ФАГОТЕРАПИЯ АНТИБИОТИКОРЕЗИСТЕНТНОЙ ПНЕВМОНИИ: ИММУНОМОДУЛЯЦИЯ ИЛИ ПЕРЕРАСПРЕДЕЛЕНИЕ?

Бочкарёва С.С.1, Федорова И.М.1, Ершова О.Н.2, Котелева С.И.1, Капустин И.В.1, Бляхер М.С.1, Новикова Л.И.1, Алешкин А.В.1, Воробьев А.М.1

1 ФБУН «Московский научно-исследовательский институт эпидемиологии и микробиологии имени Г.Н. Габричевского» Роспотребнадзора, Москва, Россия
2 ФГАУ «Национальный медицинский исследовательский центр нейрохирургии имени академика Н.Н. Бурденко» Министерства здравоохранения РФ, Москва, Россия

Резюме. Наше сообщение касается наблюдений, сделанных в ходе лечения пневмонии индивидуально подобранными бактериофагами у больных с ИСМП, находящихся на ИВЛ.

Обследовано 19 пациентов, находящихся на ИВЛ, состояние которых осложнилось антибиотикоустойчивой пневмонией.

Лечение больных было дополнено фаготерапией, бактериофаги были подобраны индивидуально для каждого больного с учетом микробной этиологии заболевания (Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii).

Иммунофенотипирование лимфоцитов крови проведено с помощью 2-3-параметровой проточной цитометрии. Функциональная активность лейкоцитов крови оценивалась по их способности продуцировать при культивировании IFNα и IFNγ. Уровень продукции интерферонов в собранных после культивирования супернатантах количественно оценивался как по их концентрации (метод ИФА, реагенты ЗАО «Вектор-Бест-Европа»), так и по их биологической активности.

Статистическая обработка результатов проведена с использованием программы Statistica 6 по не-параметрическому U-критерию Манна–Уитни.

В ходе успешной фаготерапии пневмонии индивидуально подобранными бактериофагами в крови пациентов отмечается преодоление лимфопении (в случаях, если она была) и увеличение как количества, так и функциональной активности лимфоцитов периферической крови у всех больных.

Зависимость между микробной нагрузкой (моно- или микст-инфекция, количество КОЕ возбудителей пневмонии, потребность в повторных курсах фаготерапии) и степенью дефицита в тех или иных субпопуляциях лимфоцитов не была выявлена.

Достигнутая после одного курса фаготерапии активация иммунной системы сохранялась по крайней мере в течение 3 недель после прекращения введения фагов.

Ключевые слова: фаготерапия, влияние на иммунную систему, активированные T-лимфоциты, NK-клетки, IFNγ, IFNα, антибиотикорезистентная пневмония

Адрес для переписки:
Федорова Ирина Михайловна
ФБУН «Московский научно-исследовательский институт эпидемиологии и микробиологии имени Г.Н. Габричевского» Роспотребнадзор
125212, Россия, Москва, ул. Адмирала Макарова, 10.
Тел.: 8 (903) 107-60-67.
Факс: 8 (495) 452-18-30.
E-mail: vestnik-07@mail.ru

Address for correspondence:
Fedorova Irina M.
G.N. Gabrichevsky Research Institute for Epidemiology and Microbiology
125212, Russian Federation, Moscow,
Admiral Makarov str., 10.
Phone: 7 (903) 107-60-67.
Fax: 7 (495) 452-18-30.
E-mail: vestnik-07@mail.ru

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PHAGE THERAPY IN ANTIBIOTIC RESISTANT PNEUMONIA: IMMUNOMODULATION OR REDISTRIBUTION?

