Медицинская иммунология
2021, Т. 23, № 4,
стр. 641-646

Краткие сообщения
Short communications

Medical Immunology (Russia)/
Meditsinskaya Immunologiya
2021, Vol. 23, № 4, pp. 641-646

РОЛЬ ГЛИКОДЕЛИНА В РЕГУЛЯЦИИ ДИФФЕРЕНЦИРОВКИ МИЕЛОИДНЫХ СУПРЕССОРНЫХ КЛЕТОК

Заморина С.А.[1, 2], Тимганова В.П.[2], Бочкова М.С.[2], Шардина К.Ю.[2], Ужвиюк С.В.[2], Храмцов П.В.[1, 2], Кропанева М.Д.[2], Раев М.Б.[1, 2]

[1] ФГБОУ ВО «Пермский государственный национальный исследовательский университет», г. Пермь, Россия
[2] Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук – филиал ФГБУН «Пермский федеральный исследовательский центр Уральского отделения Российской академии наук», г. Пермь, Россия

Резюме. Гликоделин (PP14, PAEP, альфа-2-микроглобулин, димерный гликопротеин с молекулярной массой от 42 до 56 кД) рассматривается как маркер рецептивности репродуктивной ткани. Невзирая на то, что иммунодепрессивные эффекты гликоделина хорошо известны, его роль в регуляции миелоидных супрессорных клеток (MDSC) не изучена. MDSC представляют собой гетерогенную популяцию незрелых миелоидных клеток, которые при патологических состояниях приобретают супрессорный фенотип, подавляя иммунный ответ.

Известно, что MDSC играют важную роль в поддержании иммунной толерантности в период беременности и при трансплантации. Наша гипотеза состоит в том, что гликоделин способен индуцировать формирование MDSC, поскольку уровень этих клеток во время успешной беременности повышается, в то время как спонтанное прерывание беременности и развитие преэклампсии ассоциировано с низким уровнем циркулирующего гликоделина. Таким образом, целью работы являлось изучение роли рекомбинантного гликоделина в физиологических концентрациях, в регуляции дифференцировки MDSC.

Для генерации MDSC *in vitro* мононуклеарные клетки из периферической крови добровольцев-доноров сепарировали путем центрифугирования на градиенте плотности 1,077 г/см^3 (Ficoll-Hypaque, Sigma-Aldrich). Затем полученные клетки культивировали в 24-луночном планшете в концентрации 1×10^6 клеток/мл в ППС с внесенными в среду цитокинами IL-6 (20 нг/мл), GM-CSF (40 нг/мл) в течение 14 суток при 5% CO_2 и 37 °C. Замена среды в культуре производилась на 7-е сутки, тогда же повторно вносили цитокины, а на 11-е сутки вносили рекомбинантный гликоделин (MyBioSource. Inc., США) в физиологических концентрациях (0,2; 2 мкг/мл), фармакологическая концентрация была 50 мкг/мл. В культурах оценивали уровень M-MDSC ($Lin^-HLA\text{-}DR^-CD33^+CD11b^+CD14^+CD66b^-$) и PMN-MDSC ($Lin^-HLA\text{-}DR^-CD33^+CD11b^+CD14^-CD66b^+$) методом проточной цитометрии (CytoFlexS (Beckman Coulter)) при помощи антител R&D Systems по стандартной методике. Статистическую обработку данных проводили в программе GraphPad Prizm 6 при помощи критерия Фридмана.

Установлено, что гликоделин не оказывал существенного влияния на жизнеспособность клеток, оцениваемую методом проточной цитометрии (PI). Высокая концентрация GdA (50 мкг/мл), которая

Адрес для переписки:

Заморина Светлана Анатольевна
ФГБОУ ВО «Пермский государственный национальный исследовательский университет»
614990, Россия, г. Пермь, ул. Букирева, 15.
Тел.: 8 (342) 239-64-35.
Факс: 8 (342) 237-16-11.
E-mail: mantissa7@mail.ru

Address for correspondence:

Zamorina Svetlana A.
Perm State National Research University
614990, Russian Federation, Perm, Bukirev str., 15.
Phone: 7 (342) 239-64-35.
Fax: 7 (342) 237-16-11.
E-mail: mantissa7@mail.ru

Образец цитирования:

