The Role of Clycodelin in Conversion of CD11b+ Cells to MDSCs and Regulation of Their Functional Activity

K. Yu. Shardina[a, *], **S. A. Zamorina**[a, b], **M. S. Bochkova**[a, b], **V. P. Timganova**[a],
S. V. Uzhviyuk[a], **and M. B. Raev**[a, b]

[a] *Institute of Ecology and Genetics of Microorganisms, Branch of Perm Federal Research Center, Ural Branch,*
Russian Academy of Sciences, Perm, 614081 Russia
[b] *Faculty of Biology, Perm State National Research University, Perm, 614068 Russia*
***e-mail: Shardinak@gmail.com**

Abstract—Glycodelin (Gd) has pronounced immunomodulatory properties and participates in the development of immune tolerance during pregnancy. The role of recombinant Gd in physiological (0.2 and 2 µg/mL) and superphysiological (10 µg/mL) concentrations in the regulation of differentiation and functional activity of human myeloid-derived suppressor cells (MDSCs) was investigated in vitro. MDSCs were obtained from peripheral blood CD11b+ cells of healthy donors by two-step induction (IL-1β + granulocyte−monocyte colony-stimulating factor (GM-CSF) and lipopolysaccharide). The effect of Gd on the level of polymorphonuclear MDSCs (PMN-MDSCs) and monocyte MDSCs (M-MDSCs) was assessed. The intracellular level of indoleamine 2,3-dioxygenase (IDO) and arginase 1 (Arg1), as well as the cytokine profile in cultures of these cells, was measured. In general, the conversion of CD11b+ cells into MDSCs has the following features: as a result of cytokine induction, predominantly M-MDSCs are generated, but not PMN-MDSCs, and the level of Arg1 is practically not detected. It was found that Gd increased the number of M-MDSCs at concentrations of 2 and 10 µg/mL. It was shown that Gd did not affect the content of Arg1, but increased the number of MDSCs expressing IDO (10 µg/mL). Gd also modulated the cytokine profile of CD11b+ cells (at a physiological concentration of 2 µg/mL), suppressing IL-19, IL-26, and TWEAK/TNFsF12 production and, at a supraphysiological concentration, the production of IFN-α2 and IL-26.

Keywords: arginase 1, glycodelin, immune tolerance, indoleamine 2,3-dioxygenase, cell culture, myeloid suppressor cells, cytokines

DOI: 10.1134/S1990519X24700342

INTRODUCTION

Immune tolerance during pregnancy is the lack of an immune response in the mother to the developing fetus and placenta. Initially, it was believed that maternal−fetal tolerance is based on the regulation of the T-cell component of immunity (Saito et al., 2010), but it is now clear that this phenomenon is much more complex and affects innate immunity.

Innate immune cells (macrophages, innate lymphoid cells, natural killer (NK) cells) represent the largest population of decidual leukocytes and play an important role in reproduction. On the one hand, they counter the infectious threat to a healthy pregnancy, and on the other hand, they are involved in trophoblast invasion and spiral artery remodeling, controlling embryo implantation and normal development of the placental bed (Weng et al., 2003). Meanwhile, some innate immune cells, such as antigen-presenting cells, interact with T-cells and suppress the immune response to maintain an optimal immunological microenvironment for pregnancy development

at the maternal−fetal interface (Li et al., 2021b). Relatively recently, it became known that myeloid-derived suppressor cells (MDSCs), being innate immune cells, are generated during normal pregnancy (Ostrand-Rosenberg et al., 2017). The increase in MDSCs in the mother's body is mostly due to the subpopulation of polymorphonuclear (PMN) MDSCs (PMN-MDSCs); however, the concentration of monocyte (M) M-MDSCs also increases, albeit less significantly. In addition to being found in peripheral blood, MDSC levels have been shown to increase in both the placental decidua and the intervillous space (Köstlin-Gille, 2020). The level of MDSCs is also elevated in newborns in the first 2 months, and their number declines to the adult level by the sixth month of life (Gantt et al., 2014). Moreover, during pregnancy, MDSCs are found in the umbilical cord blood (Rieber et al., 2013).

It is now clear that MDSCs are involved in the maintenance of immune tolerance. The key mechanisms for the implementation of suppressor activity

are the expression of a number of surface markers, intracellular expression of arginase-1 (Arg1), indolemin-2,3-dioxygenase (IDO), and inducible NO synthase (iNOS) enzymes, as well as the production of a number of anti-inflammatory cytokines (Li et al., 2021a). The MDSC phenotype at this stage is defined as Lin$^-$HLA-DR$^-$CD33$^+$CD11b$^+$. Since the cell generation of cells in vitro is a complex methodological task, the development of protocols for obtaining MDSCs is still underway.

