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ANALYSIS OF IMMUNOGYSTOCHEMICAL EXAMINATION OF RESPIRATORY ORGANS IN EXPERIMENTAL BRONCHOECTASIS

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Abstract:

Immunohistochemical methods for the study of lung tissue in bronchiectatic disease in experimental animal conditions are necessary for a deeper understanding of the cellular and molecular mechanisms of chronic respiratory diseases and the development of new approaches to their diagnosis and therapy.

Keywords: bronchi, lungs, chronic inflammation, mucous membrane, rabbit

INTRODUCTION. The study of lung tissue in bronchiectasis in animals is important for understanding the cellular and molecular factors of chronic respiratory diseases, as well as for developing new methods for their diagnosis and treatment. The purpose of the study is to study the structural and functional organization of the bronchial mucosa and to identify patterns of inflammation and remodeling of the bronchi in bronchiectasis. The study used 120 white male rabbits: 10 rabbits of the control group and 86 with a model of experimental bronchiectatic disease, which were withdrawn from the experiment 3,4,5,6,7, 8, 9, 10, 11 and 12 months after the reproduction of the pathology. Lung tissue was stained using various methods and immunohistochemical studies were performed with antibodies to various markers. The results showed that in a model of chronic lung inflammation, inflammatory and destructive processes are observed for 3-12 months, the thickness of all layers of the bronchial wall and the number of positive cells for markers CD-3, CD-20, CD-163, Bcl-2 and Ki increase -67. The highest values were reached at month 12, and CD-163 expression increased at month 9. So, the study revealed the progression of chronic inflammation and activation of T- and B-dependent components of the immune response, accompanied by remodeling of the bronchial wall and metaplasia of the bronchial epithelium.

According to literature data, the most common causes of bronchiectasis are pneumonia (19%), primary immunodeficiency (17%), recurrent aspiration, including inhalation of foreign bodies (10%) and primary ciliary dyskinesia (7%). However, in more than 30% of cases, the disease remains idiopathic [1,7].

Early diagnosis and management of patients with bronchiectasis is challenging. Timely diagnosis based on instrumental research methods, adequate conservative therapy with control of microbiological monitoring of infection will help reduce the chronicity of the process and disability of the patient, will allow long-term remissions of a chronic disease, and will also reduce the mortality rate of this category of patients [3,6].

The integrity of the airway epithelium is important for epithelial tissue function and the regulation of lung inflammation. The subsequent disruption of intercellular communication compromises the functional integrity of the airway. These changes underlie pathological conditions associated with lung and heart diseases [4,7].

The epithelium is a barrier between the environment and the body. It is selective and ensures the passage of only soluble molecules and ions into the paracellular space. This action is mainly regulated by intercellular junctions: tight junctions, adherens junctions and desmosomes [2,5].

AMs protect the alveolar space from the absorption and release of foreign substances. In this process, AMs play an important role in the regulatory mechanisms of innate and adaptive pulmonary immunity [6]. In fact, the mechanism of the immunological response to inhalation pollution is associated with intercellular interactions and cellular communication between lung epithelial cells and AM. In this context, the exchange of extracellular vesicles (exosomes, microvesicles and apoptotic bodies) containing proteins, nucleic acids and lipids plays an important role in immunological communication [7].

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So, the study of chronic inflammation of lung tissue in experimental conditions on animals is necessary for a deeper understanding of the cellular and molecular mechanisms of chronic respiratory diseases and the development of new approaches to their therapy. Moreover, extremely important importance is given to the morphological characteristics of changes in the tissue of the lungs and bronchi, developing in dynamics, as the experimental pathology progresses. The purpose of this study was to study the structural and functional organization of the bronchial mucosa and to identify patterns of inflammation and remodeling of the bronchi in a model of bronchiectasis.