С.А. Заморина, В.П. Тимганова, М.С. Бочкова, К.Ю. Шардина, С.В. Ужвиюк, П.В. Храмцов, М.Д. Кропанева, М.Б. Раев «Роль гликоделина в регуляции дифференцировки миелоидных супрессорных клеток» // Медицинская иммунология, 2021. Т. 23, № 4. С. 641-646.
doi: 10.15789/1563-0625-ROG-2209

For citation:

S.A. Zamorina, V.P. Timganova, M.S. Bochkova, K.Yu. Shardina, S.V. Uzhviyuk, P.V. Khramtsov, M.D. Kropaneva, M.B. Rayev "Role of glycodelin in regulation of myeloid-derived suppressor cell differentiation", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2021, Vol. 23, no. 4, pp. 641-646.
doi: 10.15789/1563-0625-ROG-2209
DOI: 10.15789/1563-0625-ROG-2209

является фармакологической, не оказывала достоверного эффекта на дифференцировку MDSC. В то же время гликоделин в концентрациях, соответствующих нормальной беременности (0,2; 2 мкг/мл), повышал процент MDSC в индуцированных культурах мононуклеарных клеток человека.

При анализе субпопуляций установлено, что этот эффект был обусловлен повышением уровня PMN-MDSC, в то время как уровень M-MDSC достоверно не изменялся. Данный результат можно интерпретировать как фетопротективный эффект гликоделина, поскольку повышение уровня G-MDSC связывают с подавлением иммунного ответа на патернальные антигены. Известно, что уровень PMN-MDSC увеличивается в периферической крови здоровых беременных женщин на всех стадиях беременности по сравнению с небеременными, тогда как число M-MDSC остается без изменений.

В то же время у пациенток с выкидышем более чем на 30% снижено количество MDSC в крови и эндометрии, особенно в первом триместре. В период физиологической беременности PMN-MDSC накапливаются в плаценте, но при спонтанном аборте их количество уменьшается. Плацентарные PMN-MDSC эффективно подавляют Т-клеточный ответ, одновременно поляризуя $CD4^+$-лимфоциты в фенотип Th2. Вероятно, PMN-MDSC играют важную роль в индуцировании и поддержании толерантности к антигенам плода, что позволяет рассматривать их как перспективную мишень терапевтического манипулирования при осложнениях беременности. В целом, мы впервые продемонстрировали влияние GdA на процесс дифференцировки MDSC.

Ключевые слова: гликоделин, миелоидные супрессорные клетки, дифференцировка, беременность, культивирование клеток, иммунная толерантность

ROLE OF GLYCODELIN IN REGULATION OF MYELOID-DERIVED SUPPRESSOR CELL DIFFERENTIATION

Zamorina S.A.[a, b], Timganova V.P.[b], Bochkova M.S.[b], Shardina K.Yu.[b], Uzhviyuk S.V.[b], Khramtsov P.V.[a, b], Kropaneva M.D.[b], Rayev M.B.[a, b]

[a] *Perm State National Research University, Perm, Russian Federation*
[b] *Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Abstract. Glycodelin (PP14, PAEP, alpha-2-microglobulin, dimeric glycoprotein with molecular weight of 42 to 56 kDa) is considered as a reproductive tissue receptivity marker. Despite that glycodelin immunosuppressive effects are well-known there still remains uncovered its role in myeloid suppressor cell (MDSC) regulation. MDSC represent the heterogeneous population of immature myeloid cells that acquire suppressor phenotype while inhibiting the immune response under the pathological states. MDSC are known to play an essential role in supporting the immune tolerance in pregnancy and at transplantation. Our hypothesis suggests that glycodelin is capable of inducing the MDSC formation as the level of these cells is elevated during the successful pregnancy, whereas the spontaneous abortion and progression of eclampsia are associated with low circulating glycodelin. Therefore, the aim of the work was to analyze the role of recombinant glycodelin in physiological concentrations in regulation of MDSC differentiation. Peripheral blood mononuclear cells of donor volunteers were separated via centrifugation on density gradient of 1,077 g/cm^3 (Ficoll-Hypaque, Sigma-Aldrich) to obtain MDSC generation *in vitro*. Then cells obtained were cultured in 24-well plate at a concentration of 1×10^6 cell/ml in complete medium with cytokines IL-6 (20 ng/ml), GM-CSF (40 ng/ml) therein for 14 days at 37 °C and 5% CO_2. Medium replacement was made by 7th day in culture followed by cytokine re-introduction, and on the 11th day recombinant glycodelin in physiological concentrations (0,2; 2 mkg/ml) was applied while the pharmacological concentration was 50 mkg/ml. The M-MDSC ($Lin^-HLA\text{-}DR^-CD33^+CD11b^+CD14^+CD66b^-$) and PMN-MDSC ($Lin^-HLA\text{-}DR^-CD33^+CD11b^+CD14^-CD66b^+$) level was evaluated in cultures using flow cytometry (CytoFlexS (Beckman Coulter)) and "R&D Systems" antibodies according to standard protocol. Statistical data processing was realized with GraphPad Prizm software using Friedman test. It was found that glycodelin did not significantly affect cell viability being assessed with flow cytometry (PI). It was revealed that high GdA concentration (50 mkg/ml) being pharmacological did

