**ПУЛЬМОНОЛОГИЯ/PULMONOLOGY**

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# CYTOKINE IMBALANCE IN EXPERIMENTAL BACTERIAL PNEUMONIA

Research article

**Kostiushko A.V.[1], Chagina Y.A.[2], Zaretskaia K.B.[3,] *, Priadko Y.A.[4], Ivanova A.Y.[5]**

[1] ORCID : 0000-0001-9401-1023;
[2] ORCID : 0000-0002-9142-4532;
[3] ORCID : 0000-0003-3608-7251;
[4] ORCID : 0009-0002-8749-3743;
[1, 2, 3, 4, 5] Pacific State Medical University, Vladivostok, Russian Federation

* Corresponding author (kseniya2002zar[at]mail.ru)

**Abstract**

The experiment with mice revealed differences in systemic and local cytokine levels, as well as the dynamics of their production, depending on the etiology of pneumonia. Enterobacterial pneumonia was characterized by increased systemic production of interferon-gamma (IFN-γ) and interleukin-10 (IL-10). The level of IFN-γ remained unchanged in Escherichia coli pneumonia, while IL-10 was significantly lower than its levels in the blood serum of intact animals. The local cytokine level in pneumonia caused by Enterobacter spp. were characterized by activation of IL-10 and suppression of IFN-γ production in 2 weeks post-infection. There was an increasing local predominance of IL-10 concentration in mice infected with E. coli. Thus, Enterobacter spp. and E. coli exert a pronounced immunosuppressive effect on the pulmonary defense factors, and it must be considered when selecting methods of pathogenetic therapy for nosocomial pneumonia.

**Keywords:** pneumonia, cytokine, IFN-γ, IL-10, experiment.

# ЦИТОКИНОВЫЙ ДИСБАЛАНС ПРИ БАКТЕРИАЛЬНОЙ ПНЕВМОНИИ В ЭКСПЕРИМЕНТЕ

Научная статья

**Костюшко А.В.[1], Чагина Е.А.[2], Зарецкая К.Б.[3,] *, Прядко Ю.А.[4], Иванова А.Ю.[5]**

[1] ORCID : 0000-0001-9401-1023;
[2] ORCID : 0000-0002-9142-4532;
[3] ORCID : 0000-0003-3608-7251;
[4] ORCID : 0009-0002-8749-3743;
[1, 2, 3, 4, 5] Тихоокеанский государственный медицинский университет, Владивосток, Российская Федерация

* Корреспондирующий автор (kseniya2002zar[at]mail.ru)

**Аннотация**

В эксперименте на мышах установлены различия между уровнями системных и локальных цитокинов и динамикой их выработки в зависимости от этиологии пневмонии. Энтеробактериальная пневмония характеризовалась повышенной системной продукцией γ-интерферона и интерлейкина-10. При пневмонии, вызванной Escherichia coli, уровень интерферона не изменялся, а интерлейкина-10 – был значимо ниже его величин в сыворотке крови интактных животных. Локальный уровень цитокинов при пневмонии, вызванной Enterobacter spp., характеризовался активацией интерлейкина-10 и депрессией выработки γ-интерферона спустя 2 недели после заражения. При заражении мышей E. coli регистрировалось нарастающее локальное преобладание концентрации интерлейкина-10. Таким образом, Enterobacter spp. и E. coli оказывают выраженное иммунодепрессивное влияние на легочные факторы защиты, что необходимо учитывать при выборе методов патогенетической терапии нозокомиальной пневмонии.

**Ключевые слова:** пневмония, цитокин, ИФН-γ, ИЛ-10, эксперимент.

## Introduction

Hospital-acquired infections are one of the most actual problems in global healthcare. Incidences, mortality rates, and treatment costs of these infections continue to escalate, despite the infection control practices and significant advancements in diagnostic and therapeutic technologies [2], [7]. This trend underscores the profound medical and social significance of nosocomial infections. Epidemiological data indicate that 85% of hospitalized patients are colonized by pathogenic microorganisms in intensive care units (ICUs) across various countries, and more than 45% of patients have clinical signs of different infections [9], [12].

