DISTRIBUTION AND QUANTITATIVE CHANGES OF MAST CELLS IN GUINEA PIGS LUNG IN OVALBUMIN-INDUCED ALLERGIC INFLAMMATION

*S.S. Popko, V.M. Yevtushenko
ZAPORIZHZHIA STATE MEDICAL UNIVERSITY, ZAPORIZHZHIA, UKRAINE

Background. One of the most important cells in local immunity in lung are mast cells. They are involved in both innate and adaptive immune responses to inhaled allergens. The question of distribution of these both cell types in guinea pig lung in case of experimental allergic inflammation in most aspects remains open.

Objective. The aim of this research is to study the distribution and quantitative changes of mast cells in lung of guinea pigs in ovalbumin-induced allergic inflammation.

Methods. the lungs of 48 male guinea pigs have been studied using histological, morphometric and statistical methods in cases of experimental ovalbumin-induced allergic inflammation. The total number of mucosa related mast cells and perivascular mast cells in guinea pig lungs were counted.

Results. It has been established that mucosa related mast cells are normally more abundant in guinea pigs lung than perivascular ones. Maximum increase in a number of mucosa related mast cells was revealed in the early period of allergic inflammation, as evidenced by maximum increase coefficient of 1.4 in the 1st experimental group, compare to the control (P*/**<0.05). However, maximum increase in number of perivascular mast cells in 5 times was found during the late period of allergic inflammation in the 4th experimental group (P***<0.05).

Conclusion. Experimental sensitization and challenge with ovalbumin leads to statistically significant increase in average number of both types of mast cells but predominantly the latter ones. It has been proved that cells dynamics is multidirectional.

KEYWORDS: mucosa related mast cell, perivascular mast cell, lung, guinea pig, allergic inflammation.

Introduction

Mast cells are present in vascularized tissues of almost all organs except the central nervous system and the retina. Derived from pluripotent red bone marrow stem cells, they differentiate in connective tissue from their progenitor cells under the influence of c-Kit ligand (CD117) in the presence of growth factors and various cytokines realized by the connective tissue microenvironment of the organs in which they are located and function [1, 2]. The cytoplasm of mature mast cells contains 50-200 granules of inflammatory mediators, such as histamine, heparin, numerous cytokines, chondroitin sulphate, and neutral proteases (chymase and tryptase), provided mechanisms to increase the permeability of the microvessel wall and perivascular connective tissue, angiogenesis [3]. They regulate the inflammatory process in the connective tissue, influencing the permeability of the vascular wall and the amorphous component of the intercellular substance. In addition, they are involved in implementation of allergic reactions due to the presence of FceRI receptors on immunoglobulins type E on their plasmalemma. On the other hand, microvessel endothelial cells, secreting adhesion molecules VCAM-1, ICAM-1 and ELAM-1, initiate migration of mast cells precursors from the peripheral bloodstream into the tissue where the inflammatory process takes place [4].

It is established that in lung of human and BALB/c mice in normal physiological conditions the number of mast cell progenitors is insignificant, but in cases of antigen-induced inflammation under the influence of α4β7 integrins, VCAM-1 and CXCR2 they actively migrate to lung tissue [5]. Mast cells, located in different parts of the lungs and respiratory tract, have excellent histochemical properties and express different mediators. Two phenotypes of mast cells have been studied in lung of human and small mammals: mucosa related mast cells (synthesize only tryptase) and perivascular mast cells (synthesize tryptase, chymase, and carboxypep-
The object of the experimental study was lung removed from 48 sexually mature male guinea pigs, kept in standard conditions of the vivarium of the Zaporizhzhya State Medical University. Experiments on animals were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council Directives 86/609 / EEC (1986), Laws of Ukraine № 3447-IV “On the protection of animals from cruel treatment, general ethical principles of animal experiments”, approved by the First National Congress of Ukraine on Bioethics (2001). Induction of allergic airway inflammation was carried out by subcutaneous sensitization and subsequent aeroallergization with ovalbumin (OVA) [7]. On days 1, 7, 14 of the experiment, guinea pigs were sensitized: subcutaneous injection into the interscapular region of 0.5 mg OVA (Sigma Chemical Co., USA) together with an adjuvant – aluminium hydroxide, 10 mg (AlumVax Hydroxide vaccine adjuvant, OZ Biosciences, France) diluted in 1 ml of saline. From 21 to 28 days of the experiment, the animals were aeroallergized OVA at the dose of 10 mg/ml of saline for 15 min/day using an LD-211C compressor inhaler (Little Doctor International, Singapore) in an inhalation chamber. The animals were divided into 6 groups (8 animals in each group) for investigations. The first four groups were animals, sensitized and challenged OVA, withdrawn from the experiment, respectively, on the 23rd, 30th, 36th and 44th days after its start; the 5th group – control, the animals were injected subcutaneously with 1 ml of saline and inhaled with saline; the 6th – intact group. For the purpose of rational presentation 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 development of allergic inflammatory process in lung.