not render significant effect on MDSC differentiation. Meanwhile, glycodelin in concentrations correspanding the healthy pregnancy (0,2; 2 mkg/ml) was stated to increase the MDSC percentage in induced cultures of human mononuclear cells. When analyzing the subsets it was disclosed that this effect was conditioned by the increase in PMN-MDSC level while the M-MDSC level remained significantly unchanged. This result could be interpreted as glycodelin fetoprotective effect as the increase of the PMN-MDSC level is associated with the suppression of the immune response to paternal antigens. The PMN-MDSC level is known to be elevated in peripheral blood of healthy pregnant women at all the stages of pregnancy as compared to non-pregnant subjects whereas the M-MDSC amount remains unaltered. Meanwhile, patients with miscarriage demonstrated more that by 30% lowering in the MDSC amount in blood and endometrium and in I trimester, in particular. During the physiological pregnancy PMN-MDSC accumulate in placenta, but at spontaneous abortion their number is found to be declined. Placental PMN-MDSC efficiently suppress the T-cell response while concurrently polarizing the $CD4^+$ lymphocytes in Th2 phenotype. PMN-MDSC are suggested to play an essential role in inducing and supporting the tolerance to fetal antigens that allows considering these as promising target of therapeutical manipulation in pregnancy complications. As a whole, we have originally demonstrated the GdA effect on MDSC differentiation.

Keywords: glycodelin, myeloid-derived suppressor cells, differentiation, pregnancy, cell culture, immune tolerance

The reported study was funded by the RFBR according to the research project No. 19-29-04055 mk.

Introduction

In 1976, D.D. Petrunin, Ju.S. Tatarinov et al. initially isolated and identified glycodelin as a novel placental antigen that was called chorionic α2-microglobulin [8]. Subsequently, while accumulating new data on the protein location and properties, its name changed from placental α2-microglobulin, fertility α2-microglobulin (AMGF), and finally to specific α2-microglobulin.

Outside Russia, several independent researchers described glycodelin as a placental protein 14 (PP14), pregnancy-associated endometrial α2-globulin (α2-PEG), endometrial protein 15 (EP15), progestagen-dependent endometrial protein (PEP), α-uterine protein (AUP), and progesterone-associated endometrial protein (PAEP). In 1995, A. Dell et al. suggested to define the protein as a "glycodelin" describing its unique property: a sex-dependent glycosylation to overcome confusion in terms.

Glycodelin (GdA) is considered as the receptivity marker for reproductive tissue. In 2018, glycodelin was found to prevent experimental graft rejection [2] that favors its pharmacological application. Schneider et al. [9, 10] suggested a possible usage of glycodelin in lung transplantation. Despite that, immunosuppressive effects of glycodelin are well-known [1]. Its role in the regulation of cell functions shaping immune tolerance against allogenic graft remains unclear.

In particular, the role of glycodelin in the regulation of key (with regard to evolutionary biology) cell population myeloid-derived suppressor cells (MDSC) is still uncertain. MDSC represents a heterogeneous population of immature myeloid cells acquiring suppressor phenotype under the pathological states and repressing the immune response [3].

Recent research works demonstrated the MDSC key role in supporting immune tolerance in transplantation [12]. There is a hypothesis that glycodelin might be capable of inducing the MDSC formation because their level is elevated in successful pregnancy. In contrast, spontaneous abortion and preeclampsia progression are associated with low circulating glycodelin level.

