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DYNAMICS OF QUANTITATIVE CHANGES OF DIFFUSE LYMPHOID TISSUE CELLS IN BRONCHI AND LUNGS OF GUINEA PIGS SENSITIZED WITH OVALBUMIN

Popko S.S.  ✉, Yevtushenko V.M. Dynamics of quantitative changes of diffuse lymphoid tissue cells of bronchi and lungs of guinea pigs sensitized with ovalbumin.

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ABSTRACT. Background. There is a progressive increase of respiratory allergic diseases nowadays in the world, made it necessary to study the participation of the components of the immune system in these processes. From the point of view of modern aspects about the organs of the immune system, interesting are the patterns of morphogenesis and function of bronchus associated lymphoid tissue, occupied a special place in the immunological protection of the body due to the large area of contact with various antigens. Morphogenesis and reactive changes in the local immunity in respiratory system in conditions of allergic inflammation remain urgent issue of modern morphology. **Objective.** To study the changes in diffuse lymphoid tissue of bronchi and lungs of guinea pigs sensitized with ovalbumin. **Methods.** We have studied the lung of 48 guinea pigs, using histological, immunohistochemical, morphometric, statistical methods, under conditions of experimental ovalbumin-induced allergic inflammation, assessed the average number of lymphocytes, macrophages and plasma cells in the diffuse lymphoid tissue. **Results.** The average number of lymphocytes in diffuse lymphoid tissue of bronchi and lungs increased from the 23rd day of observation and remained at a high level until the end of the experiment, the maximum was during the early period of the development of allergic inflammation, the increasing coefficient was 4.7. The average number of plasma cells also acquired maximum elevation in the early period of allergic process, the increasing coefficient was 2.0. The most significant average number of macrophages was on the 23rd day of observation with same increasing coefficient. Among all types of immunocompetent cells of diffuse lymphoid tissue in bronchi and lungs, T-lymphocytes prevailed during the experiment elevated almost by 5 times. **Conclusions.** In the early period of development of experimental ovalbumin-induced allergic inflammation, the specific resistance of the respiratory system manifests itself in the form of activation of local links of cellular and humoral adaptive immunity, as evidenced by the dynamics of changes in the average number of lymphocytes (the maximum increasing coefficient 4.7 in the 1st experimental group), macrophages and plasma cells (maximum increasing coefficient 2.0 in the 1st experimental group) of diffuse lymphoid tissue of bronchi and lungs of guinea pigs.

Key words: diffuse lymphoid tissue, lung, ovalbumin, allergic inflammation, guinea pig.

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Background

There is a progressive increase in inflammatory and allergic diseases of the respiratory system nowadays in the world, made it necessary to study the participation of immune system components in these processes [1]. The high frequency of allergic reactions of the respiratory tract in adults and children is associated with insufficiency of the barrier function of the respiratory tract and lungs, caused by morphological and functional immaturity [2, 3]. From the point of view of modern ideas about immune system

organs, interesting are patterns of morphogenesis and function of bronchus associated lymphoid tissue. It is not abundant among all organs of peripheral immune system, but occupies a special place in the immunological protection of the body due to the large area of contact with various antigens [4, 5]. In studies devoted to the investigation of the local immunity of the respiratory system in condition of allergic inflammatory process, scientists mainly pay attention to the issues of its immunological manifestations [6]. At the same time, morphogenesis and

reactive changes in the structures involved in the formation of local immunity of the respiratory organs in condition of allergic inflammation are presented in a generalized manner and insufficiently fully substantiated by the data of specific studies.

Therefore, the **objective** of our work is to determine the changes that occur in the diffuse lymphoid tissue of the bronchi and lungs of guinea pigs sensitized with ovalbumin.

