DOI 10.26724/2079-8334-2022-1-79-218-222
UDC 611.24.018:577.112.85]:616.2-018.73-002-021.5-092.9:599.324.7

S.S. Popko, V.M. Yevtushenko
Zaporizhzhia State Medical University, Zaporizhzhia

DYNAMICS OF GLYCOPROTEINS DISTRIBUTION IN LUNGS OF GUINEA PIGS WITH EXPERIMENTAL ALLERGIC INFLAMMATION

e-mail: kluchkosv@gmail.com

At present, the question of the reaction of the components of the local link of the innate immunity of the respiratory system under conditions of allergic inflammation in most aspects remains open. The study was carried out on male guinea pigs (animals with experimental ovalbumin-induced airways allergic inflammation). Morphological changes were investigated in structural components of the lung. We have found that an increase in the number of PAS-positive goblet cells is accompanied by hypersecretion of mucus and surfactant, functional hyperactivity of type II pneumocytes and alveolar macrophages in the early stages of the development of airways allergic inflammation ($p<0.05$). Adaptive immune response manifests in the late period of development of allergic inflammation by an increase in the number of lymphoid nodules. Identified changes reflect the manifestations of nonspecific and specific resistance of the lung, represented by protective and compensatory changes in its structural components. Our study suggests that the epithelium of the airways and the respiratory part of the lungs are important modulators of the inflammatory and immune responses of the respiratory system due to the effect of allergens.

Key words: animals, asthma, goblet cells, ovalbumin, PAS reaction surfactant.

С.С. Попко, В.М. Євтушенко

ДИНАМІКА РОЗПОДІЛУ ГЛІКОПРОТЕЇНІВ У ЛЕГЕНЯХ МОРСЬКИХ СВИНОК З ЕКСПЕРИМЕНТАЛЬНИМ АЛЕРГІЧНИМ ЗАПАЛЕННЯМ

В даний час питання про реакцію компонентів місцевої ланки вродженого імунітету дихальної системи на алергічне запалення в більшості аспектів залишається відкритим. Дослідження проводили на самцях морських свинок (тварин з експериментальним овальбумін-індукованим алергічним запаленням дихальних шляхів). Досліджено морфологічні зміни структурних компонентів легені. Нами встановлено, що збільшення кількості PAS позитивних келихоподібних клітин супроводжується гіперсекрецією слизу та сурфактанту, функціональною гіперактивністю пневмоцитів II типу та альвеолярних макрофагів на ранніх стадіях розвитку алергічного запалення дихальних шляхів ($p<0.05$). Адаптивна імунна реакція проявляється в пізньому періоді розвитку алергічного запалення збільшенням кількості лімфоїдних вузликів в умовах алергічного запалення. Виявлені зміни відображають прояви неспецифічної та специфічної резистентності легень, представленої захисно-компенсаторними змінами її структурних компонентів. Наше дослідження свідчить про те, що епітелій дихальних шляхів та компоненти респіраторного відділу легень є важливими модуляторами запальних та імунних реакцій дихальної системи у відповідь на алерген.

Ключові слова: тварини, астма, келихоподібні клітини, овальбумін, ШИК реакція, сурфактант.

The study is a fragment of the research project "Immunomorphological features of internal organs under the influence on the body of endo and exogenous factors", state registration No. 0118U004250.

