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THE POSSIBILITY OF USING ANTI-HUMAN MONOCLONAL ANTIBODY CD3 AS PAN T-CELL MARKER IN GUINEA PIGS

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Abstract

The present study **was aimed** to evaluate the possibility of using anti-human monoclonal antibody CD3 as pan T-cell marker in the guinea pigs' trachea and lung in early and late manifestations of the allergic inflammatory process.

Materials and methods. We have studied the distribution and quantitative changes of CD3-positive lymphocytes in trachea and lung of guinea pigs using histological, immunohistochemical, statistical methods in conditions of experimental inflammatory process.

Results. Our results revealed the applicability of anti-Human monoclonal antibody CD3 (Clone SP7, «DAKO», Denmark) cross-reaction with T-cells of guinea pigs' tracheas and lungs. The most statistically significant elevation of the number of CD3-positive lymphocytes, in comparison with the control group ($p^*/^{**} < 0.05$), observed in the experimental group III in the late stages of experimental inflammatory process. The elevation of the number of CD3-positive lymphocytes persists even after the termination of the allergen action, which indicates the continuation of the reaction of pulmonary local adaptive immunity to the allergen.

Conclusions. The results of our study may be useful in conditions of the deficiency of guinea pig-specific tests. The immunohistochemical assessment of guinea pigs' trachea and lungs proved the possibility to use anti-Human monoclonal antibody CD3 as a panT-cell marker in guinea pigs. We demonstrated the activation of adaptive immune response (T-cells), represented by their immunohistochemical changes, predominantly in the late stages of experimental inflammatory process.

Keywords: experimental inflammation, CD3, guinea pig, lung, trachea.

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1. Introduction

Until now, the attention of scientists is drawn to the problems of reactivity of the local immune system in airways under the influence of environmental factors, including allergenic ones [1, 2]. Viruses or allergens could disrupt the airway epithelium, secreted pro-inflammatory cytokines that provoke the migration of immune cells inside the lungs [3]. There are scientific data experimentally demonstrating the structure and functions of bronchus associated lymphoid tissue (BALT) of airways and pulmonary interstitium in normal conditions and after antigen exposure [4, 5]. Nevertheless, there are currently conflicting reports on the role of BALT in the development of airways experimental inflammation in conditions of allergic reaction [6]. Some authors postulate that there is no obvious link between BALT and the development of allergic inflammation, confirming scientific data [7]. Other studies suggest that although the presence of lymphoid nodules and diffuse lymphoid tissue in respiratory system does not correlate with the allergic process, their reactivity increases in patients with bronchial asthma [8, 9]. Previous study had reported that the expansion of the lymphatic network was limited to areas of BALT in conditions of airways inflammation [10]. The dysbalance of T-lymphocytes leads to airways chronic inflammation, accompanied by a systemic inflammatory process [11]. When modelling allergic inflammation of the human respiratory tract, it becomes important to select the species of animals that are similar to humans. The guinea pig possesses variety of uncommon peculiarities making it appropriate as an animal specimen for studies refers to allergic inflammation. The pharmacology of receptors and mediators in guinea pigs is comparable to human ones, the histophysiology of guinea pigs' airways and lungs is corresponding to those in humans [12, 13]. In addition, the response to antigen administration in guinea

pig includes early and late manifestations of the experimental inflammatory process, made them resemble to those in humans and distinctive from those in rats and mice [14, 15]. Nevertheless, research in guinea pigs is still limited due to the deficiency of guinea pig-specific tests. The availability of reagents and special instruments suitable for research in guinea pigs, especially when compared to humans and more traditional laboratory animals such as mice and rats, is limited. Researchers have been announced on cross-reactive monoclonal antibodies, which have been reacted with group of differentiation clusters of human cells and were contemporaneously examined for reactivity with other specimens [12]. Based on these data, we hypothesized that mAb a-Hu CD3 antigen can be also specific for activated T-lymphocytes in guinea pigs.

Consequently, there has been little interest in developing better models using allergens in guinea pigs. However, there is a broad range of specific tests for mice, available to analyze tissue responses. Unfortunately, mice have become the reagent system of option for experimental studies, although allergic inflammation models in guinea pigs are superior in most crucial points [13, 16]. To close the translation gap between preclinical asthma models and the biology of human affliction, the guinea pig must be renowned as an important reagent specimen.

