РЕГУЛЯЦИЯ ЦИТОКИНОВОГО ПРОФИЛЯ NK-КЛЕТОК ФАКТОРАМИ МИКРООКРУЖЕНИЯ, ХАРАКТЕРНЫМИ ДЛЯ БЕРЕМЕННОСТИ

Гребенкина П.В.[1], Михайлова В.А.[1], Беспалова О.Н.[1], Сельков С.А.[1, 2], Соколов Д.И.[1, 2]

[1] ФГБНУ «Институт акушерства, гинекологии и репродуктологии имени Д.О. Отта», Санкт-Петербург, Россия
[2] ФГБОУ ВО «Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова» Министерства здравоохранения РФ, Санкт-Петербург, Россия

Резюме. Децидуальные NK-клетки обладают отличными фенотипическими и функциональными характеристиками по сравнению с периферическими NK-клетками. Однако механизмы, лежащие в основе развития этих уникальных свойств, остаются малоизученными. Предполагается, что клетки макроокружения оказывают как прямое, так и косвенное влияние на NK-клетки в матке, модулируя их уровень «агрессии» по отношению к тканям плода, включая клетки трофобласт.

Одним из механизмов регуляции NK-клеток является выделение цитокинов. Трофобласты, как и другие элементы микроокружения, продуцируют цитокины. Связываясь с рецепторами на поверхности клеток-мишеней, они изменяют поведение NK-клеток. В результате NK-клетки сами могут выделять цитокины, которые, в свою очередь, влияют на поведение других клеток. Как упоминалось ранее, недостаточно данных о причинах и механизмах, лежащих в основе изменений характеристик NK-клеток в матке. Тем не менее эти данные могут стать основой для создания более точной клеточной модели взаимодействия между клетками плода и иммунной системой матери. Кроме того, они могут послужить основой для разработки инструментов диагностики репродуктивных проблем.

Целью исследования – изучение цитокинового профиля NK-клеток (продукция TNFα, TGF-β, IFNγ, RANTES, IL-10, VEGF) под влиянием цитокинов, связанных с беременностью, – TNFα, IFNγ, TGF-β1, IL-15, IL-18 или IL-10.

Уровни этих цитокинов в кондиционированных средах, полученных после культивирования NK-клеток, были измерены с помощью проточной цитометрии. Было обнаружено, что TGF-β1, секретируемый клетками трофобласта, обладает способностью регулировать цитокиновый профиль NK-клеток. Под его воздействием уровни IFNγ, IL-10 и RANTES в средах, полученных из культуры NK-клеток, были снижены.

Основываясь на этих результатах, можно сделать вывод, что существует система, которая контролирует активность NK-клеток через сеть цитокинов. Эти данные указывают на потенциальную возможность использования TGF-β1 для моделирования взаимодействия между NK-клетками и трофобластами *in vitro*.

Ключевые слова: NK-клетки, цитокины, беременность, трофобласт, TGF-β, межклеточные взаимодействия, микроокружение

Адрес для переписки:
Гребенкина Полина Владимировна
ФГБНУ «Институт акушерства, гинекологии и репродуктологии имени Д.О. Отта»
199034, Россия, Санкт-Петербург, Менделеевская линия, д. 3.
Тел.: 8 (905) 802-85-58.
E-mail: grebenkinap@gmail.com

Address for correspondence:
Polina V. Grebenkina
D. Ott Research Institute of Obstetrics, Gynecology and Reproductology
3 Mendeleevskaya Line
St. Petersburg
199034 Russian Federation
Phone: +7 (905) 802-85-58.
E-mail: grebenkinap@gmail.com

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REGULATION OF THE CYTOKINE PROFILE OF NK CELLS BY THE MICROENVIRONMENT FACTORS TYPICAL FOR PREGNANCY

Grebenkina P.V.[a], Mikhailova V.A.[a], Bespalova O.N.[a], Selkov S.A.[a, b], Sokolov D.I.[a, b]