The urgency of the problem of allergic respiratory diseases is growing steadily in Ukraine and around the world [3]. The intensity of the manifestation of reactions of the structural components of the lungs in the allergic inflammatory process primarily depends on the local immune and neuroendocrine systems of the respiratory system [10]. It is the local link of innate and adaptive immunity that ensures the resistance of the respiratory system to the action of various microenvironmental factors [7]. An important part of the innate nonspecific immunity in the lung is the secretion by goblet cells of mucus in the mucous membrane of large and middle-size bronchi and secretion of the surfactant. There are two types of cells in the lung secreted it – type II pneumocytes in alveoli and exocrine bronchiolar cells, which are abundant in the epithelium of terminal and respiratory bronchioles. Exocrine bronchiolar cells are cells with anti-inflammatory immunomodulating effect, synthesized surfactant components, named SP-A, SP-B, SP-D [11]. These hydrophobic proteins prevent bronchiole wall adhesion and airway collapse at this level [13]. The secretory product of bronchiolar exocrinocytes, the CC16 protein (uteroglobin), regulates the immune response in the lungs to various infectious agents and allergens [11, 12]. The surfactant contains neutral glycoproteins and glycolipids. It is phagocytosed and processed by alveolar macrophages. With the increase of the amount of surfactant, type II pneumocytes and alveolar macrophages acquire a histological pattern of "surfactant overload", which gives a positive PAS reaction upon histochemical staining [6, 9]. At present, the question of structural and functional changes and reactivity of the components of the local link of the innate immunity of the respiratory system with allergic inflammation remains open in most aspects.

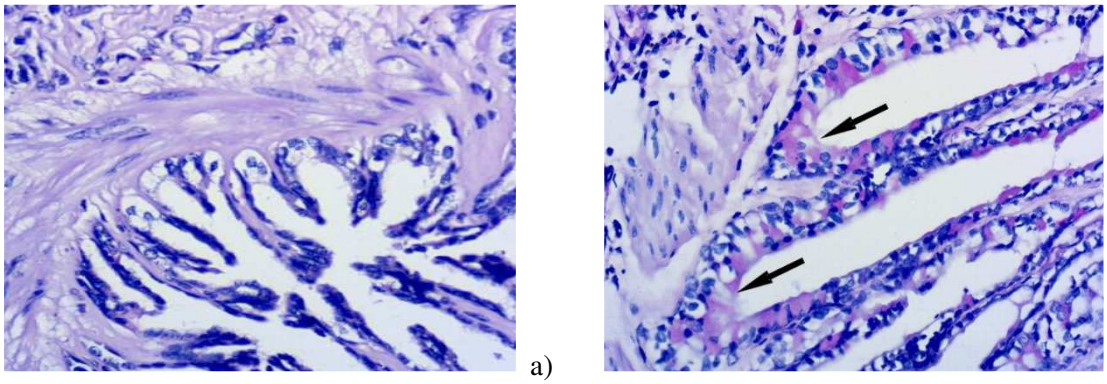
The purpose of the study was to establish the morphological changes in dynamics of neutral glycoproteins distribution as part of local innate immunity in the lung of guinea pigs with experimental allergic inflammation.

Materials and methods. The object of the experimental study was the lung removed from 48 sexually mature male guinea pigs, kept in standard conditions of the vivarium of Zaporizhzhia State Medical University. The experimental protocol followed the published guidelines (Strasbourg, 1986; Kyiv, 2006). Induction of allergic airway inflammation was carried out by subcutaneous sensitization and subsequent ovalbumin (OVA) inhalations. The animals were divided into 6 groups (8 animals in each group) for the study. The first four groups were animals, sensitized and aeroallergen OVA, withdrawn from the experiment, respectively, on the 23, 30, 36 and 44 days after its start; 5 – control group, the animals were injected subcutaneously with 1 ml of saline and inhaled with saline; 6 – intact group. For the purpose of rational presentation of the obtained data and their interpretation, we conditionally distinguish the early (23^{rd}, 30^{th} days of the experiment) and late (36th and 44th days after the start of the experiment) periods of the allergic inflammatory process development in the lung.

The animals were sacrificed from the experiment by an overdose of thiopental anaesthesia according to the established terms (23rd, 30th, 36th and 44th days of the experiment). Sections were stained using the PAS reaction with additional staining of nuclei with hematoxylin in order to assess the accumulation of glycogen and PAS-positive neutral glycoproteins. A complex of morphometric studies is carried out on a Carl Zeiss Primo Star microscope using the ZEISS ZEN 2011 software. The number of PAS-positive cells was counted in middle-size bronchi mucosa and alveoli on a unit area of 10000 μm^2 using a microscope with oil immersion technique (x1000).

