Morphological features of the respiratory part in guinea pigs lung in dynamics of experimental allergic inflammatory process

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The main function of the components of the respiratory tract is gas exchange while maintaining homeostasis in lung, given the pathogenic and non-pathogenic elements of the environment contained in the inhaled air. Morphological changes of the components of the respiratory part in lung of human and animals of adaptive nature under the influence of various factors on the body today remain insufficiently studied. The aim of the study was to investigate the morphological changes of the components of the respiratory part of guinea pigs lung in the dynamics of experimental ovalbumin-induced allergic inflammation. We used histological and electron microscopic methods to study the lungs of 48 male guinea pigs in experimental ovalbumin-induced allergic inflammation, simulated by subcutaneous sensitization and subsequent intranasal inhalation with ovalbumin. Morphological changes of the components of the respiratory tract of the lungs were determined in the early and late phases of allergic inflammatory process at the optical and submicroscopic levels. The early phase (23rd and 30th days of the experiment) was characterized by a predominance of alterative and destructive changes, consisted in the desquamation of the alveolar epithelium with a violation of the blood-gas barrier and hemomicrocirculatory bed. Type II alveolar cells had lesions of varying severity in the form of the absence of lamellar bodies or disturbances in the formation of their contents, vacuolization of the cytoplasm, mitochondrial damage. In the late phase of the development of the allergic inflammatory process (36th and 44th days of the experiment) in the respiratory part of lung were dominated adaptive and restorative changes. In addition, we observed dilation of the lung alveoli and thinning of the interalveolar septa, which is a consequence of the cascade of reactions of the local neuroendocrine and immune systems of lung as a result of allergen action. Thus, experimental ovalbumin-induced allergic inflammatory process of the respiratory tract is accompanied by structural and functional changes in the components of the respiratory part in lung of guinea pigs in stages depending on the duration of the experiment.

Key words: alveolar epithelium, surfactant, allergic inflammation, ovalbumin, guinea pig.
respiratory bronchioles [14]. After reuptake by type II alveolar cells, inactive surfactant aggregates are either catabolized or recycled by re-incorporation into the metabolic pathway [19]. A small part of surfactant is phagocytosed and cleaved by alveolar macrophages or eliminated through the respiratory tract using mucociliary clearance.

Soluble SP bind and regulate a number of effector immune cells in allergic airway inflammation. Both SP-A and SP-D inhibit the activity of the allergic inflammatory process, made them interesting molecules for the treatment of bronchial asthma [10]. The anti-inflammatory properties of alveolar macrophages are being actively studied nowadays [8, 19]. However, most of the scientific works, focused on the study of the components of the respiratory part of lung in allergic inflammation are immunological studies [2, 3, 4, 9]. The above indicates the need to clarify this urgent problem from a morphological point of view in the early and late phases of the experimental allergic inflammatory process.

The aim of this work is to study the morphological changes of the components of the respiratory part of guinea pig lung in the dynamics of experimental ovalbumin-induced allergic inflammation.

Materials and methods

Forty-eight sexually mature male guinea pigs (450 - 600 gram) were weighed and kept at vivarium of Zaporizhzhya State Medical University, with free access to OVA-free food and water. The experimental protocol was followed the published guidelines (Strasbourg, 1986; Kiev, 2006).

The study protocol was approved by the institutional review board of the Bioethics Committee of Zaporizhzhia State Medical University (Protocol № 8 of 11 Juny 2019).

Induction of airway allergic inflammatory process was performed by subcutaneous sensitization and airway challenge through nasal inhalation with OVA (0.5 mg/mL per animal) mixed with aluminum hydroxide (10 mg/mL in saline per animal) on days 0, 7 and 14. From 21 to 28 days animals were exposed for 15 min to an aerosol of OVA (10 mg/mL in saline) using a nebulizer (Little Doctor International, Singapore, LD-211C) attached to a plastic chamber [14]. Animals were assigned equally into six experimental groups of 8 guinea pigs each. Group I - IV are guinea pigs sensitized and challenged with ovalbumin (OVA) (Sigma Aldrich, USA) with alum as an adjuvant (AlumVax Hydroxide vaccine adjuvant, OZ Biosciences, France), dropped out the experiment respectively on the 23rd, 30th, 36th and 44th days after its start. Group V: guinea pigs sensitized and exposed to saline, served as control. Group VI: intact animals (norm).