[a] *D. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation*
[b] *First St. Petersburg State I. Pavlov Medical University, St. Petersburg, Russian Federation*

Abstract. Decidual NK cells exhibit distinct phenotypic and functional characteristics as compared to peripheral NK cells. However, the mechanisms underlying development of these unique properties remain poorly understood. The cells in microenvironment are known to exert both direct and indirect influence on NK cells within uterus, modulating their level of "aggressiveness" towards fetal tissues, including trophoblasts. Cytokine release presents a remote regulatory tool for the NK cells. Trophoblasts produce cytokines like as other components of the microenvironment. These cytokines bind the receptors on surface of target cells thud changing the behavior of NK cells. As a result, NK cells may release the own cytokines, which, in turn, influence the behavior of other cells. As mentioned above, there is a lack of data on causes and mechanisms behind the changes in characteristics of NK cells in uterus. Nevertheless, this data can lay the foundation for designing a more accurate cellular model of interactions between fetal cells and maternal immune system. Moreover, it may serve as a basis for developing diagnostic tools for reproductive issues.

The aim of our study was to investigate changes in cytokine profile of NK cells, in particular, their production of TNFα, TGF-β, IFNγ, RANTES, IL-10, and VEGF under the influence of cytokines associated with pregnancy, i.e., TNFα, IFNγ, TGF-β1, IL-15, IL-18, or IL-10. The levels of these cytokines in the culture media conditioned by NK cells were measured using flow cytometry. TGF-β1, produced by trophoblasts was found to have the ability of regulating cytokine secretion by NK cells. The levels of IFNγ, IL-10, and RANTES in the media derived from NK cell culture have been decreased under its influence.

On the basis of these findings, one may propose the existence of a regulatory system that controls activity of NK cells via the cytokine network. These data suggest a potential for using TGF-β1 to model *in vitro* interactions between NK cells and trophoblasts.

Keywords: NK cells, cytokines, pregnancy, trophoblast, TGF-β, intercellular communication, microenvironment

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Introduction

NK cells are found in peripheral blood, but there are also local populations of these cells with special properties. One such population is decidual NK cells, which are found in the uteroplacental contact zone. These cells have a different phenotype and function compared to NK cells in peripheral blood [6].

One of the mechanisms of long-distance regulation of natural killer (NK) cells is cytokine secretion. The primary population of cells that influence NK cells is trophoblasts, but other cells such as uterine macrophages and endometrial cells also produce cytokines in the context of maternal-fetal interaction. Cytokines bind to receptors on cell membranes, triggering signaling cascades that result in changes in NK cell function, including cytokine secretion, which in turn allows NK cells to influence other cells. The cytokines under consideration in this study were detected in the region of uterine-placental interaction – IL-15, IL-18, IL-10, TGF-β, TNFα, IFNγ, RANTES VEGF [4, 7, 8, 15]. Data on possible uterine-placental cells that produce these cytokines are presented in Table 1.

The intricate molecular mechanisms governing the regulation of natural killer (NK) cell functions by pregnancy-specific cytokines remain underexplored. Therefore, the objective of this study was to elucidate the nature of alterations in the cytokine profile of NK cells in response to microenvironmental cues.

Materials and methods

Cell cultures

This study was performed using the NK-92 cell line (ATCC, USA), which reflect the main characteristics of and natural killer cells, respectively. The cells

