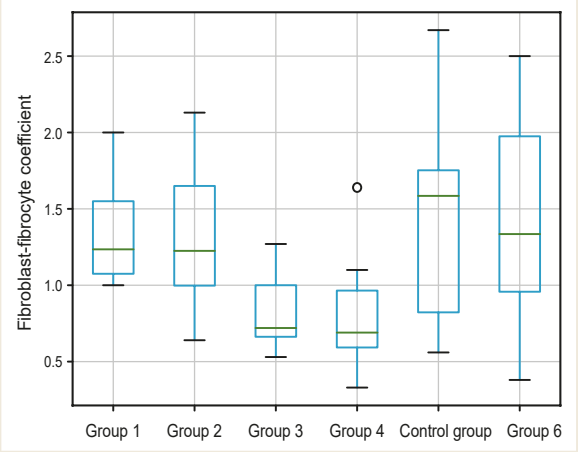

Fig. 4. Dynamics of fibroblast/fibrocyte coefficient.
*: P < 0.05 (Student's t-test); **: P < 0.05 (Whitney–Mann U-test) comparing to the control group, Me (Q_1; Q_3), M ± m (n = 8).

Prospects for further research. We are planning to study the components of ground substance of extracellular matrix and ultramicroscopic changes in the connective tissue cells of guinea pigs' lung in case of experimental sensitization with ovalbumin.

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