ОСОБЕННОСТИ ИЗМЕНЕНИЙ УРОВНЯ ЭКСПРЕССИИ РЕЦЕПТОРОВ TNFα И ФУНКЦИОНАЛЬНОГО ОТВЕТА КЛЕТОЧНЫХ ЛИНИЙ ПРИ СТИМУЛЯЦИИ РАЗЛИЧНЫМИ ДОЗАМИ ЦИТОКИНА

Лопатникова Ю.А., Жукова Ю.В., Альшевская А.А., Облеухова И.А., Киреев Ф.Д., Беломестнова И.А., Сенников С.В.

ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия

Резюме. TNFα является провоспалительным цитокином, передача сигналов которого осуществляется через рецепторы типа 1 (TNFR1) и типа 2 (TNFR2). TNFR1 обычно опосредует апоптоз, выживание клеток и секрецию цитокинов, в то время как TNFR2 избирательно опосредует выживание клеток и секрецию цитокинов. В некоторых случаях при активации рецепторов происходит изменение функционального ответа клеток на противоположный. Активация сигнальных путей имеет свои пусковые механизмы, отличающиеся при взаимодействии между разными формами цитокина и разными формами рецепторных комплексов, а также при изменении соотношения различных типов рецепторов. Изучение механизмов регуляции в системе лиганд-рецептор является приоритетной задачей многих исследований. В данной работе показано дозозависимое влияние TNFα на экспрессию цитокиновых рецепторов и изменение функционального ответа опухолевых клеточных линий различного происхождения. Для этого в исследовании проводили сравнительную оценку экспрессии и ко-экспрессии рецепторов, фаз клеточного цикла и апоптоза клеточных линий без стимуляции и стимулированных TNFα в концентрациях 5 и 50 нг/мл. Было выявлено, что для клеточной линии K562 характерны более выраженные изменения ко-экспрессии рецепторов, которые наблюдались при концентрации TNFα 50 нг/мл по сравнению как с контрольной группой, так и с группой 5 нг/мл. Снижение относительного содержания клеток, экспрессирующих только TNFR1, сочеталось со снижением процента клеток в апоптозе, что подтверждает литературные данные о роли данного рецептора в развитии апоптоза. При этом изменений плотности экспрессии для этой клеточной линии не наблюдалось. Для клеточной линии ZR75-1 наибольшее количество эффектов было выявлено также для концентрации TNFα 50 нг/мл. Увеличение относительного со-

Адрес для переписки:

Сенников Сергей Витальевич
ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии»
630099, Россия, г. Новосибирск, ул. Ядринцевская, 14.
Тел.: 8 (383) 222-19-10.
E-mail: sennikovsv@gmail.com

Address for correspondence:

Sergey V. Sennikov
Research Institute of Fundamental and Clinical Immunology
14 Yadrintsevskaya St
Novosibirsk
630099 Russian Federation
Phone: +7 (383) 222-19-10.
E-mail: sennikovsv@gmail.com

Образец цитирования:

Ю.А. Лопатникова, Ю.В. Жукова, А.А. Альшевская, И.А. Облеухова, Ф.Д. Киреев, И.А. Беломестнова, С.В. Сенников «Особенности изменений уровня экспрессии рецепторов TNFα и функционального ответа клеточных линий при стимуляции различными дозами цитокина // Медицинская иммунология, 2023. Т. 25, № 3. С. 459-464.
doi: 10.15789/1563-0625-FOC-2718

For citation:

Yu.A. Lopatnikova, Yu.V. Zhukova, A.A. Alshevskaya, I.A. Obleukhova, F.D. Kireev, I.A. Belomestnova, S.V. Sennikov "Features of changes in the expression level of TNFα receptors and the functional response of cell lines upon stimulation with various doses of cytokine", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 3, pp. 459-464.
doi: 10.15789/1563-0625-FOC-2718

DOI: 10.15789/1563-0625-FOC-2718

держания клеток, экспрессирующих только TNFR2, сочеталось с увеличением апоптоза, однако плотность экспрессии данного типа рецептора была низкой, что могло повлиять на переключение сигнальных путей в сторону проапоптотических. Таким образом, наше исследование позволило выявить особенности изменений экспрессии и ко-экспрессии рецепторов TNFα, характерных для клеточных линий различного происхождения, а также изменение функционального ответа клеток в ответ на стимуляцию различными дозами цитокина. Все это позволяет расширить представления о регуляторных механизмах в системе цитокин-рецептор.