TABLE 1. LIST OF CYTOKINES ANALYZED IN THE RESEARCH AND THEIR EFFECTS

Cytokine	Cell-producer	Effect
IL-15	Trophoblast cells, endometrial cells, placental macrophages	Regulation of effector functions of NK cells, stimulation of invasion and proliferation of trophoblast cells
IL-18	Decidual stromal cells	Induces cytotoxicity proteins expression by uterine, natural killer (uNK) cells
IL-10	NK cells, trophoblast cells	Inducing the effector functions of NK cells, stimulating the expression of the protective molecule HLA-G on the surface of trophoblast cells, anti-invasive effect on trophoblast cells
TGF-β	NK cells, trophoblast cells, T regulatory cells	Reduction of cytotoxicity of NK cells, regulation of trophoblast cell invasion
TNFα	NK cells	Enhancement of effector functions of NK cells, invasive effect on trophoblast cells
IFNγ	NK cells, trophoblast cells	Enhancement of effector functions of NK cells, inhibition of trophoblast cell invasion
RANTES	NK cells, trophoblast cells	Stimulation of NK cell proliferation and migration, increased migration and invasion of trophoblast cells
VEGF	Decidual NK cells, endometrial cells	Regulation of vascular growth and formation at the uteroplacental contact boundary. Promotes the acquisition of an endothelial phenotype by the extracellular trophoblast, which ensures the successful embedding of the trophoblast into the experimental vessel model

were cultured together with the manufacturer's recommendations in a humid environment at 37 °C, 5% CO_2. The viability of the cells was controlled by trypan blue exclusion, it was 95±3.4%.

Cytokines

The following inductors were used: TNFα (50 U/mL), IFNγ (1000 U/mL), TGF-β1 (5 ng/mL), IL-15 (10 ng/mL), IL-18 (10 ng/mL), IL-10 (10 ng/mL) (R&D, USA). These concentrations were chosen according to the concentrations in human biological fluids, including in the area of utero-placental contact.

NK-92 cells were added to a part of the wells in 100 μL of a medium of 20,000 cells. IL-2 (500 U/mL) was added to all wells (LLC "Biotech", Russia), cytokines were added to some of the wells and then cells were cultured for 96 hours in the presence of TNFα, IFNγ, TGF-β1, IL-15, IL-18, or IL-10. After that, the analysis of their cytokine production was conducted. Four experiments were conducted with two technical repetitions in each.

Analysis of cytokine level in conditioned media obtained after cultivation of NK-92 cells

After cultivation for 96 hours in the presence of cytokines, NK-92 cells were centrifuged for 5 minutes at 2500 g, the supernatants (hereinafter "conditioned media", CM) were frozen at -20 °C and stored until the study. Then, the level of IL-10, RANTES, VEGF, TNFα, IFNγ and TGF-β1 in CM was evaluated using commercial kits for CBA (Cytometric Bead Array) (BD, USA) and a flow cytofluorimeter BD FACSCanto II (BD, USA) according to the manufacturer's instructions.

Results and discussion

The content of TNFα, TGF-β, IFNγ, RANTES, IL-10, and VEGF was evaluated in CM obtained after cultivation of NK-92 cells for 96 hours in the presence of cytokines. We found that NK-92 cells on the baseline level secreted IFNγ, IL-10 and RANTES and in low concentrations TGF-β, but did not secrete VEGF and TNFα (Figure 1).

It has previously been shown that NK-92 cells are capable of secreting VEGF [13], TNFα [5]. During the 96-hour incubation period, it is likely that secreted cytokines are internalized by NK cells in order to regulate their properties. However, in order to confirm this hypothesis, further analysis will be required to determine the intracellular content of cytokines. The content of IFNγ, RANTES, and IL-10 in CM was reduced after cultivation of NK-92 cells in the presence of TGF-β1 when compared with the content of these cytokines after cultivation without inducers (Figure 1).