Therefore, **this work was aimed** at examining human recombinant GdA role in MDSC formation and regulation playing an important role in allograft tolerance induction .

Materials and methods

The study was conducted according to the requirements of the Declaration of Helsinki WMA (2000) and protocol to the European Council Convention on Human Rights and Biomedicine (1999); the permission from the Ethics Committee of the IEGM UB RAS of 30.08.2019 (IRB00010009) was received.

Glycodelin was used in physiological range because it corresponds to its female peripheral blood level during pregnancy. GdA peaks at 2.0 μg/ml by the end of the trimester I reaching as high as 0.2 μg/ml in the trimester II, but declining to the minimal value or even not detected in the trimester III.

Recombinant glycodelin (PAEP), (*E. coli*), MBS 718444 was used; glycodelin (PAEP) was received from the https://www.mybiosource.com/recombinant-protein/glycodelin-paep/718444.

Fractionated peripheral blood mononuclear cells (PBMC) of apparently healthy donors (n = 6, women of 28±6 years) were used. Each donor provided a written informed consent. PBMC were obtained using

density gradient (1.077 g/cm^3) centrifugation (Diacoll "Dia-M", Russia).

MDSC production *in vitro*. To obtain MDSC *in vitro* PBMC were cultured in 96-well plate at 1×10^6 cell/ml concentration in complete medium (RPMI-1640, 10% FBS, 10 mM Hepes, 2 mmol L-glutamine (ICN Ph., USA) added with penicillin-streptomycin-amphotericin (100 μL per 10 ml of medium "B.I.", Israel) for 14 days (37 °C, 5% CO_2). Recombinant cytokines IL-6 (20 ng/ml), GM-CSF (40 ng/ml) (Milteniy Biotech, Germany) were applied for MDSC induction.

A complete medium was replaced on day 7, followed by three days of exposure to recombinant glycodelin (GdA, "MyBioSource. Inc.", *E. coli*; USA) at physiological concentrations (0.2; 2.0 μg/ml) corresponding to its level in peripheral blood of pregnant women ; pharmacological concentration was equal to 50 μg/ml [7]. Thereafter, cells were cultured for another three days followed by collection using the "Accutase" according to the manufacturer's recommendations (Capricorn Scientific, Germany).

Then cells were stained to detect viability with propidium iodide (PI) (Biolegend, USA) according to the manufacturer's protocol. The percentage of live (PI^-) cells in cultures (using a gate of living cells on FSC/SSC plot) varied within 85,5-92,1%.

Following standard washing procedures, cells were stained with antibodies to determine the MDSC percentage by flow cytometry ($Lin^-HLA^-DR^-CD33^+CD11b^+$) as well as M-MDSC ($Lin^-HLA\text{-}DR^-CD33^+CD11b^+CD14^+CD66b^-$) and PMN-MDSC ($Lin^-HLA\text{-}DR^-CD33^+CD11b^+CD14^-CD66b^+$). Staining was made using antibodies (Alexa Fluor 750-HLA-DR, APC-Anti Siglec-3/CD33, Alexa Fluor 405-Anti-Integrin aM/CD11b, PE-CEACAM-8/CD66b and PerCP- Anti CD14, all purchased from "R&D Systems", USA) according to methodology suggested by the manufacturer. Percentage of appropriate cell subsets (MDSC, M-MDSC, PMN-MDSC) was assessed on CytoFLEX S flow cytometer (Beckman Coulter, USA). Flow cytometry data files were analyzed using the "KALUZA Analysis 2.1 Software".

Statistical data processing was realized with GraphPad Prism 8 and the non-parametric Wilcoxon test.

Results and discussion

Experiments demonstrated that GdA resulted in elevated MDSC percentage in cytokine-induced human mononuclear cell cultures that was significant only at its low concentrations (0.2 and 2.0 μg/ml) that fits its pregnancy-related range. While analyzing the cell subsets, it was demonstrated that the rise in the MDSC level treated with GdA (0.2 and 2.0 μg/ml) resulted in significant PMN-MDSC increase, whereas the M-MDSC level was changed insignificantly. Therefore, GdA did not reliably affect the M-MDSC (Table 1). It was determined that high GdA concentration (50 μg/ml) corresponding to the pharmacological level conferred no significant effect on MDSC differentiation.