Ключевые слова: фактор некроза опухоли альфа, клеточные линии, рецепторы фактора некроза опухоли 1-го типа, рецепторы фактора некроза опухоли 2-го типа, апоптоз, плотность экспрессии рецепторов

FEATURES OF CHANGES IN THE EXPRESSION LEVEL OF TNFα RECEPTORS AND THE FUNCTIONAL RESPONSE OF CELL LINES UPON STIMULATION WITH VARIOUS DOSES OF CYTOKINE

Lopatnikova Yu.A., Zhukova Yu.V., Alshevskaya A.A., Obleukhova I.A., Kireev F.D., Belomestnova I.A., Sennikov S.V.

Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation

Abstract. TNFα is a pro-inflammatory cytokine that is signaled through type 1 (TNFR1) and type 2 (TNFR2) receptors. TNFR1 normally mediates apoptosis, cell survival, and cytokine secretion, while TNFR2 selectively mediates cell survival and cytokine secretion. But in some cases, when receptors are activated, the functional response of cells changes to the opposite. Activation of signaling pathways has its own triggers, which differ in the interaction between different forms of cytokine and different forms of receptor complexes, as well as changes in the ratio of different types of receptors. The study of the mechanisms of regulation in the ligand-receptor system is a priority task for many studies. This work shows the dose-dependent effect of TNFα on the expression of cytokine receptors and changes in the functional response of tumor cell lines of various origins. For this, a comparative assessment of the expression and co-expression of receptors, cell cycle phases and apoptosis of cell lines without stimulation and stimulated with TNFα at concentrations of 5 and 50 ng/mL was carried out. It was found that the K562 cell line was characterized by more pronounced changes in receptor co-expression, which were observed at a TNFα concentration of 50 ng/mL compared to both the control group and the 5 ng/mL group. The decrease in the relative content of cells expressing only TNFR1 was combined with a decrease in the percentage of cells in apoptosis, which confirms the literature data on the role of this receptor in the development of apoptosis. At the same time, no changes in expression density were observed for this cell line. For the ZR75-1 cell line, the largest number of effects was also found for a TNFα concentration of 50 ng/mL. An increase in the relative content of cells expressing only TNFR2 was combined with an increase in apoptosis; however, the expression density of this type of receptor was low, which could affect the switching of signaling pathways towards proapoptotic ones. Thus, our study allowed us to reveal the features of changes in the expression and co-expression of TNFα receptors characteristic of cell lines of various origins, as well as changes in the functional response of cells in response to stimulation with different doses of cytokine. All this allows us to expand our understanding of the regulatory mechanisms in the cytokine-receptor system.

Keywords: tumor necrosis factor alpha, cell lines, tumor necrosis factor receptor 1, tumor necrosis factor receptor 2, apoptosis, receptor expression density

The study was supported by a grant from the Russian Science Foundation (project No. 20-75-10051).

Introduction

TNFα is a pro-inflammatory cytokine that is signaled through type 1 (TNFR1) and type 2 (TNFR2) receptors. TNFR1 is ubiquitously expressed on most cell types. TNFR2 expression has been predominantly described for immune cells. TNFR1 and TNFR2 have structurally different intracellular domains that activate different signaling pathways. TNFR1 normally mediates apoptosis, cell survival, and cytokine secretion, while TNFR2 selectively mediates cell survival and cytokine secretion [4]. However, in some cases, when the receptors are activated, the functional response of the cell changes to the opposite. These processes depend on several possible variants of signaling, which are currently being actively studied [7]. The triggering of signaling pathways, in turn, has its own triggers. One of the activation mechanisms can be the interaction between different forms of cytokine and different forms of receptor complexes, as well as a change in the ratio of different types of receptors [2, 3, 5].