Bochkareva S.S.\textsuperscript{a}, Fedorova I.M.\textsuperscript{a}, Ershova O.N.\textsuperscript{b}, Koteleva S.I.\textsuperscript{a}, Kapustin I.V.\textsuperscript{a}, Blyakher M.S.\textsuperscript{a}, Novikova L.I.\textsuperscript{a}, Aleshkin A.V.\textsuperscript{a},
Vorobiev A.M.\textsuperscript{a}

\textsuperscript{a} G.N. Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russian Federation
\textsuperscript{b} N. Burdenko National Medical Research Center for Neurosurgery, Moscow, Russian Federation

Abstract. Our report concerns the observations made during the treatment of pneumonia with individually selected bacteriophages in HCAI patients on mechanical ventilation.

19 patients on mechanical ventilation whose condition was complicated by antibiotic-resistant pneumonia were examined.

The treatment of patients was supplemented with phage therapy, bacteriophages were selected individually for each patient, taking into account the microbial etiology of the disease (\textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumoniae}, \textit{Acinetobacter baumanii}).

Immunophenotyping of blood lymphocytes was carried out using 2-3-parameter flow cytometry. The functional activity of blood leukocytes was assessed by their ability to produce IFN\(\alpha\) and IFN\(\gamma\) during cultivation.

The level of interferons production in supernatants collected after cultivation was quantitatively evaluated both by their concentration (ELISA, reagents from “Vector-Best-Europe”, Russia) and by their biological activity.

Statistical processing of the results was carried out using the Statistica 6 program according to the nonparametric Mann-Whitney U-test.

In the course of successful phage therapy with individually selected bacteriophages overcoming of lymphopenia (if there was one) and an increase in both the number and functional activity of peripheral blood lymphocytes in all patients with pneumonia observed are noted.

The relationship between the microbial load (mono- or mixed infection, the number of CFU pathogens of pneumonia, the need for repeated courses of phage therapy) and the degree of deficiency in one or another subpopulation of lymphocytes was not detected.

Activation of the immune system achieved after one course of phage therapy was maintained for at least 3 weeks after phage administration was discontinued.

Keywords: phage therapy, effects on the immune system, activated T lymphocytes, NK cells, IFN\(\gamma\), IFN\(\alpha\), antibiotic-resistant pneumonia

Introduction

The development of pneumonia as a complication caused by health care associated infections (HCAI) (the earlier term is “nosocomial infections”) in patients who have been on artificial lung ventilation (ALV) for a long time (mechanical ventilation) is a significant problem, especially in cases of antibiotic resistance of pathogenic microflora.

The list of HCAI pathogens includes representatives of various taxonomic groups of bacteria, viruses, protozoa and fungi. More than 90\% of all nosocomial infections are of bacterial origin and, moreover, HCAI are usually caused by hospital strains of microorganisms. The incidence rate of HCAI in resuscitation departments and intensive care units is 5-10 times higher than in patients of other departments. HCAI pathogens are mostly antibiotic resistant and frequently there are multidrug resistant microorganisms [1, 14]. ALV-associated pneumonia develops in 5\% of intensive care unit patients.

The use of bacteriophages is a modern approach to the treatment of these types of complications caused by HCAI. [2]. In addition to the known specific lytic effect of phages against the corresponding target bacteria, there is literature evidence of other effects of phages in the human body, the immunomodulating effect in particular [5, 9, 10]. At the same time, the effect of phage therapy on the immune system is not well studied.

Our report concerns the observations made during the treatment of pneumonia with individually selected bacteriophages in HCAI patients on mechanical ventilation.

Materials and methods

We examined 19 patients on mechanical ventilation whose condition was complicated by antibiotic-resistant pneumonia.

The treatment of patients was supplemented with phage therapy, bacteriophages were selected individually for each patient, taking into account the microbial etiology of the disease (\textit{Pseudomonas aeruginosa}, \textit{Klebsiella pneumoniae}, \textit{Acinetobacter baumanii}).
**Results and discussion**

Before the start of phage therapy, half of the examined patients showed lymphopenia, a reduced number of cytolytic T lymphocytes (CTL), and NK cells. A decrease in the number of CTL in most patients was combined with an increase in the percentage of activated cells among them (CD3+CD8+CD38+). The percentage of activated T cells CD3+CD8+CD38+ was increased initially in patients of both groups, and the proportion of CD3+HLA-DR+ in group 2 was slightly lower than in group 1 (6.9 versus 10.9%), and after one course of phage therapy its increase was significant (15.0%, p = 0.023).