Nosocomial pneumonia ranks third in frequency among hospital-acquired infections. However, in the structure of hospital infection-related mortality, it takes the leading position, accounting for 20% of deaths in patients within intensive care units (ICUs) [2], [8]. The pathogenesis of nosocomial pneumonia is closely linked to the patient's immunological profile, while the course of the infectious process in the respiratory tract and immune reactivity are influenced by the causative agent [5]. Nosocomial pneumonia has a bacterial etiology in 90% of cases [4], [10]. Patients with "early pneumonia" without antibiotic treatment have antibiotic-sensitive strains as primary pathogens from the upper respiratory tract microbiota including Streptococcus pneumoniae, Staphylococcus spp., Haemophilus Influenzae, and certain types of Enterobacteriaceae. In contrast, "late" hospital-acquired pneumonia, which develops during or after antibiotic treatment (orantibiotic prophylaxis), is predominantly caused by hospital-acquired strains: members of the Enterobacteriaceae family (Klebsiella pneumoniae and Enterobacter spp.), Staphylococcus aureus, Acinetobacter spp., and Pseudomonas aeruginosa [1], [5], [9], [11]. The immune

response exhibits distinct features when opportunistic microorganisms act as pathogens, including enterobacteria which are among the primary etiological agents of pneumonia. Cytokines play a significant role in the inflammatory response; although their synthesis is nonspecific for various pathogens, they determine the dynamics of the pathological process. Our previous studies have revealed differences in cytokine levels in the bronchoalveolar lavage fluid of patients with nosocomial pneumonia depending on its etiology. These findings suggested that the etiological factor influences the dynamics of the cytokine cascade and the pathogenesis of lung inflammation. However, there are the multifactorial nature of pneumonia and the multifunctionality of the cytokine system, and it is challenging to isolate the direct role of the pathogen in the disease mechanism within the human body. Consequently, only experimental studies yield reliable data on the dynamics of immune inflammation in response to specific pathogens [3], [6], [13].

The aim of this study was to conduct a comparative analysis of the production of interferon-gamma (IFN-γ) and interleukin-10 (IL-10) during the pneumonia progression induced by Enterobacter spp. and E. coli in an experimental model using laboratory animals.

**Research methods and principles**

The study was conducted on 45 white outbred male mice weighing 18–20 grams. The experiment lasted 14 days. The study adhered to the requirements and conditions outlined in the International Guiding Principles for Biomedical Research Involving Animals. The conclusion of the ethical committee for conducting research using laboratory animals has been obtained. The mice once were intranasally infected by strains of Escherichia coli and Enterobacter species isolated from patients with nosocomial pneumonia. A volume of 0.05 ml was administered at a dose corresponding to the $LD_{50}$ ($1\times10^5$ CFU/ml for E. coli and $1\times10^3$ CFU/ml for Enterobacter spp.). Each group, which was removed from the experiment at the stages of the study, including the control group (intact mice), consisted of 5 animals. Cytokine production was determined by measuring their levels in the blood serum and in the lung homogenate supernatant on days 1, 7, and 14 post-infection, using enzyme-linked immunosorbent assay (ELISA) with reagents of R&D diagnostics Inc. The supernatant (liquid over precipitate) of the lung homogenate was obtained by centrifuging the homogenized lung tissue of mice in 2 ml of physiological solution at 3000 g for 10 minutes. Data analysis was performed using the Wilcoxon signed-rank test and the Mann-Whitney U Test in the SPSS 20.0 statistical software package.

**Main results**

The comparison of cytokine levels in the blood serum and in the lung tissue supernatant over time after contamination revealed unidirectional shifts in both experimental groups. It was expressed as lower systemic and local levels of IFN-γ compared to IL-10. However, the level of cytokines in the serum of mice with pneumonia caused by Enterobacter spp. was more than 20 times higher than in the control group throughout the study. The level of IFN-γ in mice with pneumonia caused by E. coli was little different from the control values, and the level of IL-10 was significantly lower than in intact animals.