Data Processing. The study results were processed by modern statistical methods of analysis with a personal computer using the standard software package Microsoft Office 2010 (Microsoft Excel) and "STATISTICA® for Windows 6.0" (StatSoft Inc., USA, license 46 No. AXXR712D833214FAN5) based on the Windows 10 operating system. The hypothesis of the normal distribution of the studied parameters was checked using the Shapiro-Wilk test and the Kolmogorov-Smirnov test of consistency. Values represent the mean (M) and standard deviation of the mean (±SD) from each animal. The statistical significance of intergroup differences according to the data obtained was established using the nonparametric Whitney-Mann U-test (p*). Differences between the compared values at the level of 95 % ($p<0.05$) were considered statistically significant.

Results of the study and their discussion. *Histochemical and histological analysis*. Histological examination of guinea pig lungs after sensitization and aeroallergization with ovalbumin revealed changes in the distribution and amount of PAS-positive material statistically confirmed. First, attention is drawn to the increase in the content of PAS-positive substances in the epithelial cover of the bronchial mucosa (fig. 1).



a) b)

Fig. 1. Intrapulmonary bronchi of guinea pig: 1a – intact group, 2b – 2^{nd} experimental group (30^{th} day after the start of the experiment). Arrows indicate PAS-positive goblet cells, increased in the number. PAS reaction. ×400.

These data of histochemical analysis indicate hypertrophy and hyperplasia of the goblet cells. The difference in the number of PAS-positive goblet cells in middle-size bronchi mucosa is not statistically verified between animals of intact and control groups. In animals of the control group, the number of PAS-positive goblet cells in middle-size bronchi mucosa reaches 3.12±0.07 at 10000 μm^2.

In experimental animals at the 23^{rd} day after the start of the experiment (early period of the development of an allergic inflammatory process in the lung) in the number of PAS-positive goblet cells in middle-size bronchi mucosa raises to 8.56±0.42 at 10000 μm^2 compared to control animals ($p<0.05$). Such changes are the result of the manifestation of nonspecific resistance mechanisms of the respiratory tract due to the action of the allergen.

In experimental animals, at the 30^{th} day after the start of the experiment (early period of the development of an allergic inflammatory process in the lung), there is a continuing tendency of a higher content of PAS-positive goblet cells in middle-size bronchi mucosa compared to control animals (9.12±0.14 at 10000 μm^2) ($p<0.05$). The number of PAS-positive goblet cells in middle bronchi mucosa in

the experimental animals on the 36th and 44th days after the start of the experiment (late period of the development of an allergic inflammatory process in the lung) was statistically significantly higher than in the control group ($p<0.05$). In all groups in experimental animals, the number of PAS-positive goblet cells had a tendency to increase compared to control animals (table 1).

Table 1

Dynamics of the content of PAS positive cells in lung of guinea pigs (M±m) (n=8)

Groups / Cells number	1st group	2nd group	3rd group	4th group	5th (control group)	6th (intact group)
PAS positive cells in alveoli	7.75±0.2*	5.5±0.11*	3.75±0.11*	3.0±0.11	2.25±0.05	2.25±0.06
Goblet cells in bronchi	8.56±0.42*	9.12±0.14*	8.42±0.34*	6.01±0.12*	3.12±0.07	3.17±0.05

Note: the statistical differences between groups' percentage points were determined using nonparametric Whitney-Mann U-test; the differences were considered reliable at $p<0.05$. *$p<0.05$, statistically significant with respect to corresponding values of the control group.

Histochemical analysis of the components of perivascular and peribronchial connective tissue in the lung of guinea pigs of the intact and control groups shows a low content of neutral glycoproteins, as evidenced by a weakly positive (++) PAS reaction. The extracellular component predominates over the cellular one (fig. 2a, 2b). The increasing of amorphic substance and thickening of fibrous structures evidenced by more intense (+++) staining during the PAS reaction, compared with the intact and control groups, was pronounced in the early period of the development of experimental ovalbumin-induced allergic inflammation in the peribronchial and perivascular connective tissue. There are also changes in the cellular component of the connective tissue as an increase in the number of lymphoid nodules (fig. 2c, 2d). Vasospasm of the resistant regions of microvessels also deserves attention (fig. 2c).

