ПЛЕОТРОПНЫЕ ИММУНОМОДУЛИРУЮЩИЕ ЭФФЕКТЫ ПЕПТИДА ARGINYL-ALPHA-ASPARTYL-LYSYL-VALYL-TYROSYL-ARGININE НА РАЗЛИЧНЫЕ СУБПОПУЛЯЦИИ НЕЙТРОФИЛЬНЫХ ГРАНУЛОЦИТОВ И ИХ ФЕНОТИП У ПАЦИЕНТОВ С COVID-19 В ЭКСПЕРИМЕНТАЛЬНОЙ СИСТЕМЕ *IN VITRO*

Городин В.Н., Матушкина В.А., Чапурина В.Н., Меняйло А.И., Ковалева С.В., Дыдышко Е.И.

ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ, г. Краснодар, Россия

Резюме. Ключевая роль нейтрофильных гранулоцитов (НГ) в патогенезе COVID-19 делает их новыми мишенями для таргетных терапевтических подходов с возможностью влияния на течение и исход болезни, восстановление негативных изменений фенотипа и функций НГ. Наиболее перспективными в лечении COVID-19 являются синтетические пептиды или полипептидные комплексы с физиологическим механизмом действия. Цель – выявить эффекты влияния гексапептида (ГП) – Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine на фенотип функционально-значимых субпопуляций НГ при среднетяжелой форме COVID-19.

Обследованы пациенты 61 (57-71) года (n = 45) в остром периоде COVID-19 – группа исследования 1 (ГИ1). Кровь пациентов ГИ1 инкубировали *in vitro* с ГП (10^6 г/л, 60 мин, Т 37 °С) – группа исследования 2 (ГИ2). Оценивали количество НГ субпопуляций $CD16^+IFN\alpha/\beta R1^+CD119^+$, $CD16^+IFN\alpha/\beta R1^+CD119^-$, $CD16^+IFN\alpha/\beta R1^+CD119^+$, $CD64^-CD16^+CD32^+CD11b^+$, $CD64^+CD16^+CD32^+CD11b^+$ и фенотип по интенсивности флюоресценции (MFI) (FC 500, Beckman Coulter, США); фагоцитарную активность НГ до и после инкубации с ГП. Группа сравнения (ГС) – 22 добровольца 58 (57-70) лет, обследованных в доковидный период.

Выявлены однонаправленные эффекты ГП *in vitro*, способствующие восстановлению фенотипа субпопуляций $CD16^+IFN\alpha/\beta R1^-CD119^+$НГ и $CD16^+IFN\alpha/\beta R1^+CD119^-$НГ до показателей ГС. Показано снижение MFI CD16 ($p < 0,05$) в обеих субпопуляциях; MFI CD119 ($p < 0,05$) в субпопуляции $CD16^+IFN\alpha/\beta R1^-CD119^+$НГ и MFI IFNα/βR1 рецепторов в субпопуляции $CD16^+IFN\alpha/\beta R1^+CD119^-$НГ. Эффекты влияния ГП на фенотип субпопуляций $CD16^+IFN\alpha/\beta R1^+CD119^+$НГ в 76% случаев прояв-

Адрес для переписки:

Матушкина Валерия Александровна
ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ
350061, Россия, г. Краснодар, ул. Дмитрия Благоева, 4.
Тел.: 8 (918) 410-22-14.
E-mail: lm.mva@yandex.ru

Address for correspondence:

Valeriya A. Matushkina
Kuban State Medical University
4 Dmitry Blagoev St
Krasnodar
350061 Russian Federation
Phone: +7 (918) 410-22-14.
E-mail: lm.mva@yandex.ru

Образец цитирования:

В.Н. Городин, В.А. Матушкина, В.Н. Чапурина, А.И. Меняйло, С.В. Ковалева, Е.И. Дыдышко «Плеотропные иммуномодулирующие эффекты пептида Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine на различные субпопуляции нейтрофильных гранулоцитов и их фенотип у пациентов с COVID-19 в экспериментальной системе in vitro» // Медицинская иммунология, 2023. Т. 25, № 4. С. 815-822.
doi: 10.15789/1563-0625-PIE-2755

For citation:

V.N. Gorodin, V.A. Matushkina, V.N. Chapurina, A.I. Menyailo, S.V. Kovaleva, E.I. Dydyshko "Pleiotropic immunomodulating effects of peptide Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine on various subsets of neutrophilic granulocytes and their phenotype in patients with COVID-19 in vitro", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 4, pp. 815-822.
doi: 10.15789/1563-0625-PIE-2755

DOI: 10.15789/1563-0625-PIE-2755

лялись снижением MFI CD16 ($p < 0,05$) и повышением MFI IFNα/βR1 и CD119 ($p_{1,2} < 0,05$), а в 24% случаев уменьшением MFI IFNα/βR1 ($p < 0,05$). Под влиянием ГП *in vitro* установлено ремоделирование фенотипов субпопуляций НГ $CD64^-CD16^+CD32^+CD11b^+$ и $CD64^+CD16^+CD32^+CD11b^+$, отвечающих за эффекторные функции, от гиперактивированных до нормальных. Изменялся фенотип НГ в субпопуляции $CD64^-CD16^+CD32^+CD11b^+$ – отмечалось снижение MFI CD16 и CD11b до показателей ГС ($p_{1,2} < 0,05$). Наблюдалась уменьшение количества $CD64^+CD16^+CD32^+CD11b^+$НГ, со сниженным MFI CD16 ($p_{1,2} > 0,05$). Восстановление фенотипа НГ, трансформированного при COVID-19, под влиянием ГП приводило и к нормализации фагоцитарной функции.