Lungs removed and fixed in 10 % neutral buffered formalin. Formalin fixed, paraffin wax embedded lung specimens were selected for histological preparation, prepared as 5-μm-thick sections and stained with hematoxylin and eosin. Histological study was carried out on Carl Zeiss Primo Star microscope equipped with the Axioscam digital microphoto attachment using the ZEISS ZEN 2011 software, using the oil immersion technique (x1000).

Electron microscopy was performed on glutaraldehyde-fixed 1x1 mm specimens of lung tissue followed by processing in a 1 % solution of osmium tetroxide. Subsequently, the pieces were dehydrated in a series of graded ethanol up to 100 % according to histological standards, acetone with additional contrasting for two hours in 2.5 % uranyl acetate at 700°C. Pouring into the block was carried out by gradual impregnation of the material with acetone oxide with Eponym (2:1, 1:1, 1:2) and poured into pure Epon. The resin polymerization was carried out in two stages at 36°C (12:00) and 56°C (24 hours). Ultra-thin (55-65 nm) sections were obtained on a "PowerTome RMC Boeckeler" ultratom and contrasted with Reynolds lead citrate for 25 minutes at room temperature. Ultrathin sections viewed on a PEM-100-01 electron microscope.

Results

In the control group of animals in the morphological view of the respiratory part of lung, we showed that the walls of the alveoli are lined by flattened type I alveolar cells, among which type II alveolar cells are localized. We also found single alveolar macrophages and eosinophilic granulocytes in the alveoli lumen. Septal cells, fibroblasts, plasma cells and eosinophilic granulocytes occupied the pulmonary interstitium in respiratory portion of lung (fig. 1a).

We have shown short microvilli and a small number of LB at the apical pole of type II alveolar cells in the control group at the submicroscopic level. Alveolar macrophages had an underdeveloped cytoplasmic membrane, formed single cell processes. There were rare secondary lysosomes in their cytoplasm. Type I alveolar cells had a more expanded nucleus-containing part protruded into the alveoli lumen and thin peripheral part of the cytoplasm. There single pinocytic vesicles in the endotheliocytes of blood capillaries in the peripheral part of their cytoplasm.

We have found local destruction and desquamation of alveolar epithelium, interalveolar septa, exposure of the basal membrane of the endothelium in blood capillaries in the early manifestation of the allergic inflammatory process on the 23rd day after the experiment in the respiratory portion of guinea pigs lung. There were abundant alveolar macrophages in the alveoli lumen with signs of increased phagocytic activity. The count of eosinophilic granulocytes was increased. In addition, we observed some microcirculatory disorders, such as plethora and stasis of blood capillaries and postcapillary venules, numerous foci of erythrocyte diapedesis in the alveoli lumen and in the pulmonary interstitium (fig. 1b).

On the 30th day after the start of the experiment morphological changes in the respiratory part of lung became less pronounced, although microcirculatory disorders in the form of plethora and stasis of blood
Fig. 1. Microscopic changes in the respiratory portion of the guinea pigs lung after sensitization and aeroallergization with ovalbumin on days 23 (1b), 30 (1c) and 44 (1d) after the start of the experiment compared with the control group (1a). 1 - alveoli lumen; 2 - type I alveolar cell; 3 - type II alveolar cell; 4 - eosinophilic granulocyte; 5 - alveolar macrophage; 6 - plasma cell; 7 - blood capillary; 8 - lumen of the terminal bronchiole; 9 - smooth myocyte in the wall of the terminal bronchiole; 10 - interalveolar pore. Stain: hematoxylin and eosin. x1000.

Fig. 2. Ultrastructural changes in the respiratory portion of the guinea pigs lung after sensitization and aeroallergization with ovalbumin on days 23 (2a) and 30 (2b) after the start of the experiment. 2a: 1 - alveoli lumen; 2 - type I alveolar cell; 3 - blood capillary; 4 - type II alveolar cell; 5 - alveolar macrophage. 2b: 1 - type II alveolar cell nucleus; 2 - alveoli lumen; 3 - lamellar body; 4 - mitochondria; 5 - cytoplasm vacuolization; 6 - fragment of the cytoplasm of alveolar macrophage. Transmission electron microphotos. 2a) x6000. 2b) x6800.
capillaries persisted (fig. 1c). In some places, the intercellular septa were thickened due to edema. We observed a trend towards renewal of the alveolar epithelium.