The regulatory continuum formed by pregnancy proteins also contributes to the development of immune tolerance, including at the MDSC level. It is known that trophoblastic β1-glycoprotein (TBG) and α-fetoprotein (AFP) are able to regulate the differentiation and functional activity of these cells in vitro (Timganova et al., 2023; Shardina et al., 2023).

A key protein of pregnancy, amniotic glycodelin (GdA) (synonyms: PP14, PAEP, and α-2 microglobulin of fertility), has pronounced immunomodulatory properties (Uchida et al., 2013). The biological activity of GdA is associated with the interaction of its unique carbohydrate chains with membrane sialic acid receptors (selectins, Ig-binding-type lectins) common for various cell types of the mother–fetal interface (Lam et al., 2011).

Thus, the aim of the study was to investigate the effect of recombinant Gd on MDSC differentiation in vitro, assessing a number of indicators of their functional activity.

MATERIALS AND METHODS

Peripheral blood mononuclear cells (PBMCs) from apparently healthy donors (22- to 42-year-old women, $n = 7$) were used in this work with their consent.

The maximum concentration of GdA in the blood serum by the end of the first trimester reaches 2 µg/mL in the second trimester its concentration is 0.2 µg/mL, and it is further declines by the third trimester (Halttunen et al., 2000). In the experiments, we used physiological concentrations of recombinant Gd (source *E. coli*, MyBioSource, United States), which corresponded to the level of GdA in the serum of pregnant women, as well as a concentration exceeding these values, 10 µg/mL. Despite the fact that recombinant rather native GdA was utilized, it was important to monitor the possible prospects for the effects of the entire protein for its application in various pathologies in which the manipulation of the MDSC level is important.

Objects of Research

PBMC were obtained by centrifugation on Diacoll density gradient (ρ = 1.077 g/cm^3, Dia-M). CD11b$^+$ cells were isolated from donor blood PBMCs by immunomagnetic separation for further cultivation. MACSiBead magnetic spheres with CD11b antibodies and an MS separation column (Miltenyi Biotec, Germany) were applied.

MDSC Cultivation

The obtained cells were seeded into 96-well plates at concentration of 10^6/mL in complete growth medium (RPMI-1640, 10% FBS, 10 mM HEPES (ICN Ph., United States), 2 mM L-glutamine (ICN Ph., United States), and 100 µg/mL penicillin–streptomycin–amphotericin (100 µL per 10 mL medium; BI, Israel). On the first day, 20 ng/mL GM-CSF (Miltenyi Biotec, Germany) were added to the culture plates after which the cells were cultured in a CO$_2$ incubator for 2 days. After the first stage completion, the cells were activated by addition of cytokine IL-1β (20 ng/mL; Miltenyi Biotec, Germany) and LPS (0.1 µg/mL; Sigma-Aldrich, United States). The next day, Gd was added at concentrations of 0.2, 2.0 or 10.0 µg/mL. After the addition of activation molecules and Gd, the cells were cultured for another 3 days under the same conditions. In general, the cells were cultured for 7 days, after which the cells were harvested with accutase, with additional washing of the wells with cold Dalbecco's phosphate-buffered saline (DPBS) (Thermo Fisher Scientific, United States) for better harvesting.

Flow Cytometry

After cultivation for 7 days, the cells were stained with antibodies labeled with fluorochromes (HLA-DR–Alexa Fluor 750, anti-CD33–APC, anti-CD11b–Alexa Fluor 405, anti-CD66b–PE, and anti-CD14–PerCP; R&D Systems, United States). To exclude the possible presence of lymphocytes and NK cells, anti-CD19–AF700, anti-CD56–AF700, and anti-CD3–AF700 (Lin) were used. FMO (fluorescence minus one) samples were used as controls to determine negative populations. Experiments were performed on a Cytoflex S flow cytometer (Beckman Coulter, United States).

Gating was done according to side scattering (SSC) and forward scattering (FSC) data. Living cells (ZA$^-$) were identified with Zombie Aqua dye (ZA; BioLegend, United States), and the region of cells not carrying linear markers (Lin) and HLA-DR was defined in this gate. A two-parameter plot was then generated for CD33 and CD11b, showing MDSC population (Lin$^-$HLA-DR$^-$CD33$^+$CD11b$^+$). To analyze the subpopulation composition in MDSC gate, CD66b (PMN-MDSC) and CD14 (M-MDSC) markers were additionally determined.