Положительные эффекты влияния ГП *in vitro* на фенотипы субпопуляций, участвующих в противовирусной защите, и функции НГ при COVID-19 открывают перспективы для создания новых методов иммунотерапии с включением гексапептида для восстановления дисфункций НГ.

Ключевые слова: COVID-19, нейтрофильные гранулоциты, субпопуляции, фенотип, иммуномодуляция, гексапептид

PLEIOTROPIC IMMUNOMODULATING EFFECTS OF PEPTIDE ARGINYL-ALPHA-ASPARTYL-LYSYL-VALYL-TYROSYL-ARGININE ON VARIOUS SUBSETS OF NEUTROPHILIC GRANULOCYTES AND THEIR PHENOTYPE IN PATIENTS WITH COVID-19 *IN VITRO*

Gorodin V.N., Matushkina V.A., Chapurina V.N., Menyailo A.I., Kovaleva S.V., Dydyshko E.I.

Kuban State Medical University, Krasnodar, Russian Federation

Abstract. The key role of neutrophilic granulocytes (NG) in the pathogenesis of COVID-19 makes them new targets for therapeutic approaches and of influencing the course and outcome of the disease, restoring changes in the phenotype and functions of NG. Synthetic peptides or polypeptide complexes of action are the most promising in the treatment of COVID-19. Aim: to reveal the effects of the influence of the hexapeptide (HP) – Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine on the phenotype of functionally significant NG subsets in moderate COVID-19.

The study examined patients 61 (57-71) years old ($n = 45$) in the acute period of COVID-19 – study group1 (SG1). *In vitro*, samples SG1 were incubated with HP (10^6 g/L, 60 min, 37 °C) – study group2 (SG2). The number of NG subsets was evaluated: $CD16^+IFN\alpha/\beta R1^+CD119^+$, $CD16^+IFN\alpha/\beta R1^+CD119^-$, $CD16^+IFN\alpha/\beta R1^+CD119^+$, $CD64^-CD16^+CD32^+CD11b^+$, $CD64^+CD16^+CD32^+CD11b^+$ and phenotype by membrane receptor expression density (MFI) (FC 500, Beckman Coulter, USA); NG phagocytic activity was tested before and after incubation with HP. The comparison group (GS) – of 22 volunteers examined in the pre-COVID period.

It was revealed that unidirectional effects of HP *in vitro* contributing to the restoration of the phenotype of subsets $CD16^+IFN\alpha/\beta R1^-CD119^+$, $CD16^+IFN\alpha/\beta R1^+CD119^-$ to CG indicators. There was a decrease in MFI CD16 ($p < 0.05$) in both subsets; MFI CD119 ($p < 0.05$) in the $CD16^+IFN\alpha/\beta R1^-CD119^+$NG subset, MFI IFNa/βR1 in the $CD16^+IFN\alpha/\beta R1^+CD119^-$NG subset. The effects of HP on the phenotype of $CD16^+IFN\alpha/\beta R1^+CD119^+$NG subsets in 76% of cases were manifested by a decrease in MFI CD16 ($p < 0.05$), an increase in MFI IFNα/βR1 and CD119 ($p_{1,2} < 0.05$), and in 24% of cases a decrease in MFI IFNα/βR1 ($p < 0.05$). HP *in vitro* remodeling of the phenotypes subsets $CD64^-CD16^+CD32^+CD11b^+$ and $CD64^+CD16^+CD32^+CD11b^+$ were established, providing the usefulness of effector functions from hyperactivated to normal. In the $CD64^-CD16^+CD32^+CD11b^+$ subset, there was a decrease in MFI CD16 and CD11b to the indicators CG ($p_{1,2} < 0.05$). Recovery of the NG phenotype under the influence of HP led to the restoration of the phagocytic function of NG.

Positive effects of HP *in vitro* on the phenotypes of subsets actively and NG functions in COVID-19 open up prospects for the creation of new methods of immunotherapy to restore NG dysfunctions.