Materials and methods

The object of the experimental study was lung, removed from 48 sexually mature male guinea pigs weighing 450 - 600 g, which were kept in standard conditions of the vivarium of the Zaporizhzhya State Medical University. All manipulations were carried out in compliance with the basic principles of working with experimental animals in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles for Animal Experiments adopted by the First National Congress on Bioethics (Kiev, 2001), the Law of Ukraine "On the protection of animals from cruelty" (from 21.02.2006).

Allergic airway inflammation induced by subcutaneous sensitization and followed challenging by intranasal inhalation with ovalbumin (OVA) (Sigma Aldrich, USA) [7]. Guinea pigs were sensitized by subcutaneous injections into the interscapular region of ovalbumin (0,5 mg/mL) with alum (10 mg/mL in saline) as an adjuvant (AlumVax Hydroxide vaccine adjuvant, OZ Biosciences France) on days 0, 7 and 14. From 21 to 28 days of the experiment, guinea pigs were challenged for 15 min with inhalation of either OVA (10 mg/mL in saline) via a nebulizer (Little Doctor International, Singapore, LD-211C) coupled to a plastic box. The animals divided into 6 groups (8 animals in each group). The first four groups are animals sensitized and challenged OVA, withdrawn from the experiment, respectively, on the 23rd, 30th, 36th and 44th days after its start; 5 - control group, received injections and challenged with saline only; 6 - intact group. For the purpose of rational demonstration of the obtained data and their interpretation, we conditionally distinguish the early (23rd, 30th days of the experiment) and late (36th and 44th days after the start of the experiment) periods of the development of allergic inflammatory process in lung. The animals were withdrawn from the experiment by an overdose of thiopental anesthesia (50 mg/kg) according to the established terms (23rd, 30th, 36th and 44th days of the experiment).

Histological sections stained with hematoxylin-eosin. To set up immunohistochemical reactions, we used monoclonal antibodies Mo a-Hu CD68 Antigen, Clone PG-M1 (DAKO, Denmark) - to determine macrophages, Mo a-Hu CD3 Antigen, Clone SP7 (DAKO, Denmark) - to determine T - lymphocytes. The sections were viewed and photographed

by the binocular light microscope (Primo Star, Zeiss, Germany). We evaluate the average number of lymphocytes, macrophages and plasma cells of diffuse lymphoid tissue of bronchi and lung per 5000 μm^2 .

The research results were processed by modern statistical methods of analysis on 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). The matplotlib library (BSD License) for moving Python programs used for the visualization of the represented data. We use the Shapiro-Wilk test and the Kolmogorov-Smirnov test of consistency testing the hypothesis about the normal distribution of the studied parameters. We use the Kolmogorov - Smirnov homogeneity criterion testing the hypothesis that two independent samples belong to the same distribution law. Values represent the mean (M) and standard deviation of the mean (\pm SD). The statistical significance of intergroup differences according to the obtained data established using the parametric Student's t-test (p^*) and the nonparametric U-Whitney-Mann test (p^{**}). The obtained indicators were compared between the median and interquartile range Me (Q1; Q3). Differences between the compared values at the level of 95% ($p < 0.05$) were considered statistically significant.

Results and discussion

The lymphoid component in guinea pigs lung is represented by diffuse lymphoid tissue in the pulmonary interstitium (fig. 1b) and lymphoid nodules in adventitial layer of intrapulmonary bronchi and blood vessels (fig. 1a). Among immunocompetent cells in the composition of diffuse lymphoid tissue are determined small, medium and large lymphocytes, plasma cells, macrophages (fig. 1c). Single plasma cells are found subepithelially, in loose fibrous connective tissue of respiratory mucosa of bronchi, nearby secretory units of serous-mucous bronchial glands and in pulmonary interstitium nearby blood vessels (fig. 1c). Most often CD3 positive lymphocytes are detected in pulmonary interstitium and interalveolar septa (fig. 1d). CD68 positive activated macrophages in most cases are detected in interalveolar septa of pulmonary acinus and in pulmonary interstitium.