Assessment of Arg1 and IDO Levels in MDSCs

In addition to surface markers, the intracellular content of Arg1 and IDO enzymes was assessed by cell permeabilization and monoclonal antibodies to IDO and Arg1 (R&D Systems, United States). The level of enzymes in PMN-MDSCs and M-MDSCs was also estimated by flow cytometry.

Analysis of the Cytokine Profile in CD11b$^+$ Cell Cultures

The level of cytokines was determined in cell culture supernatants with a Bio-Plex Pro Human Inflammation panel 1, 37-plex commercial kit (Bio-Rad, United States) according to the manufacturer's instructions. The results were recorded with a Bio-Plex MAGPIX multiplex analysis system (BioRad, United States). The data were processed with the Belysa software. The five-parameter logistic (5PL) analysis method was used to plot standard curves. The results were expressed in pg/mL.

Statistical Analysis

The GraphPad Prism 8 software was used to process the results. The Friedman test, the nonparametric analogue of repeated analysis of variance, was used to compare dependent samples. Differences were considered statistically significant at $p < 0.05$. Data are presented as median (Me), lower, and upper quartiles (Q1−Q3). To assess the correlation between all measured values, Spearman's method was used.

RESULTS AND DISCUSSION

A small number of MDSCs (1−3%) are generated in cultures of peripheral mononuclear cells, and so it is logical to isolate cells according to one of the two main MDSC markers—CD33 (Lechner et al., 2010) or CD11b (Shardina et al., 2023).

MDSC isolation from CD11b$^+$ cells revealed that cytokines and LPS were able to significantly increase the level of MDSCs in our experimental system. At the same time, no effect of Gd on the conversion of CD11b$^+$ cells to MDSCs was detected (Fig. 1).

Taking it into account that MDSCs are divided into two main subpopulations (PMN-MDSCs with CD33^{low}CD11b$^+$CD14$^-$CD15$^+$/CD66b$^+$ phenotype and M-MDSCs with CD33$^+$CD11b$^+$CD14$^+$HLA-DR$^{-/low}$CD15$^-$/CD66b$^-$ phenotype), we assessed the effect of Gd on these cells.

It was found that, at the subpopulation level, Gd did not affect the PMN-MDSC pool, but significantly increased the level of M-MDSCs in the MDSC population at concentrations of 2 and 10 µg/mL (Table 1). It is important to note that, in fact, our experimental scheme does not allow the generation of granulocytic MDSCs, which may be due to two reasons: an insufficient number of granulocytic cells in PBMCs, as well
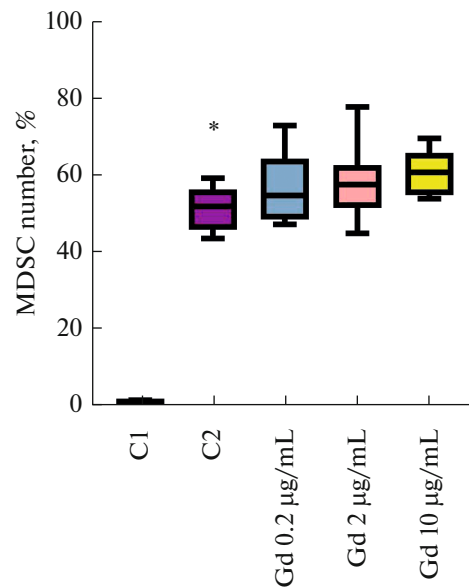
Fig. 1. Effect of glycodelin (Gd) at different concentrations on the conversion of CD11b$^+$ cells (from mononuclear cells of donor peripheral blood) to MDSC phenotype. Presented are medians, interquartile range (Q1−Q3, column borders and maximum values (vertical bars); vertically: the number of living Lin$^-$HLA-DR$^-$ cells in the gate; (C1 is control, without adding cytokines and glycodelin to the culture medium; C2 is control 2, adding only cytokines to the medium); difference is significant at $p < 0.05$: * between C1 and C2 ($n = 7$, Friedman test).

as an inappropriate set of stimulators for PMN-MDSCs used in experiments. Thus, Gd enhanced the generation of M-MDSCs from CD11b$^+$ cells at concentrations of 2 and 10 µg/mL.