Keywords: COVID-19, neutrophilic granulocytes, subsets, phenotype, immunomodulation, hexapeptide

Introduction

The ongoing COVID-19 pandemic, the emergence of new strains of SARS-CoV-2 and the lack of a specific treatment for COVID-19 are drawing attention to the search for effective immunotherapy due to the possibility of increasing immunity to the virus and reducing hyperinflammation [4]. It has been shown that the immunological phenotype of COVID-19 is characterized by an increased number of neutrophilic granulocytes (NG) and depletion of lymphocytes, the ratio of NG to lymphocytes correlates with the severity of the disease and respiratory symptoms, being a predictor of an unfavorable outcome [7, 10]. It was found that the pathophysiology of severe COVID-19 is characterized by changes in the number, phenotype and functionality of NG [6]. It is known that NG secrete cytokines and chemokines that contribute to the maintenance of inflammation in COVID-19, while ROS and NETosis are involved in tissue damage [6, 10]. Convincing evidence about the role of NG in the pathogenesis of COVID-19 make them the new targets for targeted therapeutic approaches with the possibility of influencing the development, course and outcome of the disease, restoring negative changes in the NG phenotype.

The most promising for research and use in the treatment of COVID-19 are short synthetic peptides or polypeptide complexes isolated from animal organs and tissues: inhibitors of the SARS-CoV-2 protein, immunomodulators and broncho protectors with a physiological mechanism of action [3]. It has been shown that immunomodulatory peptides contribute to the normalization of innate and adaptive immunity, the hemostasis system and cytokine synthesis and have an anti-inflammatory effect, thereby preventing the development of distress syndrome and multiple organ failure [3].

Thymopentin is an immunomodulatory pentapeptide (Arg-Lys-Asp-Val-Tyr, RKDVY, TP5), which is the active center of the thymus hormone thymopoietin [3], which is previously used to normalize immunological parameters in tumor, immunodeficiency and autoimmune diseases [11], also was used to treat COVID-19 in China. It has been shown that TP5 *in vitro* affects the functions of T cells and monocytes by activating intracellular signaling cascades [1]. The ability of TP5 to normalize the functions of the immune system (IS) in viral diseases also has been established [1]. In the context of SARS-CoV-2, timopentin is considered to be a 3-chymotrypsin-like protease (3CLpro) inhibitor [13].

In Russia, a medicinal product No. P N000106/04 is registered, the main active substance is Hexapeptide (HP) – Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine, a synthetic analogue of the active center of the thymus hormone – thymopoietin, which has all the biological activities of the native thymus hormone [9, 13].

According to the scientific data, Hexapeptide has an immunoregulatory effect on a defectively functioning immune system, regulating and restoring the T cell link, the number and functional activity of neutrophilic granulocytes, monocytes, normalizing the synthesis of cytokines; also are described effects of hepatoprotective, antioxidant properties, the ability to enhance the effectiveness of antibiotic therapy, inhibit the multidrug resistance of the body [5, 12], while its effect on neutrophilic granulocytes in COVID-19 remains unexplored.

The particular scientific interest, from our point of view, is the simultaneous assessment of NG subsets that are responsible for triggering and regulating antiviral immunity and subsets that provide effector phagocytic and microbicidal properties of NG.

The surface receptors for IFN I and II types: IFNα/β (IFNAR1) and CD119 (IFNγ – IFNGR) were chosen for the study, through which innate antiviral responses are regulated [12]. The receptors which are responsible for the effector functions of NG were also studied: CD64 (FcγRI) is a high-affinity cytoactivating receptor, its cross-linking induces phagocytosis, release of inflammatory mediators, participates in antibody-dependent cell-mediated cytotoxicity and antigen presentation; CD32 (FcγRII) is a low-affinity receptor takes part in activating the processes of phagocytosis and degranulation; CD16 (FcγRIII) is a low-affinity receptor for degranulation, oxidative burst, phagocytosis activation; CD11b (CR3, Mac-1, integrin) regulates adhesion and migration, participates in cell-mediated cytotoxicity, phagocytosis.

Aim: to evaluate the various effects of the influence of the Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine peptide on the phenotype of 5 functionally significant NG subsets in moderate course of COVID-19 *in vitro*.

Materials and methods

A study was made of peripheral blood samples of patients with COVID-19, in the acute period of the disease (7-9 days of the disease), on the 1st day of hospitalization at the Specialized Clinical Infectious Diseases Hospital of the Ministry of Health of the Krasnodar Territory. Study group 1 (SG1) included 45 patients, aged 61 (57-71) years, 58% men, 42% women with a moderate form of the disease, in accordance with clinical and laboratory severity

criteria (World Health Organization guidelines version 10 (02/08/2021)).

In vitro system, patient samples (SG1) were incubated with Hexapeptide (HP) (10^6g/L), 60 min, at 37 °C – study group 2 (SG2).