The initial level of IFNγ production in group 2 was also lower than in group 1 (1849 versus 4130 pg/ml), and after one course of phage therapy it increased significantly (7688 pg/ml, p = 0.047), whereas in group 1 the ability of leukocytes to produce IFNγ increased to a lesser extent (from 4130 to 5253 pg/ml).

To statistically evaluate the results obtained and at the same time take into account interindividual variability, the change in each parameter was analyzed by us not only in units of laboratory analysis, but also as a percentage of the initial state.

The state of the immune system in both groups was similar: the proportion of patients with lymphopenia and cell deficiency in individual subpopulations of lymphocytes was almost the same. However, some features can be noted.

The decrease in the number of T helpers and CTL was accompanied by a decrease in their ability to produce IFNα and IFNγ during cultivation (stimulants are Newcastle disease virus and PHA, respectively; doses and stimulation regimen were used in accordance with [6]). The level of interferons production in supernatants collected after cultivation was quantitatively evaluated both by their concentration (ELISA, reagents from “Vector-Best-Europe”, Russia) and by their biological activity, in accordance with [6]. The biological activity of interferons was expressed in U/ml which corresponded to the 1/titer of the abolishment of the cytopathic effect of the virus on the human embryo lung fibroblasts after their incubation with supernatants of stimulated blood cell culture. Statistical processing of the results was carried out using the Statistica 6 program according to the nonparametric Mann–Whitney U-test.

The percentage of activated T cells CD3+CD8+CD38+ was increased initially in patients of both groups, and the proportion of CD3+HLA-DR+ in group 2 was slightly lower than in group 1 (6.9 versus 10.9%), and after one course of phage therapy its increase was significant (15.0%, p = 0.023).

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Table 2 in this aspect presents the dynamics of individual indicators for a period of 3 weeks after the start of phage therapy. From 19 patients, 11 were observed by us for 3 or more weeks, including 5 people from group 1 and 6 people from group 2.

Table 2 demonstrates that immediately after the completion of one course of phage therapy, the number of lymphocytes in such subpopulations as T helpers and NK cells significantly increases. Considering whether there was a deficiency of these cells in each patient before the start of phage therapy and its level, the magnification rate was different, but on average it was 1.5 for T helpers and 1.5-2 times for NK cells.

Functional activity of lymphocytes was also registered at a higher level. The percentage of CD3+HLA-DR+ increased immediately after 1 course of phage therapy and remained at this and higher level for 3 weeks of observation. The percentage of CD3+CD8+CD38+ increased to a lesser extent, but this parameter was already initially increased in patients compared to parameters of healthy people (see Table 1).

The ability of blood lymphocytes to produce IFNγ also increased after phage therapy and continued to
increase or remained at the achieved level through- out the observation period, and the change in IFNα production did not occur in all patients and was not statistically significant.

Although the stimulating effect of bacteriophages on the immune system, shown in experiments in vivo and in vitro is known from the literature [4, 8, 10, 15], the changes that we observed in the immune status of patients after phage therapy would probably be more correctly regarded as not immunostimulation, but redistribution of lymphocytes between individual sites of the immune system.

It is believed that immune protection in the lungs is provided by both non-recirculating cells of innate immunity [3] and T cells. The role of memory T cells is to rapidly deploy a specific immune response, acti-