This work **aimed** to study the possibility of applying anti-Human monoclonal antibody CD3 as pan T-cell marker in the guinea pigs' airways and lungs in the dynamic of experimental allergic inflammation.

2. Materials and methods

Animals. 48 sexually mature male guinea pigs (500–600 gram) were weighed and kept at vivarium of Zaporizhzhia State Medical University, with free access to OVA-free food and water [17].

Ethics statement. The study protocol was approved by the institutional review board of the Bioethics Committee of Zaporizhzhia State Medical University (protocol 8 of 11 June 2019). The design of the study and all experimental procedures used in the study was performed by the principles expressed in the published guidelines (Strasbourg, 1986; Kiev, 2006). All experimental procedures were carried out in May-April 2021.

Experimental design. 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).

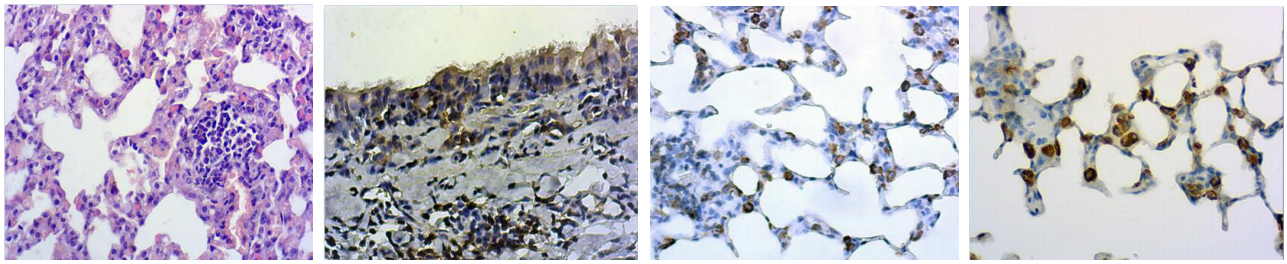
Sensitization protocol. Induction of airway allergic inflammation was performed by subcutaneous sensitization and airway challenge through nasal inhalation with OVA (0.5 mg/mL per animal) mixed with aluminium hydroxide (10 mg/mL in saline per animal) on days 0, 7 and 14. From 21 to 28 days animals were exposed for 15 min to an aerosol of OVA (10 mg/mL in saline) applying a nebulizer (Little Doctor International, Singapore, LD-211C) attached to a plastic box.

Tissue processing and immunohistochemical staining. Trachea and lungs removed and fixated immediately in 10 % formalin. Formalin fixed; paraffin wax embedded blocks were selected for histological preparation [18, 19]. 4μm histological sections were dyed by haematoxylin-eosin. Next immunohistochemistry (IHC) method was performed on paraffin sections using monoclonal antibody Mo a-Hu CD3 Antigen, Clone SP7 («DAKO», Denmark). Dewaxing and rehydration with simultaneous high-temperature unmasking the antigens were carried out by heating in the autostainer with a PT-module (Thermo Fisher Scientific, USA) in Dewax&HIER buffer H (Thermo Fisher Scientific, USA) (pH = 9.0); suppressed endogenous peroxidase activity with 3 % H_2O_2 solution and a protein block were applied. Incubation with primary antibodies was performed in concordance with the fabricator's instructions, visualization of the IHC reaction was completed applying an UltraVisionQuanto HRP+DAB System (Thermo Scientific, USA). Sections were stained with Mayer's hematoxylin and embedded in Cytoseal. Complex morphometric examinations were carried out under a Carl Zeiss Primo Star microscope equipped with a digital Axiocam for photomicrographs microphoto attachment using the ZEISS ZEN 2011 software. Following the immunohistochemical staining on the serial cross transverse sections, the total number of CD3-positive cells per unit area of 5000 μm^2 was counted applying a microscope with an oil immersion objective technique (×1000).