The number of NG, % of subsets, was estimated: $CD16^+IFN\alpha/\beta R1^+CD119^+$, $CD16^+IFN\alpha/\beta R1^+CD119^-$, $CD16^+IFN\alpha/\beta R1^+CD119^+$, $CD64^-CD16^+CD32^+CD11b^+$, $CD64^+CD16^+CD32^+CD11b^+$ and phenotype according to the expression density of membrane receptors (MFI) (FC 500, Beckman Coulter, USA); monoclonal antibodies (Mab): IFNαβR1-FITC, CD119-PE, CD16-ECD, CD64-FITC, CD32-PE, CD11b-PC5 (Beckman Coulter International S.A., France); phagocytic activity of NG in relation to S. aureus. The parameters were tested before and after the incubation with HP. The comparison group (CG) was formed from the indicators of 22 volunteers 58 (57-70) years old, examined in the pre-COVID period.

The data were analyzed using the StatSoft Statistica 10.0 program. After checking the normal distribution by the Kolmogorov–Smirnov method, they are presented as a median (Me) and a quartile interval ($Q_{0.25}$-$Q_{0.75}$). To compare groups, nonparametric criteria was used: Mann–Whitney U test. The difference in indicators was considered statistically significant at $p < 0.05$.

Results and discussion

It was established that 3 subsets of NG expressing receptors for IFN I and II types circulate in the peripheral blood of CG volunteers: $CD16^+IFN\alpha/\beta R1^-CD119^+$, $CD16^+IFN\alpha/\beta R1^+CD119^-$, $CD16^+IFN\alpha/\beta R1^+CD119^+$. It is shown that NG subset $CD16^+IFN\alpha/\beta R1^-CD119^+$ make up 93.7 (89.8-96.5) % with MFI expression density of CD16 – 39.8 (20.4-51.3) and $CD119^+$ (IFNγ) – 2.8 (2.5-3.1), which determines the $CD16^{mid}IFN\alpha/\beta R1^-CD119^{dim}$ phenotype. The subset of NG $CD16^+IFN\alpha/\beta R1^+CD119^-$, not expressing the IFNγ receptor, was 1.4 (0.5-2.3) % with an expression density according to MFI IFNα/βR1 of 3.4 (2.6-4.1) and MFI CD16 – 39.9 (22.9-54.5) with $CD16^{mid}IFN\alpha/\beta R1^{dim}CD119^-$ phenotype. The $CD16^+IFN\alpha/\beta R1^+CD119^+$ NG subset simultaneously expressing IFNα/β and IFNγ receptors accounted for only 0.9 (0.4-1.8) % of NG, but had higher MFI values of IFNα/βR1 ($p > 0.05$) and MFI CD119 ($p > 0.05$), which was reflected by the $CD16^{mid}IFN\alpha/\beta R1^{mid}CD119^{mid}$ (Table 1).

In SG1 with a moderate form of COVID-19, in 76% of cases (SG1a, n = 36), the content of NG in the studied subsets did not differ significantly from CG ($p > 0.05$), but a phenotype type changes was observed. There was 2 times increasing in MFI CD16 in all subsets ($p_{1\text{-}3} < 0.05$), MFI CD119 was 1.3 times increasing ($p < 0.05$) in the subset $CD16^+IFN\alpha/\beta R1^-CD119^+NG$, MFI IFNα/βR1 was 1.8 times increasing in the $CD16^+IFN\alpha/\beta R1^+CD119^-NG$ ($p < 0.05$) and 1.6 times increasing in the $CD16^+IFN\alpha/\beta R1^+CD119^+NG$ ($p < 0.05$) (Table 1). The identified changes are characterized by the phenotypes $CD16^{bright}IFN\alpha/\beta R1^-CD119^{mid}NG$, $CD16^{bright}IFN\alpha/\beta R1^{mid}CD119^-NG$, $CD16^{bright}IFN\alpha/\beta R1^{bright}CD119^{mid}NG$. At the same time, in 24% of cases in SG1b (n = 9) there was a 63 times increasing in the content of subset of neutrophilic granulocytes in the $CD16^+IFN\alpha/\beta R1^+CD119^-$ subset up to 88.95 (81.73-92.65) % versus 1.4 (0.53-2.35) % in CG and 1.2 (0.6-1.9) % in SG1a ($p_{1,2} < 0.05$) and 15 times reduction in NG subset in the $CD16^+IFN\alpha/\beta R1^+CD119^-$ with phenotype transformation in the $CD16^{bright}IFN\alpha/\beta R1^-CD119^{mid}NG$ subset, similar to that observed in SG1a, and $CD16^{bright}IFN\alpha/\beta R1^{bright}CD119^-NG$ with a 3 times ($p < 0.05$) increasing in MFI IFNα/βR1 (Table 1).

Innate antiviral responses are largely controlled by type I IFNs signaling through IFNAR. Elevated expression levels of IFN receptors demonstrate the readiness of NG to perceive cytokine signals and respond to them. However, given that type I IFNs enhance NETosis [2], it is possible to suggest that the significant increase in receptor expression noted in SG1b may exacerbate neutrophil infiltration and netosis.