To determine MDSCs, it is important to understand that to date, no unique marker has been found in these cells that can accurately establish cellular identity in this group. For this reason, to determine MDSCs, a number of enzymes involved in suppressing the activity and proliferation of immune cells are additionally assessed. For these purposes, in the course of research, we measured the intracellular level of two such enzymes—Arg1 and IDO. MDSCs mediate the inhibitory effect through multiple mechanisms, including depletion of L-arginine (by arginase-1 gene expression) and L-tryptophan (by IDO gene expression) (Lim et al., 2020).

It was found that Arg1 in MDSCs was expressed by a small number of cells (less than 1%; Table 2). Experiments showed that Gd did not affect the content of arginase-1 in the MDSC pool (Table 2). However, given that Arg1 is predominantly expressed by the PMN-MDSC subpopulation, the level of which has been extremely low in our experimental design, the main conclusion may be related to this. At the same time, IDO was actively expressed in MDSCs. It was demonstrated that Gd at concentration of 10 µg/mL increased IDO expression in MDSCs (Fig. 2).

Table 1. Effect of Gd on the number of cells with MDSC phenotype (PMN-MDSCs and M-MDSCs) producing Arg1 enzyme

Treatment	MDSC no., %
Control before induction (without cytokines and Gd)	0.150 (0.025−0.447)
Induction (GM-CSF, IL-1β, and LPS)	
Control (without Gd)	0.920 (0.212−1.538)
Gd, 0.2 μg/mL	0.510 (0.177−0.985)
Gd, 2 μg/mL	0.215 (0.022−1.143)
Gd, 10 μg/mL	0.905 (0.115−2.745)

Data are presented as median (Me), lower, and upper quartiles (Q1−Q3); $n = 7$; the differences are significant at $p < 0.05$: [a] compared to the control (without Gd) and [b] between the control before induction and the induction control (without Gd).

Table 2. Effect of Gd on Arg1 production in MDSCs after CD11b^{+} cell cultivation for 7 days

Treatment	Cell no., %	
	PMN-MDSC	M-MDSC
C1 (without cytokines and Gd)	0.040 (0.020−0.095)	0.090 (0.065−0.165)
Induction (GM-CSF, IL-1β and LPS)		
C2 (without Gd)	0.375 (0.020−1.713)	7.835[a] (0.767−9.608)
Gd 0.2 μg/mL	0.445 (0.140−3.405)	23.87 (17.90−29.49)
Gd 2 μg/mL	0.420 (0.202−0.982)	46.90[b] (39.11−51.18)
Gd 10 μg/mL	0.455 (0.077−2.773)	59.74[b] (50.09−72.76)

Data are presented as median (Me), lower and upper quartiles (Q1−Q3); ($n = 7$); differences are significant at $p < 0.05$: [a] compared to C1 (C1, control, without adding cytokines and glycodelin to the culture medium; C2, control 2, adding only cytokines to the medium) and [b] compared to C2.

A direct correlation was found ($r = 0.80$, $p < 0.05$) between IDO expression and MDSC number in the culture. IDO is known to catabolize tryptophan, creating a local deficiency of this essential amino acid and suppressing T-cell proliferation and function (Fallarino et al., 2002). Thus, high concentrations of Gd may contribute to the suppression of the immune response through increased IDO expression in myeloid cells.

Regarding the cytokine profile in supernatants of CD11b^{+} cells induced into the MDSC phenotype, it was shown that Gd had no effect on the following cytokines: APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, IFN-β, IFN-γ, IL-2, sIL-6Rα, IL-8, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-20, IL-22, IL-27 (p28), IL-28A/IFN-λ2, IL-29/IFN-λ1, IL-32, IL-34, IL-35, LIGHT/TNFSF14, MMP-1, MMP-2, MMP-3, osteocalcin, osteopontin, pentraxin-3, sTNF-R1, sTNF-R2, and TSLP. However, Gd (at different concentrations) reduced the production of the following cytokines: IFN-α2 (10 μg/mL), IL-26 (2 and 10 μg/mL), IL-19 (2 μg/mL), TWEAK/TNFsF12 (2 μg/mL) (Fig. 3). Thus, Gd at the physiological concentration of 2 μg/mL suppressed IL-19, IL-26 and TWEAK/TNFsF12 production, and it suppressed IFN-α2 and IL-26 production at a supraphysiological concentration