### TABLE 1. CHANGE IN THE IMMUNE SYSTEM STATE OF PATIENTS AT THE END OF ONE COURSE OF PHAGE THERAPY

| Indicator | Before phage therapy | After 5-10 days | Normal limits |
|-----------|----------------------|-----------------|---------------|
| Lymphocytes (10³/ml) | 1407 | 1698 | 1500-2800 |
| T cells (CD3⁺, 10³/ml) | 1075 | 1276 | 1100-2000 |
| T helpers (CD3⁺CD4⁺, 10³/ml) | 673 | 762 | 750-1200 |
| CTL (CD3⁺CD8⁺, 10³/ml) | 370 | 470 | 300-700 |
| CD4⁺/CD8⁺ | 2.12 | 1.83 | 1.6-3.00 |
| B cells (CD3⁺CD19⁺, 10³/ml) | 138 | 159 | 100-500 |
| NK cells (CD3⁺CD16⁺CD56⁺, 10³/ml) | 161 | 227 | 150-550 |

**Activated T cells**
- CD3⁺CD69⁺ (%) | 3.2 | 3.3 | < 10
- CD3⁺HLA-DR⁺ (%) | 8.7 | 15.5⁺ | p = 0.03 | < 10
- CD3⁺CD8⁺/CD38⁺ (%) | 15.9 | 27.1⁺ | p = 0.04 | < 10

**Concentration in serum**
- IgG, mg/ml | 9.3 | – | 7.6-18.9
- IgM, mg/ml | 1.2 | – | 0.5-3.4
- IgA, mg/ml | 2.4 | – | 0.8-3.5

**IFNγ production**
- concentration, pg/ml | 2990 | 6785⁺ | p = 0.04 | 2000-25000
- biol. activity, U/ml | 15 | 24 | 32-128

**IFNα production**
- concentration, pg/ml | 231 | 260 | 100-500
- biol. activity, U/ml | 110 | 130 | 160-640

**Note.**, significant difference from the level before phage therapy.

### TABLE 2. DYNAMICS OF THE IMMUNE SYSTEM STATE OF PATIENTS WITHIN 3 WEEKS AFTER PHAGE THERAPY

| Indicator | Average value of changes in % of the initial state |
|-----------|---------------------------------------------|
|           | just after | in 2 weeks | in 3 weeks |
| NK cells (CD3⁺CD16⁺CD56⁺) | 188⁺ | 206⁺ | 135 |
|           | p = 0.01 | p = 0.02 |  |
| T helpers (CD3⁺CD4⁺) | 149⁺ | 142 | 126⁺ |
|           | p = 0.03 | p = 0.01 |  |
| Activated T cells (CD3⁺HLA-DR⁺) | 157⁺ | 227⁺ | 211⁺ |
|           | p = 0.04 | p = 0.01 | p = 0.03 |
| Activated T cells (CD3⁺CD8⁺CD38⁺) | 110 | 120 | 180 |
| IFNγ (biol. activity) | 173⁺ | 246⁺ | 180⁺ |
|           | p = 0.04 | p = 0.03 |  |
| IFNα (biol. activity) | 108⁺ | 107 | 98⁺ |
|           | p = 0.03 |  |  |

**Note.** As for Table 1.
vate the resident elements of the immune system, and attract circulating immune cells to the lungs [7]. It was shown that the outcome of a pulmonary infection associated with *Pseudomonas aeruginosa* depends on the number of T helpers and the polarization of the immune response at their level [12], and NK cells are the main producers of IFNγ in the lungs and are rapidly activated for this purpose (within 1 day [11]).

It is possible that the decrease in antigenic load in the respiratory tract, achieved immediately with successful phage therapy, reduces the need for these cellular elements, and an additional number of lymphocytes, including activated ones, appear in the peripheral blood. The question of whether the state of the immune system after 3 weeks or more is associated with the immunomodulating effect of the bacteriophage remains open.

## Conclusion

Thus, in the course of successful phage therapy with individually selected bacteriophages overcoming of lymphopenia (if there was one) and an increase in both the number and functional activity of peripheral blood lymphocytes in all patients with pneumonia. The relationship between the microbial load (mono- or mixed infection, the number of CFU pathogens of pneumonia, the need for repeated courses of phage therapy) and the degree of deficiency in one or another subpopulation of lymphocytes was not detected.

Activation of the immune system achieved after one course of phage therapy was maintained for at least 3 weeks after phage administration was discontinued.

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