In vitro incubation of NG of study group with COVID-19 with HP revealed unidirectional modulating effects that contributed to the restoration of the phenotype of 2 subsets: $CD16^{mid}IFN\alpha/\beta R1^-CD119^{dim}NG$, $CD16^{mid}IFN\alpha/\beta R1^{dim}CD119^-NG$. A decrease in both SG2a and SG2b expression density by MFI CD16 ($p_{1,2} < 0.05$) was shown in both subsets; MFI CD119 ($p_{1,2} < 0.05$) in subset $CD16^+IFN\alpha/\beta R1^-CD119^+NG$ and MFI IFNα/βR1 receptors in subset $CD16^+IFN\alpha/\beta R1^+CD119^-NG$ to comparison group values (CG) ($p_{1,2} > 0.05$) (Table 1). The effects of HP in SG2a on the phenotype of $CD16^+IFN\alpha/\beta R1^+CD119^+NG$ subsets are consisted in a decrease in MFI CD16 ($p < 0.05$) and an increase in MFI IFNα/βR1 and MFI CD119 ($p_{1,2} < 0.05$) – $CD16^{mid}IFN\alpha/\beta R1^{bright}CD119^{bright}$. At the same time, SG2b is showed a decrease in MFI IFNα/βR1 ($p < 0.05$), an increase in MFI CD119 ($p > 0.05$), and the density of CD16 expression did not differ from the values registered in SG1a in patients with COVID-19 ($p > 0.05$) – $CD16^{bright}IFN\alpha/\beta R1^{dim}CD119^{mid}$ (Table 1).

TABLE 1. EFFECTS OF HEXAPEPTIDE INFLUENCE ON THE CONTENT AND PHENOTYPE OF SUBSETS OF NEUTROPHILIC GRANULOCYTES EXPRESSING RECEPTORS FOR IFN TYPES I AND II IN PATIENTS WITH MODERATE COVID-19, Me ($Q_{0.25}$-$Q_{0.75}$)

Indicators	Comparison group (CG)	Moderate form of COVID-19			
		Study group 1 (SG1) before incubation with HP		Study group 2 (SG2) after incubation with HP	
		SG1a, n = 36	SG1b, n = 9	SG2a, n = 36	SG2b, n = 9
$CD16^+IFN\alpha/\beta R1^-CD119^+NG$					
NG,%	93.7 (89.8-96.5)	95.6 (92.0-98.2)	15.8* ^ (14.8-16.5)	94.9 (93.0-97.0)	16.8* ♦ (16.6-17.0)
MFI CD16	39.8 (20.4-51.3)	79.0* (59.2-91.4)	67.5* (53.2-79.7)	24.8# (20.9-32.1)	13.7♦ (8.3-17.4)
MFI CD119	2.8 (2.5-3.1)	3.6* (3.3-4.3)	3.4* (3.2-3.6)	2.0# (2.3-4.2)	6.6* ♦ (5.9-7.4)
$CD16^+$ $IFN\alpha/\beta R1^+CD119^-NG$					
NG,%	1.4 (0.5-2.4)	1.2 (0.6-1.9)	88.9* ^ (81.7-92.7)	2.2 (1.4-4.0)	82.1* ♦ (32.4-90.1)
MFI CD16	39.9 (22.9-54.5)	74.8* (59.6-109.0)	66.5* (55.9-79.6)	24.8# (20.2-32.1)	68.7* (52.5-73.8)
MFI IFNα/β	3.4 (2.6-4.1)	6.1* (5.0-18.1)	11.22* (7.7-16.1)	3.7^ (2.6-5.0)	3.8 (2.3-5.1)
$CD16^+IFN\alpha/\beta R1^+CD119^+NG$					
NG,%	1.0 (0.4-1.8)	2.8 (1.5-3.0)	0.9 (0.8-2.3)	0.4 (0.2-0.5)	2.6 (1.7-7.6)
MFI CD16	39.1 (26.6-50.3)	77.5* (59.1-109.0)	66.5* (51.9-79.6)	40.3 (35.0-48.0)	80.5 (63.8-107.0)
MFI IFNα/β	5.7 (4.6-6.5)	9.2* (7.8-12.0)	3.4* ^ (3.1-3.6)	13.7* (8.3-17.4)	1.72* ♦ (1.58-1.86)
MFI CD119	3.2 (2.9-5.8)	2.5 (1.7-9.0)	3.7 (2.5-5.0)	6.6* (5.9-7.4)	7.7 (6.1-9.1)

Note. *, significant differences relative to the comparison group, $p < 0.05$; ^, significant differences between SG1a and SG1b, $p < 0.05$; #, significant differences in SG1a before and after incubation with hexapeptide, $p < 0.05$; ♦, significant differences in SG1b values before and after incubation with hexapeptide, $p < 0.05$

Testing in the CG, the number of NG (%) subsets: $CD64^-CD16^+CD32^+CD11b^+$, $CD64^+CD16^+CD32^+CD11b^+$ it was shown that the $CD64^-CD16^+CD32^+CD11b$ NG subset is major and amounts to 94.4 (89.7-95.9) %, has the $CD64^-CD16^{dim}CD32^{dim}CD11b^{mid}$ NG phenotype determined by the density of receptor expression: low MFI CD16 and MFI CD32 and medium MFI CD11b (Table 2). Also, a subset of $CD64^+CD16^+CD32^+CD11b^+NG$ – 1.2 (0.7-2.7) %, additionally expressing the CD64 receptor with an MFI of 6.1 (3.3-9.9) and having equipment of CD11b,CD32,CD16 receptors identical with the major subset – $CD64^{mid}CD16^{dim}CD32^{dim}CD11b^{mid}$ phenotype (Table 2).