IFN-α2 is a glycoprotein with antiviral and immunomodulatory activity. It is known that IFN-α 2 promotes the proliferation of naïve CD8 cells and inactivation of regulatory T lymphocytes. IL-26, like IFN-α2, is a pro-inflammatory cytokine. IL-19 has long been considered an anti-inflammatory molecule homologous to IL-10, but IL-19 is now believed to be a regulatory cytokine. On the one hand, IL-19 supports the production of Th2 cytokines, and, on the other hand, through IL-6 and TNF-α, it has pro-inflammatory properties (Savasan et al., 2012). TWEAK/TNFsF12 is a cytokine to the receptor for the TNFSF12 factor of the TNF superfamily of factors, which is capable of inducing apoptosis in cells and regulating angiogenesis (Wiley et al., 2001). Gd is

able to suppress the production of these cytokines in vitro, forming an anti-inflammatory cumulative vector.

In general, the trend towards a decreased level of a number of pro-inflammatory cytokines may fit into the idea that GD has immunoregulatory properties, which are aimed at maintaining immune tolerance.

First of all, it is worth noting that this of cytokines pattern was synthesized by $CD11b^+$ cells induced into MDSC phenotype. In our experimental design, about half of the cells were identified as MDSCs. Therefore, to reveal the relationship between cytokines and MDSCs, a correlation analysis was performed between the percentage of MDSCs and cytokines. A significant correlation between cytokines and MDSC level was not found.

However, a direct correlation between IDO expression and IL-26 production ($r = 0.800$, $p < 0.05$) and an inverse relationship between IDO level and IFN-α2 production ($r = -0.80$, $p < 0.05$) were found under the influence of 10 µg/mL Gd. There is no known relationship between IDO levels and IL-26, but IFN I can induce IDO expression in cells (Fallarino et al., 2002). In our experiments, Gd at a concentration of 10 µg/mL increased IDO level while reducing cellular IFN-α2 production, suggesting that IDO induction was not due to autogenous IFN-α2 production.

A similar effect of GdA was demonstrated by Vijayan et al. (2018): in their experiments, Gd increased the expression of IDO markers of decidual macrophages, and a block of the Siglec-7 receptor on cells neutralized the biological effect of Gd on monocyte differentiation, which indirectly indicated that Siglec-7 may operate as a GdA receptor. Interestingly, a later study aimed to find receptors for Siglec on MDSCs also in the glioma tumor microenvironment (Santegoets et al., 2019). It turned out that both subsets of MDSCs expressed Siglec-3, -5, -7, and -9, with a higher level of Siglec-3, -7, and -9 being detected on the surface of M-MDSCs and a higher level of Siglec-5 on the surface of PMN-MDSCs. A similar Siglec expression pattern was found in MDSCs from healthy donors. These studies suggest that the interaction between Gd and MDSCs occurs precisely due to the Siglec-7 receptor, the expression of which is higher in M-MDSCs.

As part of our study of the immunomodulatory ability of Gd, we examined its effect on the conversion of mononuclear cells to MDSCs. It was shown that, at concentrations corresponding to healthy pregnancy (0.2 and 2 µg/mL), Gd increased the number of MDSCs in induced cultures of human mononuclear cells. Subset analysis revealed that this effect was due to an enhanced PMN-MDSC level, while the M-MDSC level remained unchanged. This result can be interpreted as a fetoprotective effect of Gd, since an increased level of PMN-MDSCs is associated with suppression of the immune response to paternal anti-
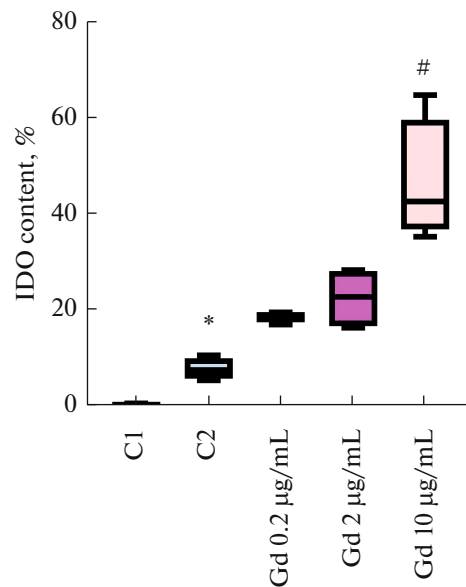
Fig. 2. Indoleamine 2,3-dioxygenase (IDO) content in MDSC cells cultivated with glycodelin (Gd) at different concentrations. Medians, interquartile range (Q1–Q3), minimum and maximum values are presented; the difference is significant at $p < 0.05$ (Friedman test): * between the induction control (C1, culture without the addition of cytokines and Gd) and C2 (culture with the addition of cytokines and Gd); # difference from C2.

gens (Zamorina et al., 2021). In the present research, we demonstrated that Gd is also able to modulate M-MDSCs, directly stimulating their suppressor activity.