In the intact group, the average number of lymphocytes is 3.75 ± 0.90 , plasma cells - 1.88 ± 0.35 , macrophages - 7.38 ± 0.62 in the field of view. In the control group, the analysis of quantitative indicators and localization of cells of diffuse lymphoid tissue of the bronchi and lungs of guinea pigs found that there is no statistically significant difference between animals of intact and control groups, which indicates that the procedure does not affect changes in number and distribution of immunocompetent cells. In the dynamics of the development of ovalbumin-induced allergic inflammation of the respiratory tract, we showed quantitative changes of immu-

nocompetent cells in diffuse lymphoid tissue of

bronchi and lungs of guinea pigs (table 1).

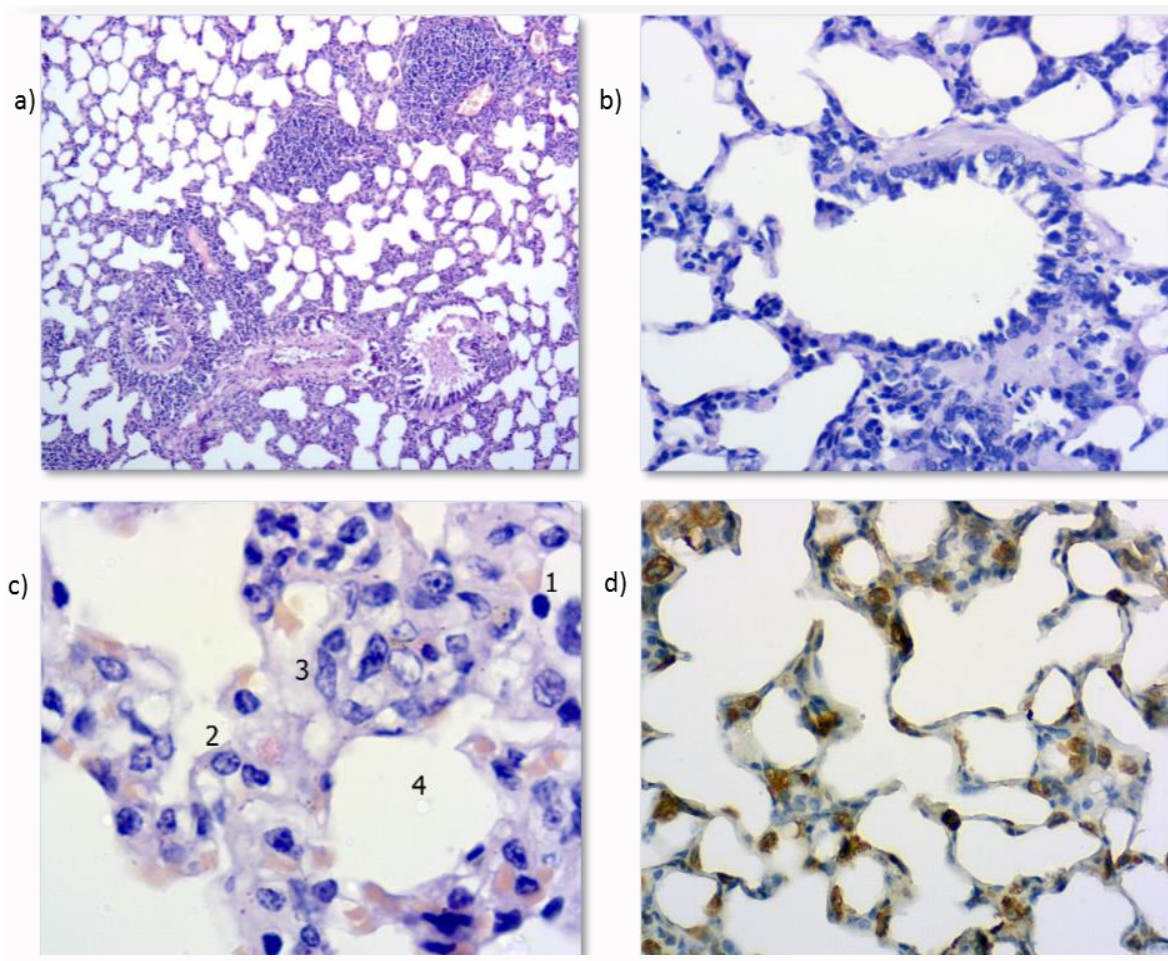


Fig. 1. Microscopic changes of bronchus associated lymphoid tissue and diffuse lymphoid tissue in guinea pigs lung after OVA-sensitization on the 30rd (1a), 36th (1b), 44th (1c, 1d) days of the experiment. 1a – increase of the number of peribronchial and perivascular lymphoid nodules; 1b – lymphocytic infiltration of pulmonary interstitium; 1c – immunocompetent cells of diffuse lymphoid tissue: 1 – lymphocyte; 2 – plasma cell; 3 – alveolar macrophage; 4 – lumen of pulmonary alveolus; 1d – elevation of the number of activated CD3 positive lymphocytes in pulmonary interstitium. Staining: 1a; 1b, 1c – G. and E. 1d – Immunohistochemical reaction with Mo a-Hu CD3 Antigen, Clone SP7. 1a - $\times 100$, 1b - $\times 400$; 1c, 1d - $\times 1000$.

Table 1

Morphometric changes in the average number of diffuse connective tissue cells per $5000 \mu\text{m}^2$ of guinea pigs lung sensitized with ovalbumin

Group	Lymphocytes	Plasma cells	Macrophages