There is a 5 times increase ($p < 0.05$) of NG of the $CD64^+CD16^+CD32^+CD11b^+$ subset with a transformed phenotype – $CD64^{mid}CD16^{bright}CD32^{mid}CD11b^{bright}$ versus $CD64^{mid}CD16^{dim}CD32^{mid}CD11b^{mid}$ in CG ($p < 0.05$, Table 2). The altered phenotype had high levels of expression of 2 membrane activation receptors: MFI CD16 -67.2 (44.4-84.4) и CD11b -39.3 (39.1-41.1) ($p_{1,2} < 0.05$), which indicates negative hyperactivation of NG. The number of NG subset $CD64^-CD16^+CD32^+CD11b^+$ did not change ($p > 0.05$), but an altered phenotype was deter-

TABLE 2. EFFECTS OF HEXAPEPTIDE (HP) INFLUENCE ON THE CONTENT AND PHENOTYPE OF $CD64^-CD16^+CD32^+CD11b^+$ AND $CD64^+CD16^+CD32^+CD11b^+$ SUBSETS OF NEUTROPHILIC GRANULOCYTES IN PATIENTS WITH MODERATE COVID-19, Me ($Q_{0.25}$-$Q_{0.75}$)

Indicators	Comparison Group (CG) n = 22	Moderate form of COVID-19 n = 45	
		SG1 before incubation with HP	SG2 after incubation with HP
$CD64^-CD16^+CD32^+CD11b^+$NG			
NG,%	94.4 (89.7-95.9)	96.2 (90.5-97.8)	92.7 (84.5-93.1)
MFI CD16	33.6 (30.0-44.3)	79.4* (64.9-87.6)	57.7* ♦ (54.7-60.0)
MFI CD32	2.7 (2.2-3.1)	2.7 (2.3-3.5)	4.7* ♦ (3.9-5.2)
MFI CD11b	32.3 (24.1-38.4)	19.3* (16.1-26.1)	26.8* (25.7-27.6)
$CD64^+CD16^+CD32^+CD11b^+$NG			
NG,%	1.2 (0.7-2.7)	5.8* (4.2-10.6)	2.7 (1.7-6.5)
MFI CD64	6.1 (3.3-9.9)	3.1 (2.1-6.6)	10.7 (5.7-14.7)
MFI CD16	28.4 (13.8-56.1)	67.2 (44.4-84.4)	15.2 (11.6-26.6)
MFI CD32	3.7 (3.1-4.2)	4.5* (4.4-6.8)	6.2 (4.9-11.1)
MFI CD11b	31.1 (24.2-38.2)	39.3* (39.1-41.1)	29.2 (22.2-32.6)

Note. *, significant differences relative to the comparison group, $p < 0.05$; ♦, significant differences in SG1 values before and after incubation with hexapeptide, $p < 0.05$.

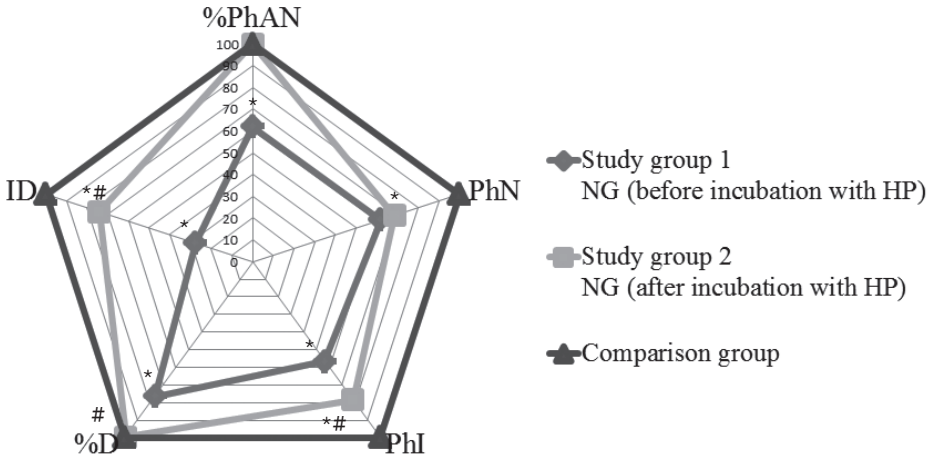

Figure 1. Effect of hexapeptide (HP) on phagocytic activities of NG in COVID-19

Note. *, differences from the CG, $p < 0.05$; #, differences from SG with moderate course COVID-19, $p < 0.05$

mined $CD64^{-}CD16^{bright}CD32^{dim}CD11b^{dim}$ NG versus $CD64^{-}CD16^{dim}CD32^{dim}CD11b^{mid}$ NG in comparison group (CG) reflects the reduced functionality of NG (Table 2). Defects in the phagocytic activity of NG were revealed: a decrease in the percentage of active phagocytic neutrophilic granulocytes (%), absorbing and killing ability (%) ($p_{1-3} < 0.05$) (Figure 1).