Another mechanism is conformational changes in the receptor, which lead to signaling switching [6]. Finally, regulation may depend on changes in the ligand-receptor ratio. When the threshold level of receptor expression density is reached, switching of signaling pathways is possible [8]. Despite a large number of hypotheses, there is no common understanding of how the functional response of TNF receptors is mediated. The study of the mechanisms of regulation in the ligand-receptor system is a priority for many studies, but there are still many questions regarding the effect of cytokine on the distribution of various types of receptors and their expression density on cells, as well as the functional response to stimulation with various doses of the cytokine. Thus, **the aim of our study** was to study the effect of stimulation with different concentrations of TNF on the co-expression and absolute number of receptors on cells in combination with the assessment of apoptosis and cell cycle phases.

Materials and methods

The object of the study is the culture of human cell lines: ZR-75-1 (breast carcinoma, ascitic fluid); and K562 (chronic myelogenous leukemia, pleural fluid). All cell lines were provided by the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg, Russia).

Cell cultivation was carried out in culture flasks (TPP, Switzerland) using RPMI-1640 medium containing 10% fetal bovine serum (FBS) (HyClone, USA), 2 mM L-glutamine (BioloT Ltd., Russia), 5×10^{-4} M 2-mercaptoethanol (Sigma-Aldrich, USA), 80 μg/mL gentamicin (KRKA, Slovenia), 10 mM HEPES buffer (Sigma-Aldrich, USA), 100 μg/mL of benzylpenicillin (JSC "Biosintez", Russia), in an incubator in a humid atmosphere at 37 °C and CO_2 concentration of 5%.

Evaluation of the dose-dependent effect of TNFα

To assess the dose-dependent effect of TNFα, cells were cultured for 72 hours in the absence and presence of recombinant TNFα at concentrations of 5 and 50 ng/mL in plates (TPP, Switzerland) at the optimal density for each cell line. Cells were cultured in an incubator in a humid atmosphere at 37 °C and 5% CO_2.

Estimation of expression density and co-expression of receptors

Quantitative expression of TNFR1 and TNFR2 receptors was assessed by flow cytometry using a commercial kit of BD QuantiBRITE PE calibration particles (BD Biosciences, USA).

Two combinations of monoclonal antibodies were used to assess co-expression of TNFα type 1 and 2 receptors (TNFR1 and TNFR2) on cells:

TNFR1-Phycoerethrin (R&D System, USA) and TNFR2-Allophycocyanin (R&D System, USA); and TNFR1-Allophycocyanin (R&D System, USA) and TNFR2-Phycoerethrin (R&D System, USA).

The relative abundance of double-negative ($TNFR1^-TNFR2^-$), double-positive ($TNFR1^+TNFR2^+$) and cells expressing only TNFR1 ($TNFR1^+TNFR2^-$) and TNFR2 ($TNFR1^-TNFR2^+$) cells was determined as the average between the two experiments. The analysis was performed on a FacsVerse flow cytometer (BD, USA).

Assessment of cell cycle phases and apoptosis

For intracellular DNA labeling, 7-aminoactinomycin D (7-ADD) (BD Biosciences, USA) was used. The analysis was performed on a FacsVerse flow cytometer (BD, USA).

Statistical analysis

Statistical processing of the results was carried out using the GraphPad Prism version 8 application package and Microsoft Excel version 2010.

Kruskal–Wallis rank analysis of variance was used to assess the difference between groups. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Co-expression of receptors

The study evaluated the dose-dependent effect of TNFα on receptor co-expression. Cell lines were incubated with TNFα at doses of 5 and 50 ng/mL and without TNFα. Evaluation of changes in co-expression was carried out after 72 hours.