MATERIAL AND RESEARCH METHODS. The study used 120 white male rabbits: 10 rabbits of the control group and 86 with a model of experimental bronchiectatic disease, which were withdrawn from the experiment 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months after the reproduction of the pathology. Lung tissue was stained using various methods and immunohistochemical studies were performed with antibodies to various markers. Immunohistochemical studies of lung micropreparations were performed using antibodies to Ki-67, Bcl-2, CD3, CD20, CD163, CK5/6 and CK8/18.

RESULTS. During the 3 - 6 months of the experiment, the level of expression of different markers in the transition from acute inflammation to chronic inflammation in the tissues of the lungs and bronchi of rabbits was different. The bronchial epithelium shows high activity for cytokeratins and immune markers, while the basal membrane shows moderate expression, especially for immune markers.

The proliferation factor is actively manifested in smooth muscle tissue of the bronchi, thus representing higher levels of interalveolar obstruction and vascular endothelium. Fibrosis areas are characterized by significant expression of inflammatory signs and cell proliferation, which indicates the development of fibrotic changes in the lungs.

The study noted a consistent increase in CD3 marker expression, which showed activation of T lymphocytes. In the 3rd month, the CD3 indicator was 14.8±1.3 cells/mm2, reaching 17.6±1.5 cells/mm2 in the 4th month, significantly different from the control group (*Figure 2,3*).

From the 5th month, the indicator rose from 21.2±1.4 cells/mm2 to 24.6±1.3 cells/mm2 in the 6th month, 33.7±1.6 cells/mm2 in the 7th month, and 34.6±1.2 cells/mm2 in the 8th month. In months 9-12, CD3 levels increased further, reaching a peak of 67.3±1.6 cells/mm2 in the 12th month, representing increased immunity.

In experimental bronchoectasis groups, the expression of the CD3 marker on inflammatory phases and respiratory tract was analyzed. In the exudation and proliferation stages, CD3 positive results were about 60.86% and 58.6% respectively (R = 0.99), meaning there is no statistical difference. CD3 expression was most commonly detected in small-caliber bronchi and lung tissue (80.6% and 80.0%), while in large and primary and medium-caliber bronchi it was 58.33% and 69.56%, respectively (*Figure 1*).

During the study, the expression of the CD20 marker grew consistently and showed T lymphocyte activation. In the 3rd month, CD20 levels were 13.7±1.6 cells/mm2, and in the 4th month, there was a statistically significant increase of 0.89 times higher than the control group, reaching 15.3±1.2 cells/mm2. In 5-9 months, the indicator increased from 18.4±1.3 to 33.7±1.4 cells/mm2. Reaching 38.6±1.5 cells/mm2 in the 10th month, 43.6±1.3 in the 11th month, and 44.6±1.8 cells/mm2 in the 12th month, further enhanced immunity (*Figure 2,3*).