Cell viability in cultures was assessed following the propidium iodide staining. It was revealed that GdA at all concentrations did not affect cell viability in culture. Gating strategy according to light scattering parameters in FSC/SSC plot was applied to analyze solely live cells. Therefore, the effects obtained are related indeed to GdA direct influence on MDSC differentiation.

More recently, it became evident that the elevation of MDSC level also accompanied pregnancy. In 2014, it was found that the PMN-MDSC level was increased in peripheral blood of healthy pregnant women at all the stages of pregnancy as compared to non-pregnant subjects, while the M-MDSC amount remained unchanged [4]. Around the same time, it was revealed that patients with miscarriage had count of blood and endometrial MDSCs by 30% lower particularly in trimester I [6].

PMN-MDSC are known to accumulate in placenta of healthy pregnant women, while their number declines in spontaneous abortion. Placental PMN-MDSCs effectively suppress T-cell response

TABLE 1. GDA EFFECT ON MDSC PERCENTAGE IN MONONUCLEAR CELL CULTURES INDUCED BY IL-6 AND GM-CSF CYTOKINES, Me ($Q_{0.25}$-$Q_{0.75}$), n = 4

Cell type/GdA concentration	Control	GdA 0.2 μg/ml	GdA 2 μg/ml	GdA 50 μg/ml
MDSC % ($CD33^+CD11b^+$)	21.5 (15.2-26.1)	32.3# (24.8-38.5)	28.2# (21.5-37.4)	22.5 (16.7-30.2)
PMN-MDSC % ($CD33^+CD11b^+CD14^-CD66b^-$)	12.6 (7.8-15.7)	17.4# (12.6-24.3)	16.8# (11.7-22.1)	15.1 (7.8-21.3)
M-MDSC % $CD33^+CD11b^+CD14^+CD66b^+$)	6.2 (3.4-7.9)	7.6 (4.6-11.3)	6.9 (5.5-10.1)	5.8 (2.8-7.6)

Note. Percentages of cells in corresponding subsets from $HLA\text{-}DR^-Lin^-$ cells are presented. #, significant differences as compared with control by Wilcoxon U test ($p < 0.05$).

with subsequent polarization of $CD4^+$-lymphocytes to Th2-phenotype [4]. PMN-MDSC are suggested to play an essential role in inducing and supporting the tolerance to fetal antigens allowing to consider them as a promising target for therapeutic manipulation under the pregnancy complications.

Meanwhile, the question about exact nature of receptor mediating GdA effects remains open. Subsequent analysis of GdA mode of action showed that the surface L-selectin molecule on monocyte-macrophage lineage cells is a binding protein for GdA. Thus, the antibody against L-selectin reduced the GdA binding and GdA-induced IL-6 production [5]. In particular, Vijayan et al. in 2018 found potential receptors for GdA on human peripheral blood monocyte membrane.

SIGLEC-7 (sialic acid-binding immunoglobulin-like lectin 7, CD328) – receptor to GdA was identified using co-immunoprecipitation and flow cytometry. GdA was found under culture conditions to improve the expression of decidual macrophage markers IDO-1 and CD209 on GdA-polarized macrophages. SIGLEC-7 receptor blocks GdA-related biological effects on monocyte differentiation [11]. However, it is not clear through which receptor this occurred, and we can only speculate.

Therefore, we have initially demonstrated that GdA can regulate the MDSC differentiation primarily by increasing its polymorphonuclear subset (PMN-MDSC). Potentially, GdA possesses the potential to manipulate the MDSC level that, in turn, serve as the immune response suppressors.