For the cell line ZR 75-1 (Figure 1), a statistically significant decrease in the percentage of double-positive cells was observed for all concentrations of

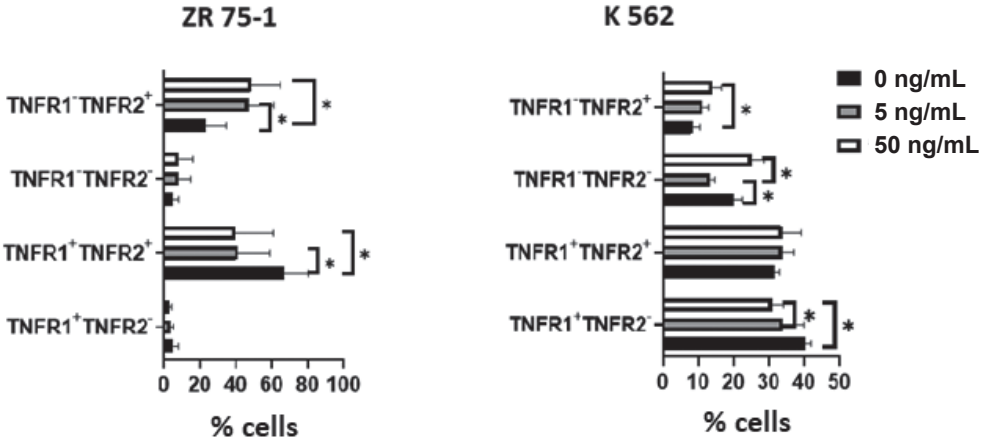

Figure 1. Co-expression of receptors on K562 and ZR 75-1 cell lines upon stimulation with TNFα at a dose of 5 and 50 ng/mL and without addition of TNFα

Note. Data are presented as medians normalized to mean values. *, indicate significant (p < 0.05, Kruskal–Wallis test for multiple comparisons).

TNFα compared with the control group from 66.9% to 41.2% at a concentration of 5 ng/mL (p = 0.0039) and up to 39.9% at 50 ng/mL (p = 0.013). Also, this cell line was characterized by an almost two-fold increase in the percentage of cells expressing only TNFR2 from 23.7% to 46.7% at a concentration of 5 ng/mL (p = 0.002) and up to 48.7% at a concentration of 50 ng/mL (0.0062) compared to the control group. At the same time, the indicator between groups with a concentration of 5 and 50 ng/mL did not have significant differences.

For the K562 cell line (Figure 1), more pronounced changes were detected. The percentage of cells expressing only TNFR1 decreased for a concentration of 50 ng/mL from 40% to 30% (p = 0.0189) compared with the control, while the percentage of cells expressing only TNFR2 increased from 8.6% to 13.9% (p = 0.0098). The percentage of cells with double-negative cells was lower at 5 ng/mL compared to controls (13.3 and 20%, respectively) (p = 0.0096) and 50 ng/mL (25.1%) (p = 0.0046). Further, the study evaluated the change in the expression density of TNFR1 and TNFR2 receptors on cells with the addition of TNF at concentrations of 5 and 50 ng/mL and without TNF.

Changes in receptor expression density were significant only for the ZR75-1 cell line (Figure 2). There was a significant decrease in the amount of TNFR2 in the 5ng/mL group, both compared with the control (2661 and 6788, respectively (p = 0.002) and compared with the 50 ng/mL group (6184 p = 0.003). For the K562 cell line, statistically significant differences were not found. Next, the functional response of cells was evaluated, during which changes in the relative numbers of cells in various phases of the cell cycle and apoptosis were evaluated.