1	$16.5 \pm 1.60^{***}$	$3.88 \pm 0.30^{*/**}$	$12.88 \pm 0.85^{*/**}$
2	$11.75 \pm 1.83^{***}$	$3.50 \pm 0.71^{**}$	$12.62 \pm 1.15^{*/**}$
3	$9.75 \pm 1.03^{***}$	1.75 ± 0.31	$10.88 \pm 0.67^{*/**}$
4	$8.25 \pm 0.88^{***}$	1.88 ± 0.40	$9.12 \pm 0.85^{**}$
5	3.50 ± 0.76	1.88 ± 0.30	6.62 ± 0.98
6	3.75 ± 0.90	1.88 ± 0.35	7.38 ± 0.62

Note. * - $p < 0.05$ (Student's t-test); ** - $p < 0.05$ (Whitney-Mann U-test) compared to the control group. $M \pm SD$.

The average number of lymphocytes increased statistically significantly from the 23rd to the 44th day of the experiment in animals of all experimental groups. Increasing coefficients of average indicators

of lymphocytes number at animals of experimental groups are high (in the 1st group - 4,7; 2nd - 3,4; 3rd - 2,8; 4th - 2,4), with the maximum increase almost by 5 times on the 23rd day of the experiment in ani-

mals of the 1st experimental group, compared to the control group of animals. The increase in the number of lymphocytes in diffuse lymphoid tissue of bronchi and lungs occurs due to migration from blood vessels and due to proliferation in lymphoid nodules. 80 - 90% of all lymphocytes of diffuse lymphoid tissue of the bronchi and lungs belong to CD3 positive T-lymphocytes. The average number of plasma cells of diffuse lymphoid tissue of the bronchi and lungs increased statistically significantly during the early period of development of ovalbumin-induced allergic inflammation in animals of the 1st and 2nd experimental groups. The maximum increase in the number of plasma cells in 2 times is showed on the 23rd day of observation, compared to the control group. The average number of macrophages doubles from the 23rd day and remains high until the 36th day of observation, compared to the control group (table 1).