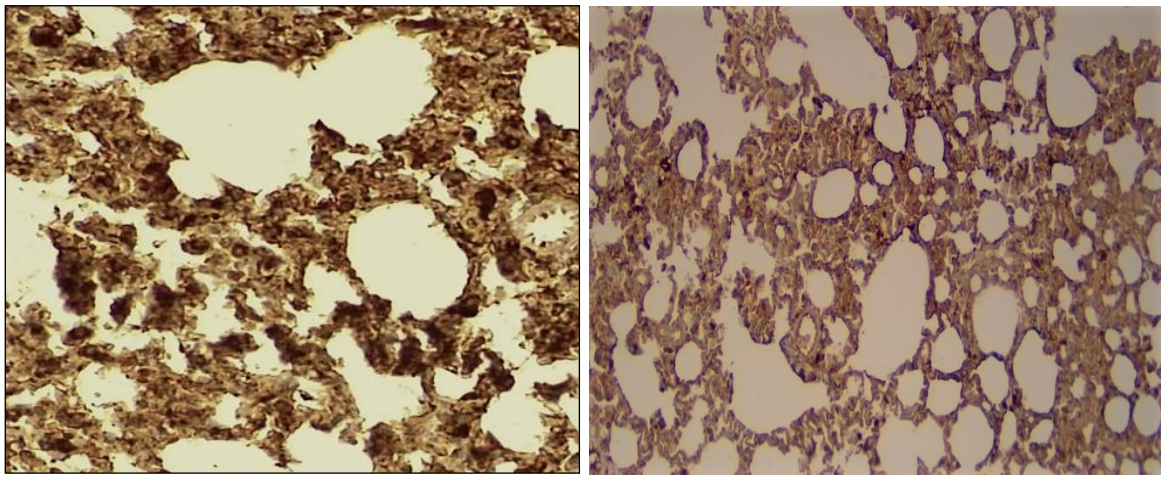

Figure 1. Lung of the main group rabbit, 6 and 9 months of study. Immunohistochemical reaction for CD3. Zoom X200.

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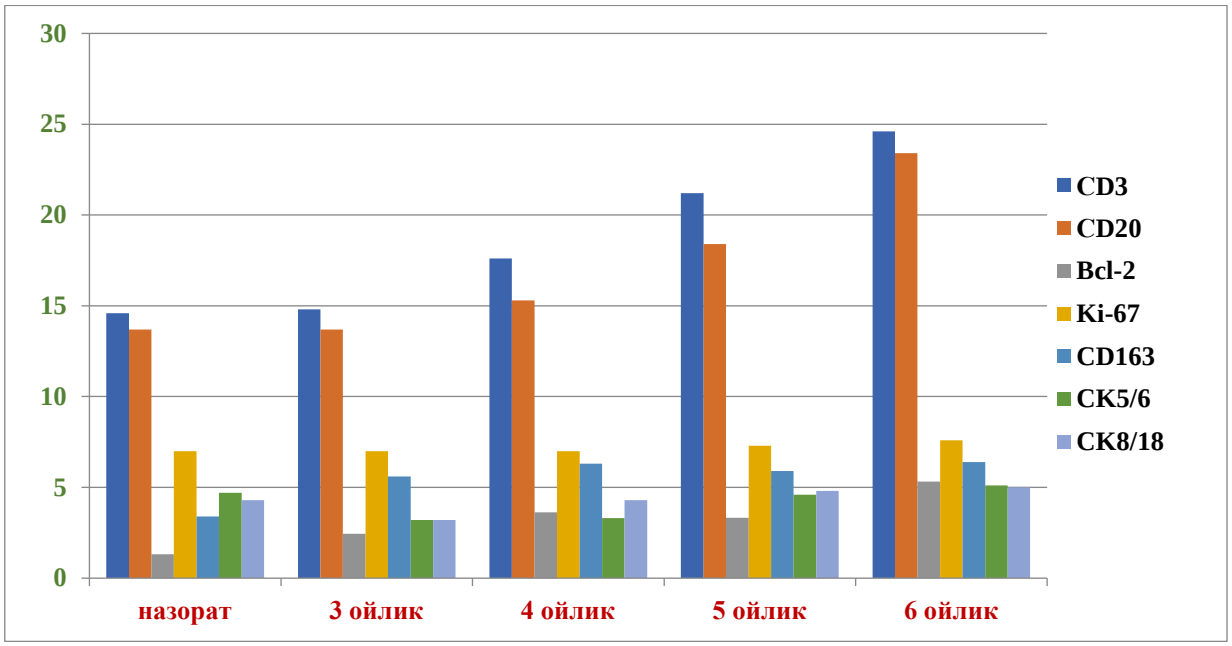

Figure 2. Laboratory animal respiratory organ structures immunogystochemical examination indicators (cell/mm2) lasting 3 to 6 months after experimental bronchoectatic disease modeling.

The study examined the expression of the CD20 marker in different stages of inflammation and in the respiratory tract of bronchoectasis rabbits. In the exudation phase, CD20 expression was 73.91% and in the proliferation phase 61.90%, the difference was not statistically significant (R=0.44). The highest expression of CD20 in the respiratory tract was detected in the middle bronchi (91.66%) and was noted to be higher (56.25% and 72.72%) in both the large bronchi and lung tissue, with slightly lower (74.28%) in the small bronchi. The differences between the different parts did not matter statistically (R=0.08). These results suggest that CD20 expression may change depending on inflammatory localization.