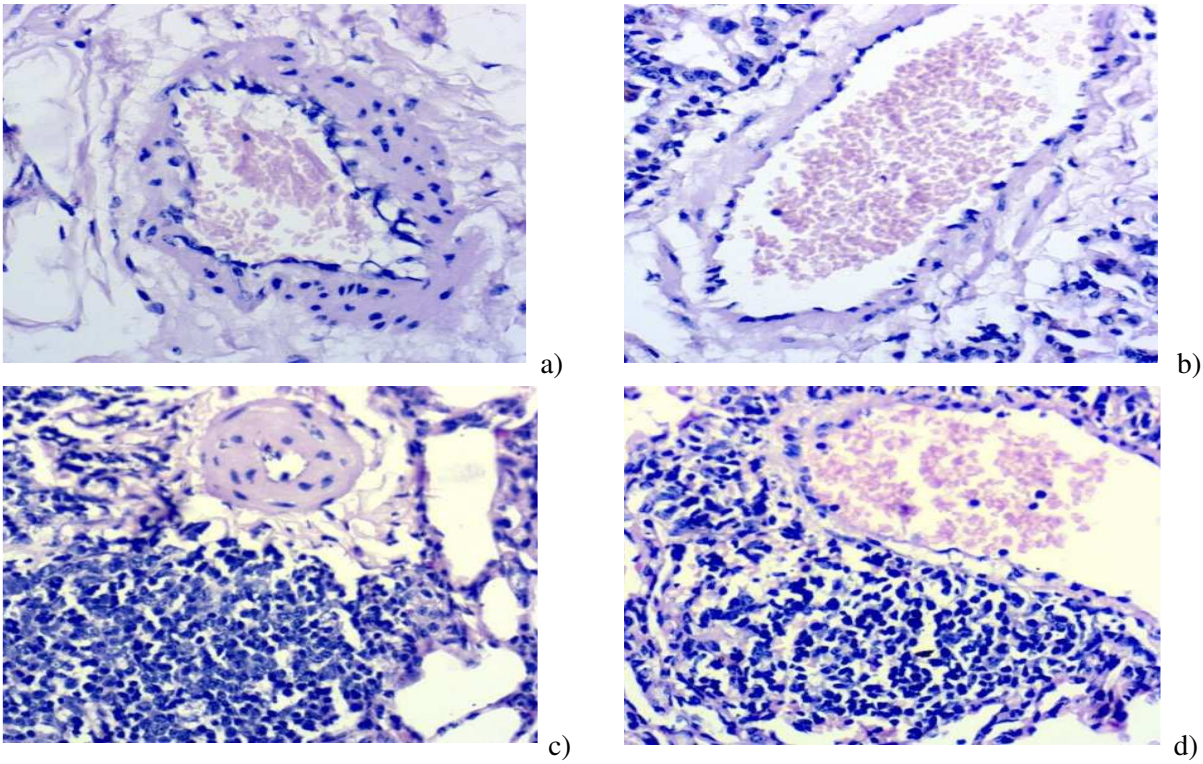


Fig. 2. Guinea pigs' lungs. Weakly positive PAS-reaction (++) of the perivascular connective tissue in the control group (2a – arteriole; 2b – venule). Perivascular lymphoid nodule around arteriole (2c), venule (2d) in 3rd experimental group (36th day of the experiment). PAS reaction. ×400.

It should also be noted changes in the distribution of neutral polysaccharides and glycoproteins in the perivascular and peribronchial connective tissue in the experiment. Most extracellular proteins are glycoproteins. As known, glycoproteins are an integral part of the structural components of the extracellular matrix, especially in connective tissue (collagen, elastin, mucins, mucous secretion).