The effects of exposure HP *in vitro* are shown in Table 2. There was a decrease in the number of NG in the subset $CD64^{+}CD16^{+}CD32^{+}CD11b^{+}$ to the values of the comparison group ($p > 0.05$). Parallel the phenotype of the subset is changed with a decrease in MFI CD16 to comparison group values ($p > 0.05$) $CD64^{bright}CD16^{dim}CD32^{mid}CD11b^{bright}$. The receptor equipment of NG in the main subset has changed $CD64^{-}CD16^{+}CD32^{+}CD11b^{+}$, there was a decrease in MFI CD16 and an increase in MFI CD32 ($p > 0.05$) and a decrease in MFI CD11b ($p_{1-3} < 0.05$) to the values of CG – $CD64^{-}CD16^{mid}CD32^{mid}CD11b^{mid}$NG. The revealed remodeling of the NG phenotype transformed during COVID-19 under the influence of HP also led to the restoration of the phagocytic function of NG (Figure 1).

Conclusion

Thus, a positive effect of the Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine peptide on the phenotypes of functionally significant subsets actively involved in antiviral protection and on the function of NG in moderate COVID-19 was shown *in vitro*. Unidirectional modulating effects were revealed that contributed to the restoration of the phenotype of regulatory subsets $CD16^{+}IFN\alpha/\beta R1^{-}CD119^{+}$, $CD16^{+}IFN\alpha/\beta R1^{+}CD119^{-}$, expressing receptors for IFN I II types to the indicators of the comparison group. Subset phenotype remodeling noted $CD64^{-}CD16^{+}CD32^{+}CD11b^{+}$ NG, $CD64^{+}CD16^{+}CD32^{+}CD11b^{+}$ NG ensure the usefulness of effector functions from hyperactivated to normal, which is confirmed by the restoration of the defective phagocytic activity of NG. The data obtained open up prospects for the creation of new methods of immunomodulatory therapy for the restoration of NG dysfunctions in COVID-19 with the inclusion of the hexapeptide Arginyl-alpha-Aspartyl-Lysyl-Valyl-Tyrosyl-Arginine, which is the active ingredient of a Russian registered medicinal product.

References

1. Afeltra A., Galeazzi M., Basso P., Pietrucci A., de Pità O., Ferri G.M., Porzio F., Bonomo L. Immune imbalance in the synovial fluid of rheumatoid arthritis patients: Effects of intra-articular injection of thymopentin. *J. Boil. Regul. Homeost. Agents, 1991, Vol. 5, pp. 71-75.*

2. Cicco S., Cicco G., Racanelli V., Vacca A. Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs): Two potential targets for COVID-19 treatment. *Mediators Inflamm., 2020, Vol. 2020, 7527953.* doi: 10.1155/2020/7527953.

3. Khavinson V., Linkova N., Dyatlova A., Kuznik B., Umnov R. Peptides: prospects for use in the treatment of COVID-19. *Molecules, 2020, Vol. 25, no. 19, 4389.* doi: 10.3390/molecules25194389.

4. Liu Y., Zhou X., Liu X., Jiang X. The immunology and immunotherapy for COVID-19. *Expert Rev. Mol. Med., 2021. Vol. 23, e24.* doi: 10.1017/erm.2021.30.

5. Markova T.P., Chuvirov D.G. Immunotherapy with imunofan to the treatment of children with recurrent respiratory deseasis and mycoplasma pneumoniae infection. *Effective Pharmacotherapy, 2022. Vol. 18, no. 12, pp. 12-18.* (In Russ.)

6. Masso-Silva J., Moshensky A., Lam M., Odish M., Patel A., Xu L., Hansen E., Trescott S., Nguyen C., Kim R., Perofsky K., Perera S., Ma L., Pham J., Rolfsen M., Olay J., Shin J., Dan J.M., Abbott R. K., Ramirez S., Alexander T.H., Lin G.Y., Fuentes A.L., Advani I., Gunge D., Pretorius V., Malhotra A., Sun X., Duran J., Hepokoski M.,. Crotty Sh., Coufal N.G., Meier A., Crotty A.L.E. Increased peripheral blood neutrophil activation phenotypes and neutrophil extracellular trap formation in critically Ill coronavirus disease 2019 (COVID-19) patients: A case series and review of the literature. *Clin. Infect. Dis., 2022, Vol. 74. pp. 479-489.*

7. McKenna E., Wubben R., Isaza-Correa J.M., Melo A.M., Mhaonaigh A.U., Conlon N., O'Donnell J.S., Ní Cheallaigh C., Hurley T., Stevenson N.J., Little M.A., Molloy E.J. Neutrophils in COVID-19: Not Innocent Bystanders. *Front. Immunol., 2022, Vol. 13, 864387.* doi: 10.3389/fimmu.2022.864387.