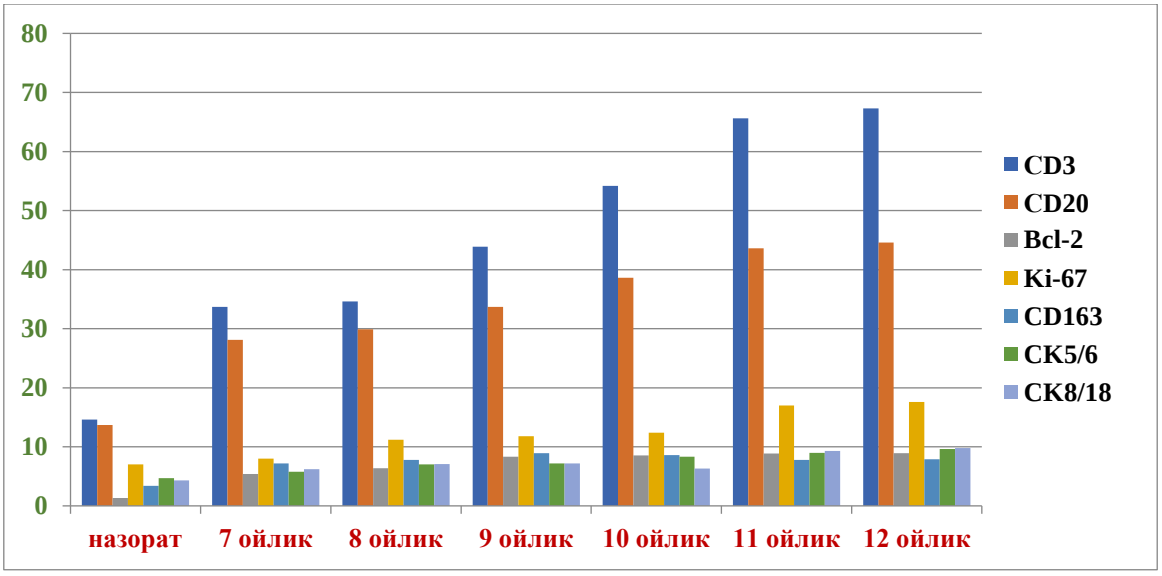

Figure 3. Laboratory animal respiratory organ structures immunogystochemical examination indicators (cell/mm2) 7-12 months after the modeling of experimental bronchoectatic disease.

In studies, the expression of the CD-163 macrophage marker gradually increased, peaking at 6 months of follow-up, a chronic lung inflammation that may indicate long-term inflammation and renewal of the bronchial walls in Model rabbits.

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The highest staining levels of CD-163 were mainly observed in the fibrosis areas of the bronchial wall and interalveolar SEPTA.

During the study, the expression of the marker CD163 grew consistently and showed macrophage activation. In the 3rd month, CD163 levels increased from 5.6±1.1 cells/mm2 to 6.3±1.3 cells/mm2 in the 4th month, recording a statistically significant increase. Between 5-8 months, the indicator reached 5.9±1.4 to 7.8±1.8 cells/mm2, indicating continued macrophage activation. In the 9-12 months, however, CD163 grew to 7.9±1.3 cells/mm2, further strengthening the phagocytic system's activity (*Figure 2,3*)..

The study analyzed the expression of the CD163 marker in the respiratory tract in rabbits with different stages of inflammation and bronchoectasis. Positive CD163 expression was 75.05% in exudation phase and 78.50% in proliferation phase; no statistically significant difference was observed between phases (R=0.54). Expression of CD163 in the respiratory tract was 75.0% in the large bronchi, 80.9% in the middle bronchi, 78.3% in the small bronchi and 80.7% in the lung tissue, no significant difference was detected (R=0.97) (*Figure 4*).