The morphological changes detected at the optical level are confirmed by the results of electron microscopic examination. Swelling and thickening of the peripheral portion of cytoplasm of type I alveolar cells, stasis, plethora and deformation of erythrocytes of blood capillaries were demonstrated (fig. 2a). Type II alveolar cells had lesions of varying severity. Absence of LB or disturbances in the formation of their content, cytoplasm vacuolization, mitochondria damage. Less frequently we observed destruction and necrosis of type II alveolar cells. Abundant lysosomes and heterophagosomes, residual bodies were found in the cytoplasm of alveolar macrophages (fig. 2b).

In the late phases of the allergic inflammatory process on the 36th and 44th days after the start of the experiment in the respiratory portion of the guinea pigs lung, we have shown thinning of the interalveolar septa and, at the same time, alveoli expansion (fig. 1d), correlated with emphysematous lesions. In local places, plethora and stasis of blood capillaries were noticeable, although less pronounced, compared with the early phases of the allergic inflammatory process.

Discussion

Morphological analysis of the respiratory part of guinea pigs lung made it possible to identify the phases of the allergic inflammatory process. The early manifestations (23rd and 30th days of the experiment) were characterized by the predominance of destructive changes. We demonstrated desquamation of the alveolar epithelium with a violation of the air-blood barrier on the background of a violation of hemomicrocirculatory bed in the early phases of the allergic inflammatory process. Similar changes in the lungs under the influence of various pathological factors were found by other scientists [6, 7, 11]. The revealed changes can be explained by the rapid degranulation of mastocytes with the release of histamine and leukotrienes, which, in addition to bronchospasm and mucus hyperproduction, caused alterative-exudative changes in the components of the respiratory part of the lungs [1]. Damage to type II alveolar cells is especially significant, since it is known that they ensure the regeneration of both their population and type I alveolar cells [10, 17]. However, their severe disorders in the form of necrosis were rarely observed, more often signs of damage to their mitochondria and LB were revealed. Accordingly, it can be assumed that there was a disturbance in the surfactant system, in turn, its enhanced exocytosis as a manifestation of the innate resistance of the lungs to damage caused by allergen, which is in agreement with other scientists [20]. We have also assumed that type II alveolar cells, after the action of the allergen, were involved in the regeneration of the alveolar epithelium, respectively, their secretory function decreased, and the proliferative function prevailed. Alterative changes in the alveolar epithelium were accompanied by elevated number and phagocytic activity of alveolar macrophages, which confirmed their anti-inflammatory properties in conditions of the allergic inflammatory process [3, 9]. In the late phases of the experimental allergic inflammatory process in the respiratory part of the lungs adaptive-renewal changes prevailed. Despite the renewal of the alveolar epithelium, we observed expansion of the alveoli and thinning of the interalveolar septa, which was a consequence of a cascade of reactions of the local neuroendocrine and immune systems in lung [5, 12, 15]. Latest lead to remodeling of the airways and the hemomicrocirculatory bed of lung, which was confirmed by other scientists [16, 18].

We are going to study electron microscopic changes in the elements of the hemomicrocirculatory bed of guinea pigs lung in the early and late phases of allergic inflammatory process.

Conclusions

Experimental ovalbumin-induced allergic inflammatory process was accompanied by structural and functional changes in the components of the respiratory part of the guinea pigs lungs, which were different in the early and late phases of the inflammatory process, depending on the duration of the experiment.

References


Морфологічні особливості респіраторного відділу легень морських свинок в динаміці експериментального алергічного запального процесу

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Основна функція компонентів респіраторного відділу легень полягає в газообміні та забезпеченню гомеостазу в легенях, враховуючи патогенні та непатогенні елементи навколишнього середовища, які містяться у повітрі, що вдихається. Морфологічні зміни компонентів респіраторного відділу легень мають адаптаційний характер при дії на організм різноманітних чинників на сьогоднішній день залишаються недостатньо дослідженими. Мета роботи – дослідити вплив патогенных та непатогенних чинників на респіраторний відділ легень морської свинки на різних етапах алергічного запального процесу.

В ході експериментальних досліджень зміни компонентів респіраторного відділу легень морської свинки в динаміці експериментального алергічного запального процесу виявлено на різних етапах його розвитку.

Ключові слова: компоненти респіраторного відділу, морська свинка