The changes of the connective tissue components are observed due to the development of allergic inflammation. In the course of further histochemical analysis in animals of the experimental groups in the lung parenchyma, an increase in the content of PAS-positive cells was observed, which are localized in the wall of the alveoli and respiratory bronchioles, as well as in the interstitial tissue of the interalveolar septa (fig. 3).

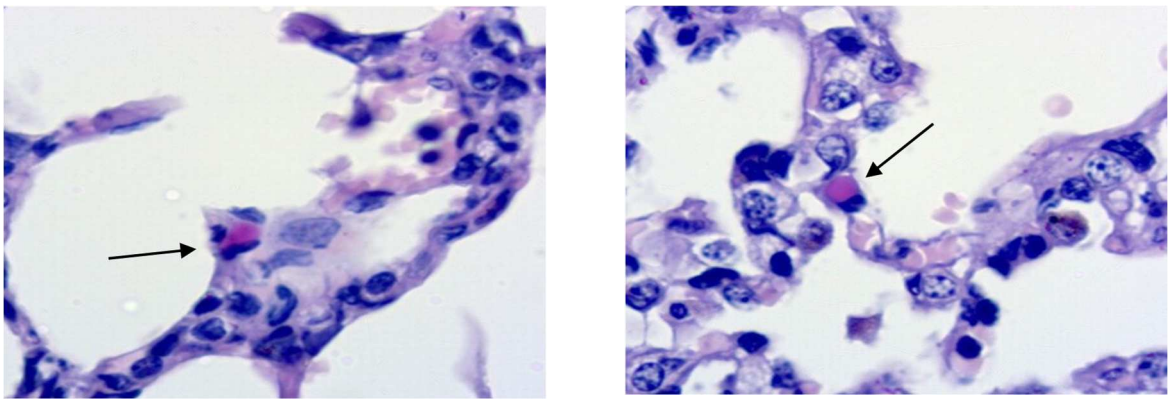


Fig. 3. PAS positive cells in the alveolar wall. A – 1st experimental group; B – 3d experimental group. PAS reaction. ×1000.

PAS-positive cells in the alveolar wall are lung alveolar cells type II and alveolar macrophages. There are neutral glycoproteins as a part of surfactants in their cytoplasm. PAS-positive cells in the interstitial tissue of the lung parenchyma are glycogen-containing cells which are young alveolar macrophages differentiated from blood monocytes. They accumulate glycogen as an energy substrate for intensive biosynthetic processes occurring with their differentiation.

The difference in the number of PAS-positive cells in the respiratory part of the lung is not statistically verified ($p>0.05$) between animals of intact and control groups. In animals of the control group in the number of PAS-positive cells in the respiratory part of the lung of guinea pigs reaches 2.25±0.05 at 10000 μm^2. In experimental animals at the 23rd day after the start of the experiment (early period of the development of an allergic inflammatory process in the lung) in the number of PAS-positive cells in alveoli and interalveolar septa increases statistically up to 7.75±0.2 at 10000 μm^2 compared to control animals ($p<0.05$).

In experimental animals, at the 30th day after the start of the experiment (early period of the development of an allergic inflammatory process in the lung), there is a continuing tendency of a higher content of PAS-positive cells in alveoli and interalveolar septa compared to control animals (5.5±0.11 at 10000 μm^2) ($p<0.05$). The number of PAS-positive cells in alveoli and interalveolar septa in the experimental animals at the 36rd day after the start of the experiment (late period of the development of an allergic inflammatory process in the lung) is statistically significantly higher than in the control group (3.75±0.11 at 10000 μm^2) ($p<0.05$). At the 36th days after the start of the experiment, the number of PAS-positive cells in the respiratory part of the lung is gradually decreased and approaches the indices of the intact and control groups by the 44th day of the experiment.

The difference in the number of PAS-positive cells in alveoli and interalveolar septa of the lung is not statistically verified ($p>0.05$) between animals of 4th experimental and control groups. In all groups in experimental animals, a number of PAS-positive cells in alveoli and interalveolar septa has a tendency to raise compared to control animals.