Thus, the average number of lymphocytes in diffuse lymphoid tissue of the bronchi and lungs increased from the 23rd day of observation and remained high until the end of the experiment, and the maximum was during the early period of allergic inflammation, their increasing coefficient was 4.7. The average number of plasma cells also gained maximum increase during the early observation period and the increasing coefficient was 2.0. The average number of macrophages was maximum on the 23rd day of observation. Among all types of immunocompetent cells of diffuse lymphoid tissue of bronchi and lungs during the experiment, lymphocytes underwent a maximum increase of almost 5 times. The dynamics of quantitative changes of immunocompetent cells of diffuse lymphoid tissue of bronchi and guinea pig lungs shows a more active course of inflammatory alternative process in the respiratory tract and pulmonary interstitium in early stages of experiment in conditions of OVA-sensitization and challenge. Our results are confirmed by other scientific studies, showed morphological changes in diffuse lymphoid tissue of the bronchi and lungs of mice, rats and guinea pigs in

case of allergic inflammatory process [8-10].

The mechanism of morphological changes in diffuse lymphoid tissue represented the reactivity and interaction of cells of nonspecific immune defense, primarily cells of epithelial cells of respiratory mucosa, such as bronchiolar exocrinocytes and pulmonary neuroendocrine cells, as well as innate lymphoid cells type 2, which in agreement with other researchers [11-14]. Their interaction under the influence of the allergen causes the activation of immunocompetent cells of the adaptive link of local humoral immunity, which ends with the synthesis of IgE, accumulated subsequently on the plasmalemma of mast cells, initiating their repeated degranulation upon repeated exposure to the allergen.

Conclusion

In the early period of the development of experimental ovalbumin-induced allergic inflammation (23rd and 44th day of the experiment) specific resistance of the respiratory system is manifested in the form of activation of local cellular and humoral adaptive immunity, as evidenced by the dynamics of changes in the average number of lymphocytes (maximum increasing coefficient 4.7 in the 1st experimental group), macrophages and plasma cells (maximum increasing coefficient 2.0 in the 1st experimental group) in diffuse lymphoid tissue of guinea pigs bronchi and lungs.

Prospects for further research

We are planning to study the electron microscopic changes of immunocompetent cells of bronchus associated lymphoid tissue in conditions of experimental ovalbumin-induced allergic inflammation.

Sources of financing

This study is a part of the research work of Zaporizhzhya State Medical University "Immunomorphological characteristics of internal organs under the influence of endo and exogenous factors on the body" (state registration № 0118U004250).

Conflicts of interest

All authors declare no conflicts of interest in this paper.

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Попко С.С., Євтушенко В.М. Динаміка кількісних змін клітин дифузної лімфоїдної тканини бронхів і легень морських свинок, сенсibiliзованих овальбуміном.

РЕФЕРАТ. Актуальність. На сьогоднішній день у світі спостерігається прогресивне зростання алергічних захворювань органів дихальної системи, що обумовлює необхідність дослідження участі компонентів імунної системи в цих процесах. З погляду на сучасні уявлення про органи імунної системи особливий інтерес викликають закономірності морфогенезу і функції лімфоїдної тканини, асоційованої з бронхами, яка займає особливе місце в імунологічному захисті організму завдяки великій площі контакту з різноманітними антигенами. Морфогенез та реактивні зміни місцевого імунітету органів дихання при алергічному запаленні залишаються актуальним питанням сучасної морфології. **Мета** - визначення змін, які відбуваються в дифузній лімфоїдній тканині бронхів і легень морських свинок, сенсibiliзованих овальбуміном. **Методи.** Гістологічним, імуногістохімічним, морфометричним, статистичним методами досліджували середню кількість лімфоцитів, макрофагів та плазмоцитів у складі дифузної лімфоїдної тканини бронхів і легень самців 48 морських свинок після ініціації експериментального овальбумін-індукованого алергічного запалення дихальних шляхів. **Результати.** Середня кількість лімфоцитів дифузної лімфоїдної тканини бронхів і легень збільшувалася з 23-ої доби спостереження і трималася на високому рівні до кінця експеримента, а максимальною була протягом раннього періоду розвитку алергічного запалення, коефіцієнт їх збільшення становив 4,7. Середня кількість плазмоцитів максимального збільшення набувала також протягом ранніх строків спостереження і коефіцієнт збільшення становив 2,0. Середня кількість макрофагів максимальною була на 23-тю добу спостереження з аналогічним коефіцієнтом збільшення. Серед всіх видів імунокомпетентних клітин дифузної лімфоїдної тканини бронхів і легень під час експерименту максимального збільшення майже у 5 разів зазнавали Т-лімфоцити. **Підсумок.** У ранній період розвитку експериментального овальбумін-індукованого алергічного запалення специфічна резистентність дихальної системи проявляється у вигляді активації локальних ланок клітинного та гуморального адаптивного імунітету, про що свідчить динаміка змін середньої кількості лімфоцитів (максимальний коефіцієнт збільшення 4.7 в 1-ій експериментальній групі), макрофагів та плазмоцитів (максимальний коефіцієнт збільшення 2.0 в 1-ій експериментальній групі) дифузної лімфоїдної тканини бронхів і легень морської свинки.