References

1. Cui J., Liu Y., Wang X. The roles of glycodelin in cancer development and progression. *Front. Immunol., 2017, Vol. 8, 1685.* doi: 10.3389/fimmu.2017.01685.
2. Dixit A., Balakrishnan B., Karande A.A. Immunomodulatory activity of glycodelin: implications in allograft rejection. *Clin. Exp. Immunol., 2018. Vol. 192, no. 2, pp. 213-223.*
3. Gabrilovich D.I. Myeloid-derived suppressor cells. *Cancer Immunol. Res., 2017, Vol. 1, pp. 3-8.*
4. Köstlin N., Hofstädter K., Ostermeir A.L., Spring B., Leiber A., Haen S., Abele H., Bauer P., Pollheimer J., Hartl D., Poets C.F., Gille C. Granulocytic myeloid-derived suppressor cells accumulate in human placenta and polarize toward a Th2 Phenotype. *J. Immunol., 2016, Vol. 3, pp. 1132-1145.*
5. Lee C.L., Lam E.Y., Lam K.K., Koistinen H., Seppala M., Ng E.H., Yeung W.S., Chiu P.C. Glycodelin-A stimulates interleukin-6 secretion by human monocytes and macrophages through L-selectin and the extracellular signal-regulated kinase pathway. *J. Biol. Chem., 2012, Vol. 287, no. 44, pp. 36999-37009.*
6. Nair R.R., Sinha P., Khanna A., Singh K. Reduced myeloid-derived suppressor cells in the blood and endometrium is associated with early miscarriage. *Am. J. Reprod. Immunol., 2015, Vol. 73, no. 6, pp. 1046–7408.*
7. Ochanuna Z., Geiger-Maor A., Dembinsky-Vaknin A., Karussis D., Tykocinski M.L., Rachmilewitz J. Inhibition of effector function but not T cell activation and increase in FoxP3 expression in T cells differentiated in the presence of PP14. *PLoS One, 2010, Vol. 5, no. 9, e12868.* doi: 10.1371/journal.pone.0012868.
8. Petrunin D.D., Griaznova I.M., Petrunina Yu.A., Tatarinov Yu.S. Immunochemical identification of human placental organ specific alpha2-globulin and its concentration in amniotic fluid. *Bulletin of Experimental Biology and Medicine, 1976, no. 7, pp. 803-804.* (In Russ.)
9. Schneider M., Meister M., Muley T. Glycodelin as diagnostic and prognostic marker and for monitoring treatment of lung diseases. European patent application, Bulletin, 2016.06, EP2982765A1.
10. Schneider M.A., Granzow M., Warth A., Schnabel P.A., Thomas M., Herth F.J., Dienemann H., Muley T., Meister M. Glycodelin A new biomarker with immunomodulatory functions in non-small cell lung cancer. *Clin. Cancer Res., 2015, Vol. 21, no. 15, pp. 3529-3540.*
11. Vijayan M., Lee C-L., Chiu P.C.N., Lee K.F. P56 Glycodelin-A polarised human macrophages exhibit characteristics and functions similar to decidual macrophages. *Am. J. Reprod. Immunol., 2018, Vol. 80, pp. 81-82.*
12. Zhang W., Li J., Qi G., Tu G., Yang C., Xu M. Myeloid-derived suppressor cells in transplantation: the dawn of cell therapy. *J. Transl. Med., 2018, Vol. 16, 19.* doi: 10.1186/s12967-018-1395-9.

Авторы:

Заморина С.А. *– д.б.н., профессор ФГБОУ ВО «Пермский государственный национальный исследовательский университет»; ведущий научный сотрудник лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Authors:

Zamorina S.A., *PhD, MD (Biology), Professor, Perm State National Research University; Leading Research Associate, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences Perm, Russian Federation*

Тимганова В.П. *– к.б.н., научный сотрудник лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Timganova V.P., *PhD (Biology), Research Associate, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Бочкова М.С. *– к.б.н., научный сотрудник лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Bochkova M.S., *PhD (Biology), Research Associate, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Шардина К.Ю. *– инженер лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Shardina K.Yu., *Engineer, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Ужвиюк С.В. *– инженер лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Uzhviyuk S.V., *Engineer, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Храмцов П.В. *– к.б.н., доцент ФГБОУ ВО «Пермский государственный национальный исследовательский университет»; младший научный сотрудник лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Khramtsov P.V., *PhD (Biology), Associate Professor, Perm State National Research University; Junior Research Associate, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Кропанева М.Д. *– инженер лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Kropaneva M.D., *Engineer, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Раев М.Б. *– д.б.н., профессор ФГБОУ ВО «Пермский государственный национальный исследовательский университет»; ведущий научный сотрудник лаборатории экологической иммунологии, Институт экологии и генетики микроорганизмов Уральского отделения Российской академии наук, г. Пермь, Россия*

Rayev M.B., *PhD, MD (Biology), Professor, Perm State National Research University; Leading Research Associate, Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russian Federation*

Поступила 21.12.2020
Принята к печати 11.05.2021

Received 21.12.2020
Accepted 11.05.2021