Statistically significant differences in the number of PAS-positive cells in the respiratory part of the lung of guinea pigs at the first three groups, from those of the intact and control group were obtained according to the results of the study. The number of PAS-positive cells is raised by 250 % in comparison with the control group ($p<0.05$) in the early period of development of experimental ovalbumin-induced allergic inflammation on the 23rd day after the start of the experiment.

The obtained dynamics of a number of goblet cells and PAS-positive cells in the airways and the respiratory part of the lung explains the most pronounced manifestations of nonspecific mechanisms of resistance of the respiratory system in the early stages of the development of allergic inflammation. It consists of hypertrophy and an increase in the number of goblet cells, an increase in the functional activity of type II pneumocytes, surfactant and activation of alveolar macrophages. This tendency of histochemical changes is confirmed by studies by other scientists [7, 14]. Innate immunity provides a non-specific defense mechanism against numerous pathogenic microbes, immediate recognition and response to pathogens. Relatively little is known about the role of innate immunity in the pathology of allergic airway inflammation until the recent discovery of innate lymphoid cells (ILCs), which produce large amounts of type 2 cytokines due to stimulating by airway epithelial cells cytokines (IL-25, IL-33, TSLP thymic stromal lymphopoietin) [4]. Firstly these cells were discovered by scientists in 2010. Studies in mice have shown that ILCs2 are major sources of IL-5 and IL-13 and that ILCs2 may play a role in asthma induction. The cytokine IL-5 activates eosinophils, increasing their number and their secretion of cytokines leukotriene C4 and activating platelet factor. The latter, in turn, increase mucin secretion by goblet cells and stimulate

hypertrophy and contraction of the smooth muscle component of the bronchi and blood vessels [5, 8]. IL-13 stimulates goblet cell hyperplasia and mucus secretion. Our results confirm these data. The neuroendocrine system of the lungs consists of pulmonary neuroendocrine cells (PNECs). PNECs act by secreting a calcitonin gene-related peptide (CGRP), activating ILCs2 and eliciting a subsequent immune response. In addition, PNECs act through the neurotransmitter γ-aminobutyric acid (GABA – γ-Aminobutyric Acid), causing goblet cell hyperplasia [12]. It turned out that ILCs2 are direct target cells for the implementation of signals from PNECs. This fact is confirmed by the results of studies that show that ILCs2 express the CGRP coreceptors Calcrl and Ramp1 and the GABA Gabrr1 receptor [1]. In the late period of development of experimental ovalbumin-induced allergic inflammation (36th and 44th days of the experiment) manifestations of specific resistance of the respiratory system in the form of activation of local links of cellular and humoral adaptive immunity prevail. This fact is confirmed by an increase in the number of perivascular and peribronchial lymphoid nodules in the lung of guinea pigs. Scientists have proven the undeniable participation of PNECs in initiating a Th2 immune response in OVA allergization through the results of a study in which the introduction of a mixture of CGRP and GABA into the airways of Ascl1 mutant mice lacking PNEC, recreates the immune response in experimentally induced allergic inflammation [2]. The results obtained by our study are evidence of the functioning of neuroimmunological modules PNECs-ILCs2 from the histophysiological point of view [5].

Conclusions

1. The number of PAS-positive cells is raised by 250% in comparison with the control group ($p<0.05$) in the early period of development of experimental ovalbumin-induced allergic inflammation on the 23rd day after the start of the experiment.

2. The histological and histochemical analysis of guinea pigs' lungs on the 23rd, 30th, 36th and 44th days of experimental ovalbumin-induced allergic inflammation made it possible to establish the morphological manifestations of nonspecific resistance of the lungs, which are represented by protective and compensatory changes in their structural components.

3. The epithelium of the airways and the respiratory part of the lungs, the secretory products of the epithelial cells of the lungs and components of the connective tissue are important modulators of the inflammatory and immune responses of the lungs due to the effect of allergens.