Statistical methods. Data were presented as mean±standard deviation [SD] for all parameters. The data were analyzed using the standard software package Microsoft Office Excel 2010 and STATISTICA 6.0 (StatSoft Inc., USA, license 46 No. AXXR712D833214FAN5), the libraries SciPy (BSD License), NumPy (BSD License), pandas-profiling (MIT License), pandas (BSD License). We used the library Matplotlib (BSD License) for the Python programming language to visualize the processed data. The hypothesis of the normal data distribution verified applying the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Statistical significance between different groups were calculated using the Student t-test (p*) for continuous variables and Whitney-Mann U-test (p**) for variables with abnormal distribution. The data were expressed as the median (interquartile range) Me (Q1; Q3). Expressed data were compared between median and interquartile range Me (Q1; Q3). The results were considered reliable at a level of $p < 0.05$.

3. Results

Lymphocytes and plasma cells provide a specific cellular and humoral response in airways and lungs. Lymphocytes occupy connective tissue of the tracheal and bronchial wall and pulmonary interstitium. They are part of lymphoid nodules (mainly B-lymphocytes) and diffuse lymphoid tissue (mainly T-lymphocytes) **(Fig. 1, *a, b*)**. We have demonstrated CD3 positive lymphocytes predominantly found in the perivascular connective tissue in trachea **(Fig. 1, *b*)** and in the pulmonary interstitium **(Fig. 1, *c, d*).**



a *b* *c* *d*

Fig. 1. Distribution of lymphocytes in guinea pigs' trachea and lungs after OVA-sensitization and challenge on the 36th (1c), 44th (1b, 1d) days of the experiment. 1a – lymphoid nodules in pulmonary interstitium of animals of intact group; 1b – abundant of CD3 positively lymphocytes of diffuse lymphoid tissue of trachea; 1c, 1d – abundant of CD3 positive lymphocytes of diffuse lymphoid tissue in the pulmonary interstitium. Staining: 1a – Haematoxylin and Eosin; 1b, 1c, 1d – immunohistochemical identification of CD3-positive lymphocytes by mAb a-Hu CD3 Antigen, Clone SP7 («DAKO», Denmark). 1a, 1b – × 400.1c, 1d – ×1000

We have not demonstrated statistically reliable differences between the parameters of the number of CD3 positive lymphocytes in trachea and pulmonary interstitium in animals of the intact and control groups ($p^{*/**} > 0.05$). There was a trend to the elevation in the number of CD3-positive lymphocytes in the early stages of the experimental inflammatory process in guinea pigs' trachea and lung. We found that the mean number of CD3-positive cells in trachea in the experimental group I is by 2 times upward than in the control group ($p^{*/**} < 0.05$).

We observed a trend towards an elevation in the mean count of CD3-positive cells in the experimental group II (7.37 ± 1.59 at 5000 μm^2) in comparison with the control group ($p^{*/**} < 0.05$). In the late manifestation of the experimental allergic inflammatory process on the 36th day of observation, the number of CD3-positive cells was 5.75 ± 1.98 at 5000 μm^2 compared to the control group. The maximum mean number of CD3-positive cells was found in the experimental group IV on the 44th day after the start of the experiment (12.01 ± 2.72 at 5000 μm^2) in comparison with the control group ($p^{*/**} < 0.05$) **(Fig. 2)**.

We have also found a statistically reliable elevation ($p^{*/**} < 0.001$) of the number of CD3-positive lymphocytes in pulmonary interstitium from the 30th day of the experiment in animals of

the group II – 28.38±1.82 at 5000 μm^2 with the increasing coefficient 1.8, compared to the same indicators in the control group and previous group **(Fig. 2).**

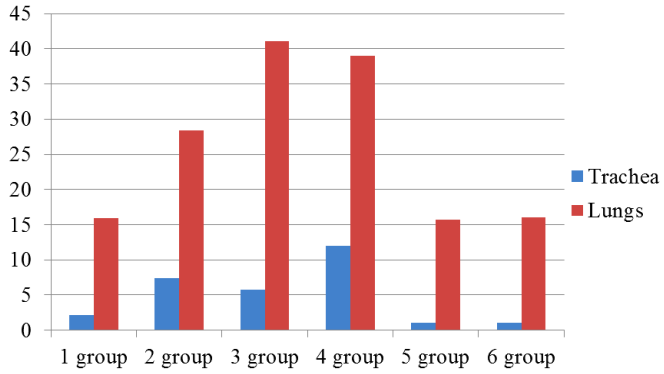


Fig. 2. The mean number of CD3-positive cells in trachea and lung of guinea pigs OVA-sensitized and challenged: * – $p < 0.05$ (Student's t-test); ** – $p < 0.05$ (Whitney-Mann U-test) compared to the control. M±SD, $n = 8$