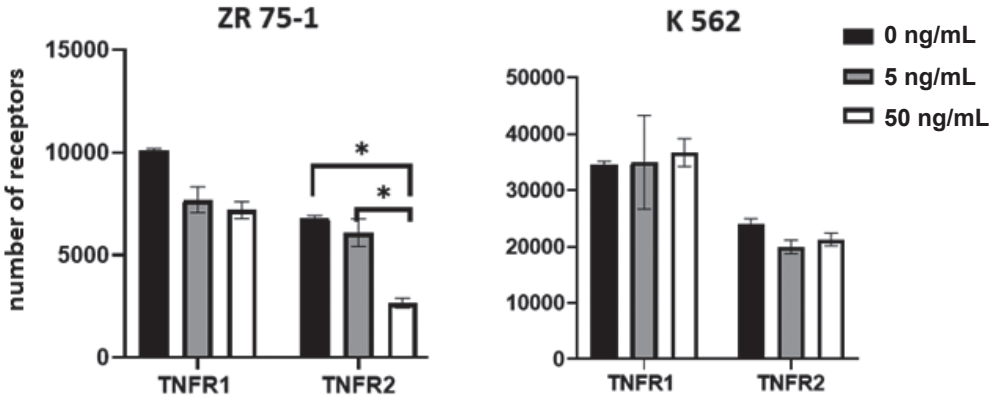

Figure 2. Density of receptor expression on K562 and ZR75-1 cell lines upon stimulation with TNFα at a dose of 5 and 50 ng/mL and without addition of TNFα

Note. Data are presented as medians. *, indicate significant (p < 0.05, Kruskal–Wallis test for multiple comparisons).

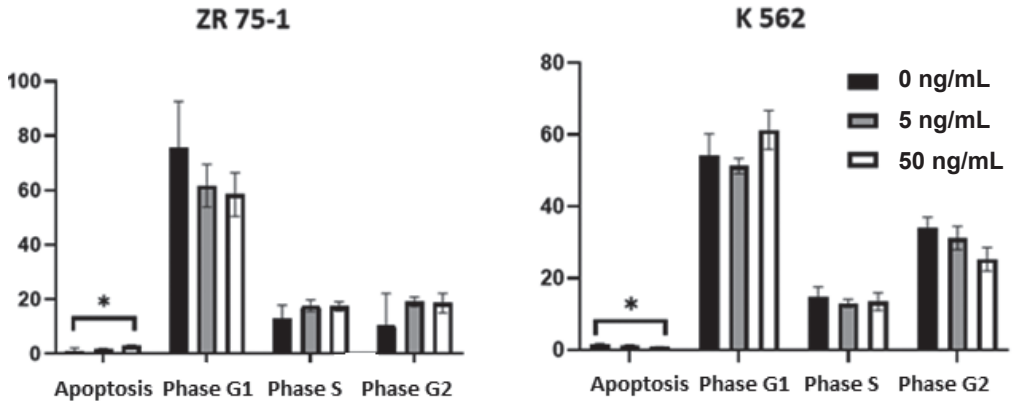

Figure 3. Phases of the cell cycle and apoptosis in K562 and ZR75-1 cell lines upon stimulation with TNFα at a dose of 5 and 50 ng/mL and without the addition of TNFα

Note. Data are presented as medians. *, indicate significant (p < 0.05, Kruskal–Wallis test for multiple comparisons).

The cell line ZR75-1 (Figure 3) was characterized by an increase in the percentage of cells in apoptosis from 1.1% to 2.8% (p = 0.0224) for the group with 50 ng TNFα stimulation compared to the control. For the K562 cell line, the opposite picture was observed; in the 50 ng group, the percentage of cells in apoptosis decreased compared to the control from 1.7 to 0.8% (p = 0.0003). As a result of the study, it was found that the K562 cell line is characterized by more pronounced changes in receptor co-expression, which were observed at a TNFα concentration of 50 ng/mL compared to both the control group and the 5 ng/mL group. The decrease in the relative content of cells expressing only TNFR1 was combined with a decrease in the percentage of cells in apoptosis, which confirms the literature data on the role of this receptor in the development of apoptosis [8]. At the same time, no changes in expression density were observed for this cell line.

For the ZR75-1 cell line, the largest number of effects was also found for a TNFα concentration of 50 ng/mL. An increase in the relative content of cells expressing only TNFR2 was combined with an increase in apoptosis; however, the expression density of this type of receptor was low, which could affect the switching of signaling pathways towards proapoptotic ones [1].

Conclusion

Our study allowed us to reveal the features of changes in the expression and co-expression of TNFα receptors characteristic of cell lines of various origins, as well as changes in the functional response of cells in response to stimulation with different doses of cytokine. All this allows us to expand our understanding of the regulatory mechanisms in the cytokine-receptor system.