References

1. Barrios J, Patel KR, Aven L, Achey R, Minns MS, Lee Y, et al. Early life allergen-induced mucus overproduction requires augmented neural stimulation of pulmonary neuroendocrine cell secretion. The FASEB Journal. 2017; 31(9):4117–28. https://doi.org/10.1096/fj.201700115r

2. Halim TYF, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 Innate Lymphoid Cells Are Critical for the Initiation of Adaptive T Helper 2 Cell-Mediated Allergic Lung Inflammation. Immunity. 2014; 40(3):425–35. https://doi.org/10.1016/j.immuni.2014.01.011

3. Hrebniak MP, Fedorchenko RA. Influence of industrial atmospheric pollution on the development of pathology of respiratory organs. Pathologia. 2019; 16(1):81–86. https://doi.org/10.14739/2310-1237.2019.1.166314.

4. Klose CSN, Artis D. Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. Nature Immunology. 2016; 17(7):765–74. https://doi.org/10.1038/ni.3489

5. McKenzie ANJ. Type-2 Innate Lymphoid Cells in Asthma and Allergy. Annals of the American Thoracic Society. 2014 Dec; 11 (Supplement 5):263–70. https://doi.org/10.1513/annalsats.201403-097aw

6. Meyerholz DK, Beck AP, Goeken JA, Leidinger MR, Ofori-Amanfo GK, Brown HC, et al. Glycogen depletion can increase the specificity of mucin detection in airway tissues. BMC Research Notes. 2018; 11(1). https://doi.org/10.1186/s13104-018-3855-y

7. Nebesna ZM, Yeroshenko GA. Histological and histochemical changes of the lungs after experimental thermal trauma. World of Medicine and Biology. 2015; 2(49):106–10.]. Available from: http://repository.pdmu.edu.ua/handle/123456789/3033

8. Nolin JD, Lai Y, Ogden HL, Manicone AM, Murphy RC, An D, et al. Secreted PLA2 group X orchestrates innate and adaptive immune responses to inhaled allergen. JCI Insight. 2017; 2(21). https://doi.org/10.1172/jci.insight.94929

9. Oliveira TL, Candeia-Medeiros N, Cavalcante-Araújo PM, Melo IS, Fávaro-Pípi E, Fátima LA, et al. SGLT1 activity in lung alveolar cells of diabetic rats modulates airway surface liquid glucose concentration and bacterial proliferation. Scientific Reports. 2016; 6(1). https://doi.org/10.1038/srep21752.

10. Popko SS, Evtushenko VM, Syrtsov VK. Influence of pulmonary neuroendocrine cells on lung homeostasis. Zaporozhye Medical Journal. 2020; 22(4):568–75. https://doi.org/10.14739/2310-1210.4.208411

11. Rokicki W, Rokicki M, Wojtacha J, Dżeljijli A. The role and importance of club cells (Clara cells) in the pathogenesis of some respiratory diseases. Polish Journal of Cardio-Thoracic Surgery. 2016; 1:26–30. https://doi.org/10.5114/kitp.2016.58961

12. Sui P, Wiesner DL, Xu J, Zhang Y, Lee J, Van Dyken S, et al. Pulmonary neuroendocrine cells amplify allergic asthma responses. Science. 2018 Mar 29; 360(6393):eaan8546. https://doi.org/10.1126/science.aan8546

13. Winkler C, Hohlfeld J. Surfactant and allergic airway inflammation. Swiss Medical Weekly. 2013; 143(w13818). https://doi.org/10.4414/smw.2013.13818

14. Yeroshenko GA, Koptev MM, Bilash SM, Shevchenko KV, Yachmin AI. Restructuring of rat lungs in acute immobilization stress and its correction. World of Medicine and Biology. 2019; 15(69):187–190. Available from: http://repository.pdmu.edu.ua/handle/123456789/11445 https://doi.org/10.26724/2079-8334-2019-3-69-187-190

Стаття надійшла 21.01.2021 р.