Studies in the chronic lung inflammation model showed a moderate increase in Ki-67 expression in rabbits over a 6-month period, indicating an increase in cell proliferation. It was noted that Ki-67 was highly stained in the interalveolar SEPTA, smooth muscle tissue, and bronchial walls, and moderate in the bronchial epithelium and pulmonary blood vessels. This growth expresses the fact that tissue remodeling and regeneration continue during the inflammatory process. By the 12th month, Ki-67 expression had become more intense and reached a higher level of proliferative activity, indicating the activity of recovery processes despite destructive changes in lung tissue.

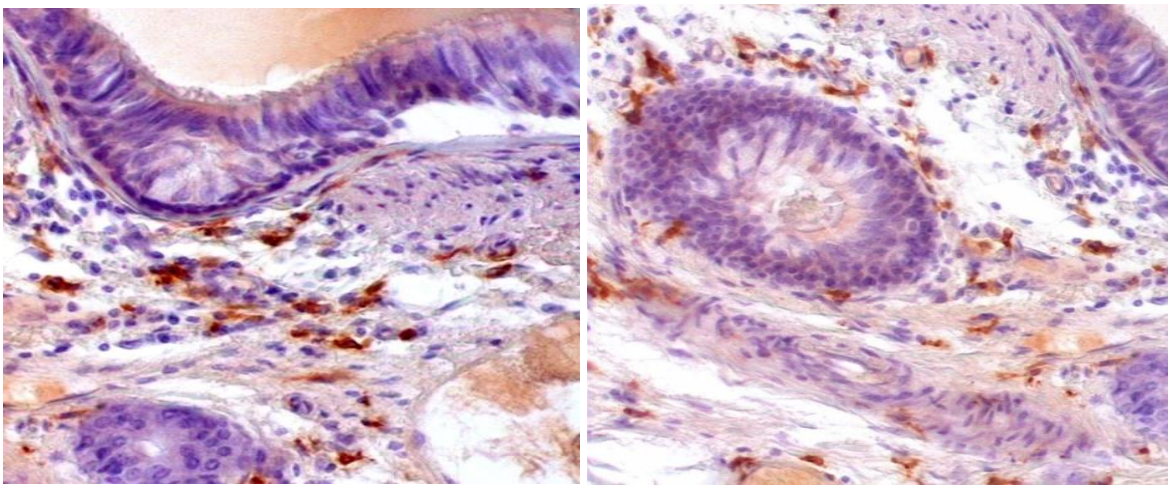

Figure 4. Small-caliber bronchi of the main group rabbit, 6 months of study. Immunogystochemical reaction for CD-163. Zoom X400.

The study showed consistent increase in Ki-67 marker expression and activation of proliferative activity in tissues. In Months 3 and 4, The Ki-67 indicator was 7.0±0.9 cells/mm2, while in months 5 and 6 it grew to 7.3±0.4 and 7.6±0.8 cells/mm2, respectively, but did not have statistical significance. From the 7th month, the indicator has grown significantly, reaching 11.2±0.6 cells/mm2 in the 8th month and a maximum of 17.0±1.3 cells/mm2 in the 11th month. In the 12th month, the highest recorded was 17.6±1.5 cells/mm2 (*Figure 2,3*).

The study analyzed Ki-67 marker expression in a model of bronchoectatic disease in rabbits in terms of inflammatory stages and anatomical location of the respiratory tract. Ki-67 expression was 75.05% in the exudation phase and 78.50% in the proliferation phase, with no statistical significance for phase differences (R=0.49). In the respiratory tract, Ki-67 was highest observed in the large bronchi (88.8%), and lowest in the small bronchi (63.0%). These results show pathological regeneration activity in tissues affected by bronchoectasis, despite the inflammatory stage and localization.

The expression level of the anti-apoptotic factor Bcl-2 increased during the study and reached its highest value during the 6-and 12-month follow-up. This condition may indicate progressive dysregulation of apoptotic processes in the bronchial epithelium of rabbits, which is observed in the model of chronic lung inflammation (Figure 5). In addition, moderate Bcl-2 staining has been observed in smooth muscle tissue of the bronchial epithelium, as well as pulmonary veins and interalveolar septa.