References

1. Cabal-Hierro L., Artime N., Iglesias J., Prado M.A., Ugarte-Gil L., Casado P., Fernández-García B., Darnay B.G., Lazo P.S. A TRAF2 binding independent region of TNFR2 is responsible for TRAF2 depletion and enhancement of cytotoxicity driven by TNFR1. *Oncotarget, 2014, Vol. 5, no. 1, pp. 224-236.*

2. Fotin-Mleczek M., Henkler F., Samel D., Reichwein M., Hausser A., Parmryd I., Scheurich P., Schmid J.A., Wajant H. Apoptotic crosstalk of TNF receptors: TNF-R2-induces depletion of TRAF2 and IAP proteins and accelerates TNF-R1-dependent activation of caspase-8. *J. Cell Sci., 2002, Vol. 115, Pt 13, pp. 2757-2770.*

3. Gough P., Myles I.A. Tumor necrosis factor receptors: pleiotropic signaling complexes and their differential effects. *Front. Immunol., 2020, Vol. 11, 585880.* doi: 10.3389/fimmu.2020.585880.

4. Kalliolias G.D., Ivashkiv L.B. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat. Rev. Rheumatol., 2016, Vol. 12, pp. 49-62.*

5. Lo C.H., Huber E.C., Sachs J.N. Conformational states of TNFR1 as a molecular switch for receptor function. *Protein Sci., 2020, Vol. 29, no. 6, pp. 1401-1415.*

6. Sennikov S.V., Alshevskaya A.A., Zhukova J., Belomestnova I., Karaulov A.V., Lopatnikova J.A. Expression density of receptors as a potent regulator of cell function and property in health and pathology. *Int. Arch. Allergy Immunol., 2019, Vol. 178, no. 2, pp. 182-191.*

7. van Loo G., Bertrand M.J.M. Death by TNF: a road to inflammation. *Nat. Rev. Immunol., 2022.* doi: 10.1038/s41577-022-00792-3.
8. You K., Gu H., Yuan Z., Xu X. Tumor necrosis factor alpha signaling and organogenesis. *Front. Cell Dev. Biol., 2021, Vol. 9, 727075.* doi: 10.3389/fcell.2021.727075.

Авторы:

Лопатникова Ю.А. *— к.б.н., старший научный сотрудник лаборатории молекулярной иммунологии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Жукова Ю.В. *— к.м.н., лаборант-исследователь лаборатории молекулярной иммунологии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Альшевская А.А. *— к.м.н., старший научный сотрудник. лаборатории молекулярной иммунологии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Облеухова И.А. *— к.м.н., старший научный сотрудник лаборатории молекулярной иммунологии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Киреев Ф.Д. *— младший научный сотрудник лаборатории молекулярной иммунологии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Беломестнова И.А. *— лаборант-исследователь лаборатории молекулярной иммунологии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Сенников С.В. *— д.м.н., профессор, заведующий лабораторией молекулярной иммунологии ФГБНУ «Научно-исследовательский институт фундаментальной и клинической иммунологии», г. Новосибирск, Россия*

Authors:

Lopatnikova Yu.A., *PhD (Bilogy), Senior Research Associate, Laboratory of Molecular Immunology, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Zhukova Yu.V., *PhD (Medicine), Research Assistant, Laboratory of Molecular Immunology, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Alshevskaya A.A., *PhD (Medicine), Senior Research Associate, Laboratory of Molecular Immunology, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Obleukhova I.A., *PhD (Medicine), Senior Research Associate, Laboratory of Molecular Immunology, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Kireev F.D., *Junior Research Associate, Laboratory of Molecular Immunology, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Belomestnova I.A., *Laboratory Assistant, Laboratory of Molecular Immunology, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Sennikov S.V., *PhD, MD (Medicine), Professor, Head, Laboratory of Molecular Immunology, Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation*

Поступила 11.04.2023
Отправлена на доработку 14.04.2023
Принята к печати 16.04.2023

Received 11.04.2023
Revision received 14.04.2023
Accepted 16.04.2023