8. Nesterova I.V., Chudilova G.A., Chapurina V.N., Kovaleva S.V., Teterin Yu.V., Barova N.K., Lyagusha D.E., Tarakanov V.A. Clinical and immunological efficacy of immunotherapeutic program after surgical treatment of children with various forms of acute peritonitis. *Medical Immunology (Russia), 2022, Vol 24, no. 3, pp. 553-572.* doi: 10.15789/1563-0625-CAI-2470.

9. Nesterova I.V., Khaidukov S.V., Nguyen T.D.L., Ronzhina A.N. Influences of hexapeptide arginyl-alpha-aspartil-lizil-valyl-tyrosil-arginin on the transformed phenotype of neutrophilic granulotsites of healthy persons in the experimental system *in vitro*. *Russian Journal of Immunology, 2017, Vol. 11 (20), no. 2, pp. 176-179.*

10. Ruan Q., Yang K., Wang W., Jiang L., Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med., 2020, Vol. 46, no. 5, pp. 846-848.*

11. Solmajer T. Design of a novel thymopoietin analogue based on conformational analyses. *Drug Des. Deliv., 1990, Vol. 6, pp. 213-221.*

12. Stegelmeier A.A., Darzianiazizi M., Hanada K., Sharif S., Wootton S.K., Bridle B.W., Karimi K. Type I interferon-mediated regulation of antiviral capabilities of neutrophils. *Int. J. Mol. Sci., 2021, Vol. 22, 4726.* doi: 10.3390/ijms22094726.

13. Wang Q., Zhao Y., Chen X., Hong A. Virtual screening of approved clinic drugs with main protease (3CLpro) reveals potential inhibitory effects on SARS-CoV-2. *J. Biomol. Struct. Dyn., 2020, Vol. 40, no. 2, pp. 685-695.*

Авторы:

Городин В.Н. *— д.м.н., профессор, заведующий кафедрой инфекционных болезней и эпидемиологии ФПК и ППС ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ, г. Краснодар, Россия*

Матушкина В.А. *— ассистент кафедры инфекционных болезней и эпидемиологии факультета повышения квалификации и профессиональной переподготовки специалистов ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ, г. Краснодар, Россия*

Чапурина В.Н. *— ассистент кафедры клинической иммунологии, аллергологии и лабораторной диагностики ФПК и ППС ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ, г. Краснодар, Россия*

Меняйло А.И. *— ассистент кафедры инфекционных болезней и эпидемиологии ФПК и ППС ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ, г. Краснодар, Россия*

Ковалева С.В. *— д.м.н., доцент кафедры клинической иммунологии, аллергологии и лабораторной диагностики ФПК и ППС ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ, г. Краснодар, Россия*

Дыдышко Е.И. *— к.м.н., доцент кафедры клинической иммунологии, аллергологии и лабораторной диагностики ФПК и ППС ФГБОУ ВО «Кубанский государственный медицинский университет» Министерства здравоохранения РФ, г. Краснодар, Россия*

Authors:

Gorodin V.N., *PhD, MD (Medicine), Professor, Head, Department of Infectious Diseases and Epidemiology of FAT and PRS, Kuban State Medical University, Krasnodar, Russian Federation*

Matushkina V.A., *Assistant Professor, Department of Infectious Diseases and Epidemiology of the Faculty of Professional Development and Professional Retraining of Specialists, Kuban State Medical University, Krasnodar, Russian Federation*

Chapurina V.N., *Assistant Professor, Department of Clinical Immunology, Allergology and Laboratory Diagnostics of FAT and PRS, Kuban State Medical University, Krasnodar, Russian Federation*

Menyailo A.I., *Assistant Professor, Department of Infectious Diseases and Epidemiology of FAT and PRS, Kuban State Medical University, Krasnodar, Russian Federation*

Kovaleva S.V., *PhD, MD (Medicine), Associate Professor, Department of Clinical Immunology, Allergology and Laboratory Diagnostics of FAT and PRS, Kuban State Medical University, Krasnodar, Russian Federation*

Dydyshko E.I., *PhD (Medicine), Associate Professor, Department of Clinical Immunology, Allergology and Laboratory Diagnostics of FAT and PRS, Kuban State Medical University, Krasnodar, Russian Federation*

Поступила 13.04.2023
Отправлена на доработку 19.04.2023
Принята к печати 21.04.2023

Received 13.04.2023
Revision received 19.04.2023
Accepted 21.04.2023