The study showed consistent increase in Bcl-2 marker expression and activation of proliferative activity in tissues. In Months 3 and 4, the Bcl-2 indicator was 2.44±0.14 and 3.63±0.06 cells/mm2, respectively, while in months 5 and 6 it increased to 3.32±0.24 and 5.32±0.08 cells/mm2, 0.24 times higher than the control group level, confirming the gradual

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increase in antiapopototic factor activity. From the 7th month, the indicator grew significantly to 5.38±0.04 cells/mm2, in the 8th month to 6.37±0.63 cells/mm2, in the 11-12 months to the maximum-8.89±0.09 and 8.93±0.3 cells/mm2 (*Figure 2,3*).

The study analyzed the expression of the Bcl-2 marker in the model of bronchoectatic disease in rabbits in terms of inflammatory stages and anatomical location of the respiratory tract. Bcl-2 expression was 68.4% in the exudation phase and 72.9% in the proliferation phase. At the same time, the differences between these phases did not turn out to be statistically significant, which is reflected by the R - value of 0.83.

In the respiratory tract, Bcl-2 was observed at the highest levels in medium-caliber bronchi (80.9%) and lung tissue (79.2%), and at the lowest in small bronchi (64.0%). These data highlight the stable expression of Bcl-2 at different stages of the inflammatory process and in different anatomical regions of the respiratory tract, which has shown activation of apoptosis and tissue repair mechanisms in bronchoectasis (*Figure 5*).

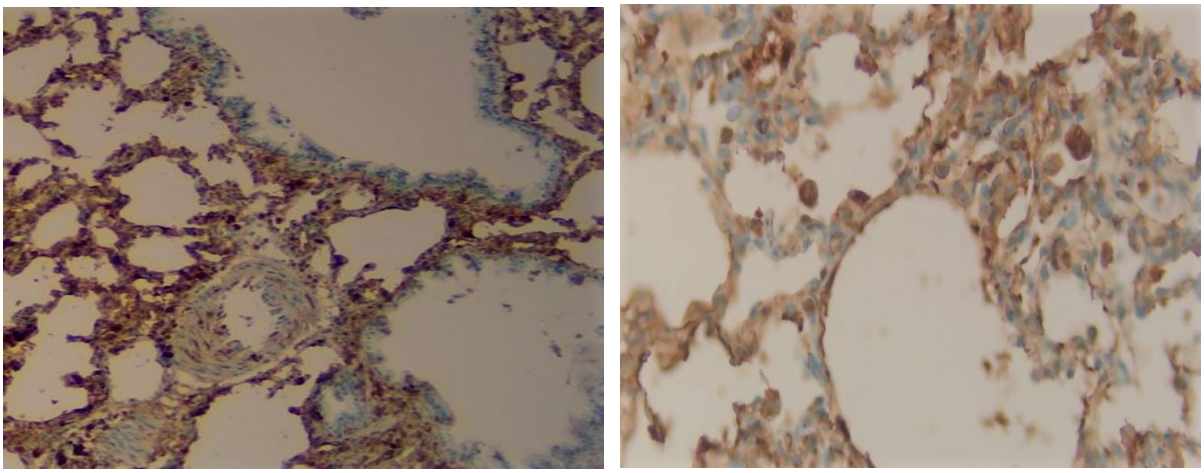

Figure 5. Lung of the main group rabbit, 6 and 9 months of study. Immunogystochemical reaction to Bcl -2. Zoom X400.

By the third month of observation, the expression of the cytokeratin CK5/6 and CK-8/18 had decreased, while by the sixth month their expression had increased.

In the immunogystochemical study of the bronchi and lungs of rabbits in the 7-12 months of modeling of bronchoectatic disease, the results of the soot were obtained.