Therefore, the aim of the research is to study distribution and quantitative changes of mast cells in lung of guinea pigs in ovalbumin-induced allergic inflammation.

Methods

The research results were processed by current 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) based on the Windows 10 operating system. Hypothesis for normal distribution of the studied parameters was checked using the Shapiro-Wilk test and the Kolmogorov-Smirnov test of consistency. The arithmetic means (M) and standard errors of the mean (± m) were calculated. The statistical significance of intergroup differences according to the data obtained was established using the parametric Student’s t-test (p *) and the nonparametric Whitney-Mann U-test (p **). The obtained data was 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

The morphometric examination of mast cells in the intrapulmonary airways and lung parenchyma of intact guinea pigs has shown that the average number of mucosa related
mast cells is 2.62±0.05, perivascular mast cells 1.38±0.07 in the field of view. Normally, in guinea pigs lung, mucosa related mast cells are more abundant, than perivascular mast cells, as evidenced by the increase factor 1.6. We have analysed the number of mast cells in the connective tissue of guinea pigs lung and have found that there was no statistically significant difference between the animals in the intact and control groups that proves that the procedure itself does not affect changes in the number of mast cells. Therefore, we compare the results of the experimental and control groups.

OVA – sensitization and challenge leads to quantitative changes in the dynamics of mast cells number of lung connective tissue. The increase in the number of mast cells of both phenotypes is determined in animals of the 1st experimental group from the 23rd day of observation in the early period of development of experimental allergic inflammation, but more significant OVA – challenge effects the number of perivascular mast cells. In addition, further dynamics of changes in their number is different in different experimental groups (Fig. 1).

The maximum average number of mucosa related mast cells, observed in the 1st experimental group, is statistically significantly higher in 1.4 times compare to the animals of the control group (P**<0.05). Further investigation has shown that starting from the 30th day of the experiment, there is a tendency for their gradual recovery to the indicators of the control group, reaching the latter on the 44th day of observation.

The average number of perivascular mast cells in animals of the 1st experimental group is by 1.6 times higher compare to the number of mucosa related mast cells, and statistically significantly higher in 3.6 times compare to the control group (P**<0.05). There is a tendency to increase in the number of perivascular mast cells with the maximum rate on the 44th day of observation, starting from the 36th day of the experiment (Fig. 2).

On the 30th day of observation the average number of mucosa related mast cells is 3.62±0.05 in the field of view, which is in 1.3 times more than the same indicator in the control group (P**<0.05). However, the average number of perivascular mast cells in animals of the 2nd experimental group on the 30th day of observation is in 1.5 times higher than the number of mucosa related mast cells, and statistically significantly higher in 3.5 times compare to the same point in the control group (P**<0.05) (Fig. 2).

We have established changes in the dynamics of mast cells of both phenotypes during the late period of development of experimental allergic inflammation in guinea pigs lung. The average number of mucosa related mast cells in the animals of the 3rd experimental group is 3.5±0.05 in the field of view, which is statistically significantly in 1.3 times higher (p**<0.05), compare to the same indicator of the control group (Fig. 1). The average number of mucosa related mast cells acquires the control indicator on the 44th day of the experiment. The average number of perivascular mast cells in the animals of the 3rd experimental group on the 36th day of observation is in 2 times higher compare to the number of mucosa related mast cells, and statistically significantly higher in 4.5 times compare to the same point in the control group.

![Fig. 1. Morphometric changes in the number of mucosa related mast cells of guinea pigs lung.](image)

Note: * - P<0.05 (Student’s t-test); ** - P<0.05 (Whitney–Mann U-test) compare to the control group. Me (Q1; Q3). The median (Me) is shown by the green line. M±m (n=8). The arithmetic mean (M) is shown by the dotted green line.
group (P*/**<0.05). The hyperplasia of perivascular mast cells (Fig. 3) is found in animals of the 4th experimental group on the 44th day after the start of the experiment (8.0±0.29 in the field of view), which is statistically significantly in 5 times higher than in the control group (P*/**<0.05).