Regarding the concentrations of Gd used in the research, it can be concluded that it is obvious that high concentrations of Gd had more pronounced effects on MDSCs, which can provide grounds for determining the immunopharmacological potential of this protein. At the same time, at the concentration of 2 µg/mL, corresponding to the second of third trimesters of pregnancy, Gd induced the generation of M-MDSCs and the cytokine profile, reducing the production of IL-19, IL-26, and TWEAK/TNFsF12.

It is important to note that other fetoplacental proteins can also stimulate MDSC differentiation in vitro: for example, we showed TBG increased their number, mainly due to M-MDSCs (Timganova et al., 2023), and AFP increased the number of M-MDSCs and IDO expression by these cells, in addition to reducing the level of IL-19 (Shardina et al., 2023).

Thus, when studying the effect of Gd on the generation of MDSCs in vitro, the following was established.

(1) Cytokine induction of $CD11b^+$ cells generated predominantly M-MDSCs but not PMN-MDSCs: the level of Arg1 was practically not detected.

(2) Gd at concentrations of 2 and 10 µg/mL increased the number of M-MDSCs in cell cultures.
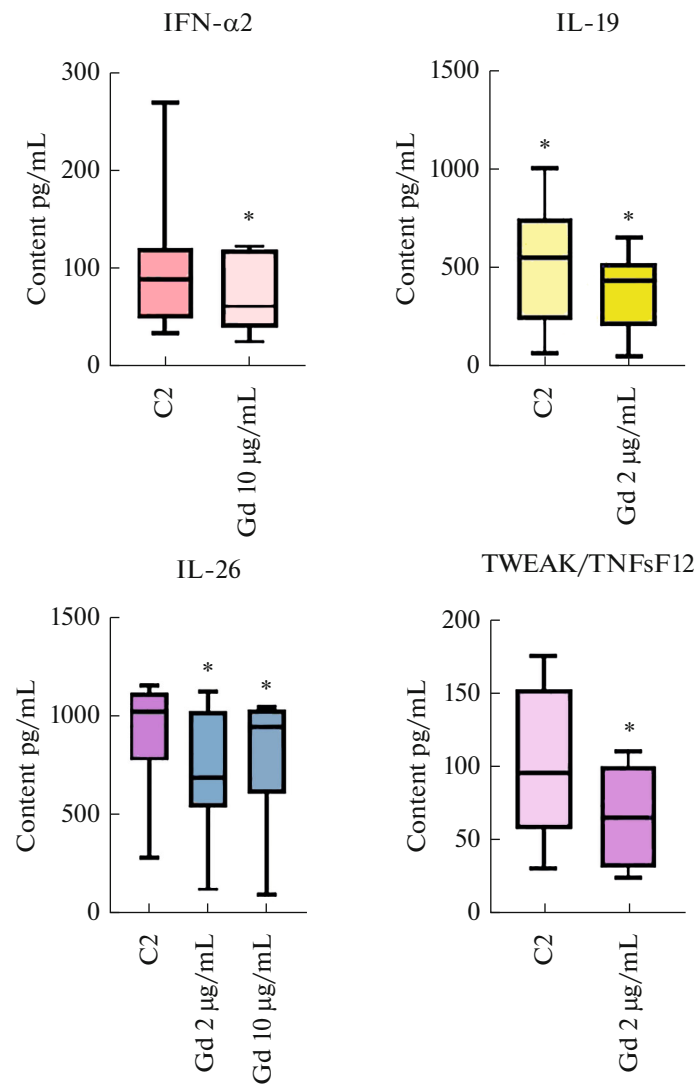
Fig. 3. Production of IFN-α2, IL-19, IL-26, and TWEAK/TNFsF12 cytokines by CD11b$^+$ cells induced into the MDSC phenotype with glycodelin (Gd) at different concentrations. The content of cytokines was measured in supernatants of cultured MDSCs. Medians, interquartile range (Q1−Q3, column boundaries), minimum and maximum values (vertical bars) are presented. * Difference in median values ($n = 7$) is significant at $p < 0.05$ compared to C2 (culture with the addition of cytokines and Gd; nonparametric Friedman test).

(3) Gd increased the number of MDSCs expressing IDO (10 μg/mL).