These changes may reflect the dynamics of metaplasia processes occurring in the epithelium of the bronchial wall and the remodeling of the bronchial wall. From the 7th to the 12th month of observation, the expression level of cytokeratin CK5/6 and CK-8/18 has increased, which may reflect the state of the epithelium of the bronchial wall and its reconstruction process, as well as the development of metaplasia. Expression of cytokeratin CK5/6 and CK-8/18 has been found to be highest with moderate expression in the bronchial epithelium and in the area of the basal membrane, as well as in parts of the fibrosis of the bronchial wall.

The study showed that the expression of the marker CK5/6 increased consistently and activated the metaplastic process in tissues. In Months 3 and 4, the CK5/6 indicator was 3.2±0.4 and 3.3±0.5 cells/mm2, showing a more reliable decrease than the control group. In the 5th month, CK5/6 levels reached 4.6±0.8 cells / mm2, which remained statistically unchanged compared to the control group. By month 6, the indicator is 5.1±0.5 cells/mm2, 0.92 times higher than the control group level (*Figure 2,3*).

From the 7th month, the indicator grew significantly to 5.8±0.9 cells/mm2, in the 8th month to 7.0±0.4 cells/mm2, in the 11-12 months to the maximum-9.0±0.4 and 9.6±0.7 cells/mm2. This is 0.48 times higher than the control values and the highest for the entire observation periods

The study analyzed the expression of the CK5/6 marker in the model of bronchoectatic disease in rabbits in terms of inflammatory stages and anatomical location of the respiratory tract. CK5/6 expression was 35.3% in the exudation phase and 73.1% in the proliferation phase. While the highest expression of CK5/6 in the mucous membranes where the respiratory organs are covered by the epithelium was observed in small-caliber bronchi (88.2%), the lowest expression was reported in medium-caliber bronchi (66.6%). This highlights the active participation of CK5/6 in the process of cell proliferation in inflammatory processes in bronchoectasis (*Figure 6*).

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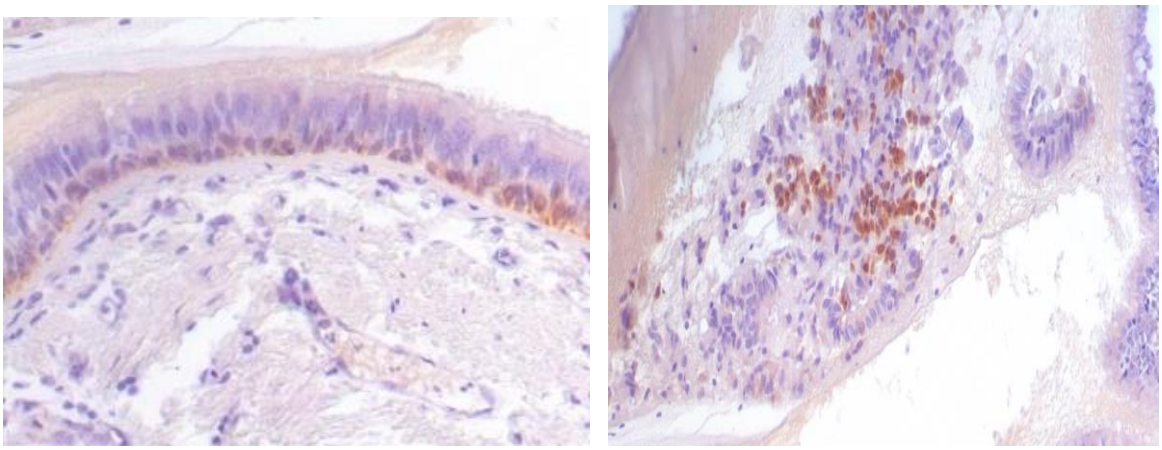

Figure 6. Small-caliber bronchi of the main group rabbit, 6 months of study. Immunogystochemical reaction for CK5/6. Zoom X400.

showed the activation of T - and B-lymphocyte-dependent components of the immune response. It was also noted that CD-163 macrophage marker, antiapoptotic factor Bcl-2, and cell proliferation marker Ki-67 peaked at a 12-month point during the observation period. Expression of tsitokeratin CK5/6 and CK-8/18 was observed to decrease in 3.4 months of observation and increase from 6 months. The highest expression of cytokeratin CK5/6 and CK-8/18 was expressed in the middle-caliber bronchial epithelium.