Ключові слова: дифузна лімфоїдна тканина, легеня, овальбумін, алергічне запалення, морська свинка.

Попко С.С., Євтушенко В.М. Динамика количественных изменений клеток диффузной лимфоидной ткани бронхов и легких морских свинок, сенсibiliзованных овальбумином.

РЕФЕРАТ Актуальность. На сегодняшний день в мире наблюдается прогрессивный рост аллергических заболеваний органов дыхательной системы, что обуславливает необходимость исследования участия компонентов иммунной системы в этих процессах. С точки зрения современных представлений об органах иммунной системы особый интерес вызывают закономерности морфогенеза и функции лимфоидной ткани, ассоциированной с бронхами, которая занимает особое место в иммунологической защите

организма благодаря большой площади контакта с различными антигенами. Морфогенез и реактивные изменения местного иммунитета органов дыхания при аллергическом воспалении остаются актуальным вопросом современной морфологии. **Цель** - определение изменений, которые происходят в диффузной лимфоидной ткани бронхов и легких морских свинок, сенсibilизированных овальбумином. **Методы.** Гистологическим, иммуногистохимическим, морфометрическим, статистическим методами исследовали среднее количество лимфоцитов, макрофагов и плазмоцитов в составе диффузной лимфоидной ткани бронхов и легких самцов 48 морских свинок после инициации экспериментального овальбумин-индуцированного аллергического воспаления дыхательных путей. **Результаты.** Среднее количество лимфоцитов диффузной лимфоидной ткани бронхов и легких увеличивалось с 23-их суток наблюдения и держалась на высоком уровне до конца эксперимента, а максимальным было в течение раннего периода развития аллергического воспаления, коэффициент увеличения 4,7. Среднее количество плазмоцитов максимального увеличивалось также в ранние сроки наблюдения с коэффициентом увеличения 2,0. Среднее количество макрофагов было максимальным на 23-е сутки наблюдения с аналогичным коэффициентом увеличения. Среди всех видов иммунокомпетентных клеток диффузной лимфоидной ткани бронхов и легких во время эксперимента наиболее выраженная реакция наблюдалась со стороны Т-лимфоцитов. **Заключение.** В ранний период развития экспериментального овальбумин-индуцированного аллергического воспаления специфическая резистентность дыхательной системы проявляется в виде активации локальных звеньев клеточного и гуморального адаптивного иммунитета, о чем свидетельствует динамика изменений среднего количества лимфоцитов (максимальный коэффициент увеличения 4.7 в первой экспериментальной группе), макрофагов и плазмоцитов (максимальный коэффициент увеличения 2.0 в первой экспериментальной группе) диффузной лимфоидной ткани бронхов и легких морской свинки.

Ключевые слова: диффузная лимфоидная ткань, легкое, овальбумин, аллергическое воспаление, морская свинка.