(4) At a physiological concentration (2 μg/mL), Gd modulated the cytokine pattern of CD11b$^+$ cells, suppressing the production of IL-19, IL-26, and TWEAK/TNFsF12: at a supraphysiological concentration (10 μg/mL), it inhibited the production of IFN-α 2 and IL-26.

Thus, fetoplacental proteins are able to directly regulate the generation of MDSCs, which, under pregnancy conditions, contributes to the development of immune tolerance.

ABBREVIATIONS AND NOTATION

AFP	α-fetoprotein
LPS	lipopolysaccharide
MPC	peripheral blood mononuclear cells
TBG	trophoblastic β-1-glycoprotein
Arg1	arginase-1
GdA	amniotic glycodelin
Gd	recombinant glycodelin
DPBS	Dalbecco's phosphate buffered saline solution

GM-CSF	granulocyte—monocyte colony-stimulating factor
IDO	indoleamine 2,3-dioxygenase
IL	interleukin
MDSC	myeloid-derived suppressor cell
M-MDSC	monocytic MDSC
PMN-MDSC	polymorphonuclear MDSC

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experiments were performed in accordance with the principles of biomedical ethics as set out in the 1964 Declaration of Helsinki and its subsequent amendments. They were also approved by the Ethics Committee of the Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences (Perm, Russia) (IRB00010009) dated February 15, 2022, protocol no. 15. Each study participant gave voluntary written informed consent after receiving explanations about the potential risks and benefits, as well as the nature of the upcoming research.

Animal experiments were done in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (http://oacu.od.nih.gov/regs/index.htm).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

Fallarino, F., Grohmann, U., Vacca, C., Bianchi, R., Orabona, C., Spreca, A., Fioretti, M.C., and Puccetti, P., T cell apoptosis by tryptophan catabolism, *Cell Death Differ.*, 2002, vol. 9, no. 10, p. 1069.
https://doi.org/10.1038/sj.cdd.4401073

Gantt, S., Gervassi, A., Jaspan, H., and Horton, H., The role of myeloid-derived suppressor cells in immune ontogeny, *Front. Immunol.*, 2014, vol. 5, p. 103650.
https://doi.org/10.3389/fimmu.2014.00387

Halttunen, M., Kämäräinen, M., and Koistinen, H., Glycodelin: a reproduction-related lipocalin, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.*, 2000, vol. 1482, nos. 1—2, p. 149.
https://doi.org/10.1016/S0167-4838(00)00158-8

Köstlin-Gille, N. and Gille, Ch., Myeloid-derived suppressor cells in pregnancy and the neonatal period, *Front. Immunol.*, 2020, vol. 11, p. 584712.
https://doi.org/10.3389/fimmu.2020.584712

Lam, K.K.W., Chiu, P.C.N., Lee, C.-L., Pang, R.T.K., Leung, C.O.N., Koistinen, H., Seppala, M., Ho, P.-C., and Yeung, W.S.B., Glycodelin-A protein interacts with Siglec-6 protein to suppress trophoblast invasiveness by down-regulating extracellular signal-regulated kinase (ERK)/c-Jun signaling pathway, *J. Biol. Chem.*, 2011, vol. 286, no. 43, p. 37118.
https://doi.org/10.1074/jbc.M111.233841

Li, K., Shi, H., Zhang, B., Ou, X., Ma, Q., Chen, Yu., Shu, P., Li, D., and Wang, Yo., Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer, *Signal Transduction Targeted Ther.*, 2021a, vol. 6, no. 1, p. 362.
https://doi.org/10.1038/s41392-021-00670-9

Li, W.X., Xu, X.H., and Jin, L.P., Regulation of the innate immune cells during pregnancy: an immune checkpoint perspective, *J. Cell. Mol. Med.*, 2021b, vol. 25, p. 10362.
https://doi.org/10.1111/jcmm.17022

Lim, H.X., Kim, T.S., and Poh, Ch.L., Understanding the differentiation, expansion, recruitment and suppressive activities of myeloid-derived suppressor cells in cancers, *Int. J. Mol. Sci.*, 2020, vol. 21, no. 10, p. 3599.
https://doi.org/10.3390/ijms21103599

Ostrand-Rosenberg, S., Sinha, P., Figley, Ch., Long, R., Park, D., Carter, D., and Clements, V.K., Frontline science: myeloid-derived suppressor cells (MDSCs) facilitate maternal—fetal tolerance in mice, *J. Leukocyte Biol.*, 2017, vol. 101, no. 5, p. 1091.
https://doi.org/10.1189/jlb.1HI1016-306RR