Through a large-scale histological and immunogystochemical description conducted on the bronchiectasis model, the activation of immune responses related to the reconstruction of the bronchial wall in the lung tissue of rabbits was observed.

During the study, destructive changes in the lungs of rabbits, emphysema, inflammation, the presence of purulent mucus exudate and sclerotic processes in foci of inflammation were observed. In rabbits of the experimental group, hyperplasia of basal cells in the bronchial mucosa and squamous metaplasia of the surface epithelium were found.

Immunogystochemical studies of lung tissue in the chronic lung inflammation model showed activation of T-and B-lymphocyte-dependent immune responses, increased CD-163 macrophage marker and Bcl-2 anti-apoptotic factor, and elevated Ki-67 proliferation mark, all of which peaked during the 12th month.

Thus, for 3-6 months of the experiment, a gradual increase in the expression of CD3 and CD20 is observed, which indicates the activity of T and B lymphocytes in chronic inflammatory conditions. Also, the anti-apoptotic marker Bcl-2 is significantly increased, which may be associated with mechanisms to protect cells from programmed death. An increase in CD163 indicates activation of macrophages, which is characteristic of the chronic course of inflammation. Changes in SK5/6 and SK8/18 indicate changes in the bronchial epithelium associated with inflammation.

In the study, the expression of the marker CK-8/18 also underwent significant changes. This marker reflected the epithelial differentiation activist during the experiment. The experiment showed a reliable decrease in 3oy by 3.2±0.6 cells/mm2 than this control group. In the 5th month, it reached 4.8±0.5 cells/mm2 and approached the control group, but remained statistically unchanged. From the 6th month (5.0±0.8 cells/mm2), the expression of the marker CK-8/18 began to grow steadily, reaching a maximum of 9.8±0.7 cells/mm2 by 12 months. This was 0.43 times higher than the control group values (*Figure 2,3*).

In our study, 36.8% of tissues in the exudation phase showed a positive reaction to CK-8/18 when a correlation of expression of marker CK-8/18 indicating epithelial differentiation expression in respiratory organ structure by inflammatory steps was performed. During the proliferation phase, this figure was 37.5%. At the same time, the differences between these phases did not turn out to be statistically significant, which is reflected by the R - value of 0.99.

The highest expression of CK-8/18 in airway mucosal epithelia was observed in middle-caliber bronchi (87.5%), followed by large-caliber bronchi (81.8%). Expression rates in small caliber bronchi and lung tissue are 76.2% and 75.0%, respectively. The difference in expression between these indicators is not statistically significant (R-value 0.76), which indicates a similar level of expression of this marker in different parts of the respiratory system.

These results emphasize the stability of the expression of CK-8/18 in various conditions of the inflammatory process and in the anatomical parts of the respiratory system, which is evidenced by the rapid development of dysplasia and metaplasia of the mucous membranes of the respiratory tract under the conditions of bronchoectasis.

Then, during the study, the results of immunogystochemical studies of lung tissue in rabbits after the creation of experimental bronchiectasis

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During the 7-12 month period of chronic inflammation, there is a significant increase in the expression of immunogystochemical signs such as CD3, CD20, Bcl-2 and Ki-67, which increases inflammation and proliferative activity in the wall of the bronchi. Increased levels of CK5/6 and SK8/18 indicate changes in epithelial differentiation, and increased CD163 indicates macrophage activity.

CONCLUSION, it can be said that in the model of chronic lung inflammation, a comprehensive histological and immunogystochemical analysis of the lung tissue of rabbits was carried out. During the observation of the pathology model from 7 to 12 months, there was an adaptive immune response of the bronchial wall and the development of chronic inflammation, as well as the development of bronchial epithelial metaplasia.

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