Thus, the serum level of cytokines significantly increased in mice infected with Enterobacter spp. in 1 day after the start of the experiment: IFN-γ – up to 119.88±15.32 pg/ml (in the control group – 3.14±0.59 pg/ml), IL-10 – up to 257.19±30.03 pg/ml (in the control group – 15.55±0.83 pg/ml). The cytokine level tended to decrease on day 7, and it statistically significant increase on day 14: IFN-γ – up to 218.58±28.09 pg/ml, IL-10 – up to 356.31±46.48 pg/ml. When mice were infected by E. coli, the level of cytokines in the blood serum decreased in 1 day: IFN-γ – down to 2.85±0.93 pg/ml, IL-10 – down to 11.77±1.79 pg/ml. The level of IL-10 remained at the same level, and IFN-γ increased over time. Monitoring of local cytokines showed that the level of IFN-γ in the group of animals infected with Enterobacter spp. doubled in 1 day when compared with the control group (144.45±21.64 vs 74.33±6.7 pg/ml in the control group). It continued to increase by day 7, then dropped sharply by day 14 of the experiment. The level of IL-10 also significantly increased (377.97±70.76 vs 85.03±5.08 pg/ml in the control group) in this group in 1 day after contamination. The level of IL-10 did not change much by day 7, and it decreased by day 14. The level of local and systemic IFN-γ in mice infected with E. coli decreased in 1 day after contamination (to 56.97±4.96 pg/ml). It increased by day 7 and decreased by day 14. The level of IL-10 almost doubled in the supernatant by day 7 (from 103.61±8.12 to 186.08±5.13 pg/ml) and continued to be high by day 14 after contamination.

**Discussion**

The serum levels of opposing cytokines were found significantly lower in experimental pneumonia induced by E. coli than in the supernatant of lung tissue. It indicates the intensity of local inflammation. In contrast, both the supernatant of lung tissue and the serum levels of IFN-γ and IL-10 were significantly elevated in pneumonia caused by Enterobacter spp. It is important to note that the local level of cytokines decreased more than twofold by day 14 post-infection with Enterobacter spp., whereas the concentration of these cytokines increased sharply in the blood serum. The anti-inflammatory IL-10 level significantly increased in the group of mice infected with E. coli.

Differences in the cytokine profile were also observed in the ratio of pro-inflammatory and anti-inflammatory factors using the IFN-γ/IL-10 coefficient. Anti-inflammatory effects predominated in the blood serum of the control group of mice, and the ratio of regulatory cytokines was more balanced in the lung tissue. A significant increase of the regulatory coefficient in the blood serum and its decrease in the supernatants of lung tissue were observed when mice were infected with Enterobacter spp. It indicated the predominance of anti-inflammatory effect in the lung tissue throughout the observation period (Table 1).

Table 1 - Immune regulatory coefficient in experimental pneumonia

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| Experimental parameter | | IFN-γ/IL-10 (pg/ml) | | | |
|---|---|---|---|---|---|
| | | Day 1 | Day 7 | Day 14 | Control group |
| Blood serum | Enterobacter spp. | 0,47±0,09* | 0,45±0,02* | 0,61±0,05* | 0,20±0,01 |
| | E. coli | 0,24±0,03 | 0,37±0,05* | 0,41±0,07* | |
| Lung tissue supernatant | Enterobacter spp. | 0,38±0,01* | 0,42±0,05* | 0,23±0,01* | 0,87±0,03 |
| | E. coli | 0,55±0,01* | 0,38±0,09* | 0,34±0,05* | |

*Note: * The difference with the control group is statistically significant*

**Conclusion**

Thus, monitoring of enterobacterial pneumonia in the experiment made it possible to detect increased production of IFN-γ and IL-10. The level of IFN-γ differed little from the control values in pneumonia caused by E. coli, and the level of IL-10 was significantly lower than its values in the blood serum of intact animals. The differences in the dynamics of local cytokine production depended on the etiology of pneumonia. Early local activation of IL-10 and conspicuous depression of IFN-γ were observed in pneumonia caused by Enterobacter spp. in 2 weeks.

It indicated the development of a deep defect in cell mediated immunity because early and excessive production of anti-inflammatory cytokines changes mechanisms' sequence of anti-infectious defense and aggravates the course of the inflammatory process. An increasing local predominance of IL-10 was detected in mice infected with E. coli without a change in the concentration of IFN-γ over time, which indicated inadequate activation of the immune cellular component. Thus, both Enterobacter spp. and E. coli exert a pronounced immunosuppressive effect on the pulmonary defense factors, and it must be considered when selecting methods of pathogenetic therapy for nosocomial pneumonia.

**Конфликт интересов**

Не указан.

**Рецензия**

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

**Conflict of Interest**

None declared.

**Review**

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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