Rieber, N., Gille, C., Köstlin, N., Schäfer, I., Spring, B., Ost, M., Spieles, H., Kugel, H.A., Pfeiffer, M., Heininger, V., Alkhaled, M., Hector, A., Mays, L., Kormann, M., Zundel, S., Fuchs, J., Handgretinger, R., Poets, C.F., and Hartl, D., Neutrophilic myeloid-derived suppressor cells in cord blood modulate innate and adaptive immune responses, *Clin. Exp. Immunol.*, 2013, vol. 174, no. 1, p. 45.
https://doi.org/10.1111/cei.12143

Saito, Sh., Nakashima, A., Shima, T., and Ito, M., Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy, *Am. J. Reprod. Immunol.*, 2010, vol. 63, no. 6, p. 601.
https://doi.org/10.1111/j.1600-0897.2010.00852.x

Santegoets, K.C.M., Gielen, P.R., Büll, Ch., Schulte, B.M., Kers-Rebel, E.D., Küsters, B., Bossman, S.A.J.F.H., Ter Laan, M., Wesseling, P., and Adema, G.J., Expression profiling of immune inhibitory Siglecs and their ligands in patients with glioma, *Cancer Immunol., Immunother.*, 2019, vol. 68, no. 6, p. 937.
https://doi.org/10.1007/s00262-019-02332-w

Savasan, Z.A., Chaiworapongsa, T., Romero, R., Hussein, Yo., Kusanovic, J.P., Xu, Yi., Dong, Zh., Kim, Ch.J., and Hassan, S.S., Interleukin-19 in fetal systemic inflammation, *J. Maternal—Fetal Neonatal Med.*, 2012, vol. 25, no. 7, p. 995.
https://doi.org/10.3109/14767058.2011.605917

Shardina, K., Timganova, V., Bochkova, M., and Uzhviyuk, S., Generation of human myeloid-derived suppressor cells from CD11b$^+$ cells in vitro, *Science and Global Challenges of the 21st Century—Innovations and Technologies in*

Interdisciplinary Applications, Perm Forum 2022. Lect. Not. Netw. Syst., Isaeva, E. and Rocha, Á., Eds., Cham: Springer Nature Switzerland, 2023, vol. 622, pp. 539−547.
https://doi.org/10.1007/978-3-031-28086-3_49

Timganova, V.P., Shardina, K.Yu., Bochkova, M.S., Uzhviyuk, S.V., Usanina, D.I., and Zamorina, S.A., Effect of pregnancy-specific β1-glycoprotein on myeloid-derived suppressor cell differentiation, *Med. Immunol.* (Russia), 2023, vol. 25, no. 3, p. 513.
https://doi.org/10.15789/1563-0625-EOP-2838

Uchida, H., Maruyama, T., Nishikawa-Uchida, S., Miyazaki, K., Masuda, H., and Yoshimura, Ya., Glycodelin in reproduction, *Reprod. Med. Biol.*, 2013, vol. 12, no. 3, p. 79.
https://doi.org/10.1007/s12522-013-0144-2

Vijayan, M., Lee, C.-L., Chiu, P.C.N., and Lee, K.F., Glycodelin-A polarised human macrophages exhibit characteristics and functions similar to decidual macrophages, *Am. J. Reprod. Immunol.*, 2018, vol. 80, p. 81.
https://doi.org/10.1111/aji.55_12984

Weng, J., Couture, C., and Girard, S., Innate and adaptive immune systems in physiological and pathological pregnancy, *Biology*, 2003, vol. 12, no. 3, p. 402.
https://doi.org/10.3390/biology12030402

Wiley, S.R., Cassiano, L., Lofton, T., Davis-Smith, T., Winkles, J.A., Lindner, V., Liu, H., Daniel, T.O., Smith, C.A., and Fanslow, W.C., A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis, *Immunity*, 2001, vol. 15, no. 5, p. 837.
https://doi.org/10.1016/s1074-7613(01)00232-1

Zamorina, S.A., Timganova, V.P., Bochkova, M.S., Shardina, K.Yu., Uzhviyuk, S.V., Khramtsov, P.V., Kropaneva, M.D., and Rayev, M.B., Role of glycodelin in regulation of myeloidderived suppressor cell differentiation, *Med. Immunol.* (Russia), 2021, vol. 23, no. 4, p. 641.
https://doi.org/10.15789/1563-0625-ROG-2209

Translated by I. Fridlyanskaya