Discussion

Thus it has been established that in contrast to human and other small mammals (mice), mucosa related mast cells are normally more abundant in guinea pigs lung than perivascular ones. Nevertheless, in contrast to BALB/c mice, a common feature of guinea pigs lung connective tissue and human lung in cases of experimental allergic inflammation due to sensitization and challenge with OVA is a statistically significant increase of both mucosa related and perivascular mast cells. However, we have demonstrated that the mast cells number changes of different phenotypes in guinea pigs lung have different nature. Our results revealed the maximum increase in the number of mucosa related mast cells in the early period of development of experimental allergic inflammation, as evidenced by the maximum increase coefficient of 1.4 in the 1st experimental group, compare to the control. It should be noted that the degranulation of mucosa related mast cells promotes the release of heparin, increasing permeability of capillaries and improves trophic of respiratory mucosa. However, the maximum increase in the number of perivascular mast cells in 5 times was evidenced during the late period of allergic inflammatory process development in lung of animals of the 4th experimental group.

Thus, the increase of perivascular mast cells is predominantly greater than mucosa related mast cells in OVA – sensitization. This should be decisive for morphological and histochemical changes of the microcirculatory bed, lymphoid tissue cells in connective tissues of pulmonary interstitium, which were previously described [7, 9]. For instance, mast cells together with respiratory endocrinocytes help maintain homeostasis of the local lung immune system [10-13]. Moreover, mast cells are involved in both innate and adaptive immune responses to allergens. Due to the presence of heparin secreted by perivascular mast cells into the intercellular substance of connective tissue the permeability of microvessels increases; in allergic inflammation it causes migration of lymphocytes and plasma cells into the perivascular intercellular substance [14-16]. In addition, mast cells contribute to maintenance

![Fig. 2. Morphometric changes in the number of perivascular mast cells in connective tissue of guinea pigs lung parenchyma. Note: * – P<0.05 (Student’s t-test); ** – P<0.05 (Whitney–Mann U-test) compare to the control group. Me (Q1; Q3). The median (Me) – the green line. M±m (n=8). The arithmetic mean (M) – the dotted green line.](image2)

![Fig. 3. Perivascular mast cells hyperplasia (arrows show) in connective tissue of the pulmonary interstitium of guinea pigs after sensitization and challenge with ovalbumin on the 44th day of the experiment. Staining: alcian blue. ×1000.](image3)
of chronic allergic airway inflammation and are crucial in initiating immune response to the allergen, during which they transmit signals that stimulate IgE synthesis by plasma cells and differentiation of Th2 lymphocytes [17].

**Conclusion**

Normally mucosa related mast cells are the predominant mast cell type in guinea pigs lung. OVA - sensitization and challenge leads to the statistically significant increase in the number of both mast cells types: mucosa related and perivascular mast cells.

The dynamics of increase in the number of mast cells of different phenotypes in guinea pigs lung is of a different nature in case of OVA – sensitization. More significant is increase in the number of perivascular mast cells in 5 times during the late period of allergic inflammation development in the 4th experimental group.

**Conflict of Interests**

Authors declare no conflict of interest.

**Acknowledgements**

This research is a part of the research affairs of Zaporizhzhia State Medical University on “Immunomorphological characteristics of internal organs under the influence of endogenous and exogenous factors on the body”, state registration No. 0118U004250.

**Author’s Contributions**

Svitlana S. Popko – conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – reviewing and editing; Valentina M. Yevtushenko – data curation, writing – reviewing and editing.
Information about the authors
Svitlana S. Popko, PhD, Associate Professor of the Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine
ORCID 0000-0002-5533-4556, e-mail: kluchkosv@gmail.com
Valentina M. Yevtushenko, MD, Professor of the Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine
ORCID 0000-0002-6858-6488, e-mail: evtushenkovm13@gmail.com

References
   DOI: 10.1002/eji.201444546
   DOI: 10.1111/all.14318
   DOI: 10.3389/fimmu.2015.00620
   DOI: 10.3389/fimmu.2015.00394
   DOI: 10.3389/fimmu.2019.00821
   DOI: 10.1042/cs20200394
   DOI: 10.14739/2409-2932.2021.1.226851
   DOI: 10.29254/2077-4214-2021-1-159-240-244 [in Ukrainian]
   DOI: 10.1042/cs20191309
   DOI: 10.1165/rcmb.2018-0179oc
   DOI: 10.3390/cells9071694
   DOI: 10.14739/2310-1210.208411
   Available from: https://doi.org/10.5772/intechopen.86964
   DOI: 10.1038/ni.3489
   DOI: 10.1016/j.immuni.2015.06.006
   DOI: 10.3390/biomedicines7040095

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