In the late manifestation of experimental inflammatory process in the guinea pigs' lung a statistically significant rise compared to the control group ($p^{*/**} < 0.001$) and the experimental group II ($p^{*/**} < 0.01$) appeared in the experimental group III on the 36th day of observation – 41.12±2.86 at 5000 μm^2. It was by 2.6 times more than in the control group and by 1.5 times more than in the group II. On the 44th day of observation, there is a tendency for the insubstantial descent of the number of CD3-positive lymphocytes, compared with the previous observation period – 39.0±4.04 at 5000 μm^2, but it is by 2.4 times more than control ($p^{*/**} < 0.001$). It was shown that elevation of the number of CD3 positive lymphocytes persists even after the end of the action of the allergen, indicated the continuation of the reaction of pulmonary local adaptive immunity to the allergen **(Fig. 2).**

4. Discussion

The revealed data of the distribution and number of CD3-positive lymphocytes in the trachea and lungs of sensitized guinea pigs explain the most pronounced manifestations of specific mechanisms of the respiratory system resistance in early and late manifestations of allergic inflammatory process. It consists in elevation in the number and activation of CD3 positive lymphocytes. This tendency of immunohistochemical changes is confirmed in studies by other scientists [20–22]. In the belated stages of development of experimental 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 demonstrated in our study. This fact was confirmed by elevation in the number of CD3-positive lymphocytes in trachea and lung of sensitized guinea pigs and was result of epithelial-immune interaction in guinea pig airways and lung, as also evidenced by other scientists [23, 24].

Meanwhile, only a few mAb to guinea pigs' lymphoid differentiation antigens were inspected and not all of them were appropriated to the CD nomenclature. Even less were verified by immunohistochemical and molecular methods. Only a very small amount of mAb can be applied as markers of T lymphocytes in guinea pigs in comparison with human or mouse cells. Th-1 is not limited to guinea pig T cells and its expression CD2, which was rarely applied as a marker of T cells in human and has not been distinguished in guinea pigs. Previous data have shown that rabbit antisera are against recombinant CD3 can be used for selective staining of T cells, but monoclonal antibodies against CD3 of administered mice could not obtained. So the lung best marker of T lymphocytes was mAb H159 [12]. Nevertheless, our immunohistochemical assessment of guinea pigs' trachea and lung proved the possibility to use anti-human monoclonal antibody CD3 Antigen, Clone SP7 «DAKO», Denmark as a panT-cell marker in guinea pigs.

Thus, our results provide useful information for the understanding of mechanisms of epithelial-immune integration in guinea pig model of experimental ovalbumin-induced allergic inflammatory process. We postulate the necessity to conduct further immunohistochemical analysis of non-specific components (surfactant, club cells, PNECs) and specific (lymphoid structures) factors of lung resistance in experimental ovalbumin-induced allergic inflammation [25].

Study limitations. Our immunohistochemical demonstration of local immune system of OVA-sensitized guinea pigs is limited due to the deficiency of guinea pig-specific reagents and requires further analysis of eventual immunological reagents, available for guinea pigs.

Prospects for further research. We are going to determine the relative level of mRNA of transcriptional regulators of T-cell differentiation T-bet (Th1), Gata 3 (Th2), RORγt (Th17), Foxp3 (T-reg) in guinea pigs' lungs as crucial developmental factors of local and systemic immune response in the early and late phases of experimental inflammatory process.

5. Conclusion

The results of our study may be useful in conditions of the deficiency of guinea pig-specific reagents.

The immunohistochemical assessment of guinea pigs' trachea and lungs proved the possibility to use anti-Human monoclonal antibody CD3 as a panT-cell marker in guinea pigs.

We demonstrated the activation of adaptive immune response (T-cells), represented by their immunohistochemical changes, predominantly in the late stages of allergic inflammatory process. The most statistically significant elevation of the number of CD3 positive cells is observed in experimental group IV by 87.5 % ($p^{*/**} < 0.05$) in trachea and in experimental group III by 75.6 % ($p^{*/**} < 0.001$) in pulmonary interstitium, compared to control animals.

Conflicts of interest

The authors declare there is no conflicts of interest.

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