TGF-β1, a major cytokine secreted by trophoblast cells, exerts an immunosuppressive influence [11], nonetheless, it has been demonstrated that in certain instances, it may enhance the cytotoxic potency of cytotoxic cells [9]. The pro-inflammatory cytokine IFNγ enhances the effector functions of NK cells [12], has an inhibitory effect on trophoblast cells, reducing their ability to invade [1]. IL-10 it can

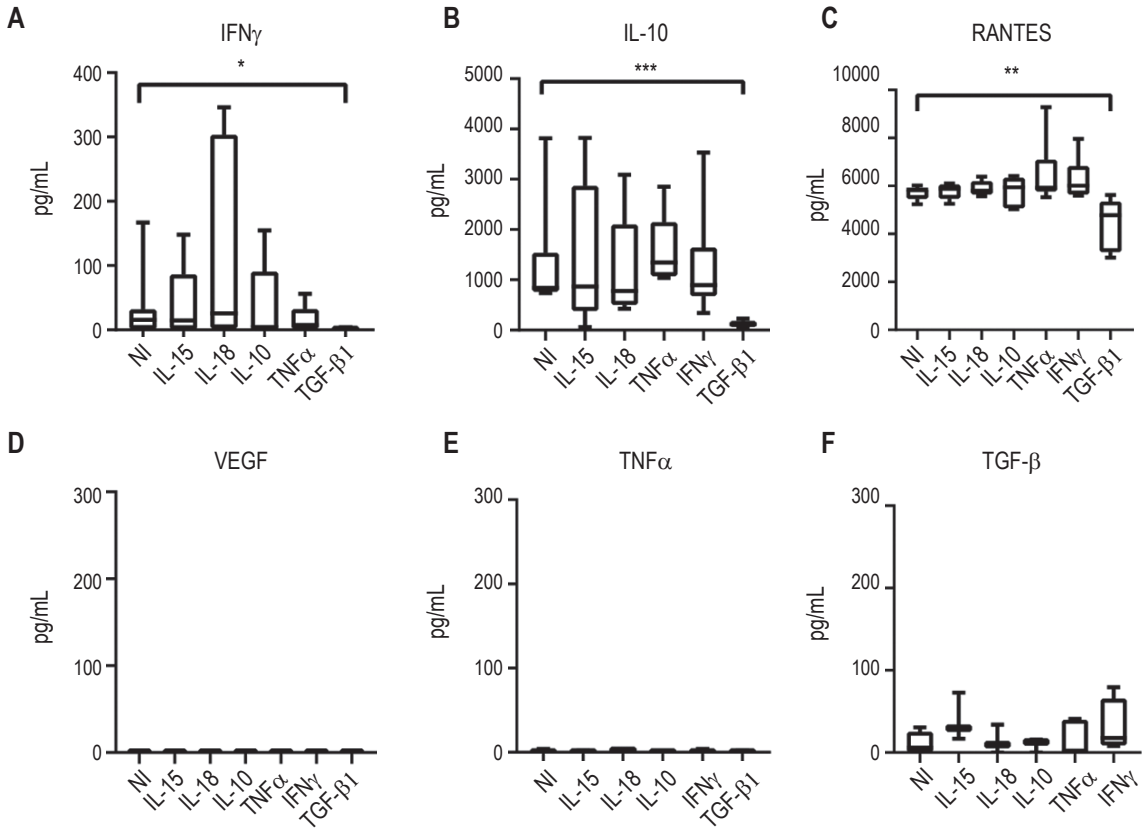

Figure 1. Content of IFNγ (A), IL-10 (B), RANTES (C), VEGF (D), TNFα (E), and TGF-β (F) in CM after cultivation of NK-92 cells in the presence of various cytokines (indicated on the abscissa axis) for 96 hours: Nl – NK-92 cells without inductors

Note. Significance of differences: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

also serve as an enhancer of the effector capabilities of NK cells [3], elicit the secretion of IFNγ, thereby augmenting the cytotoxic capacity [2]. Moreover, the cytokine exerts an anti-invasive and pro-apoptotic influence on trophoblast cells [10]. Accordingly, inhibition of IFN-y and IL-10 secretion by the action of TGF-β1 may be a mechanism used by trophoblast cells to establish a tolerant microenvironment for a semiallegenic fetus.

Nonetheless, this notion does not align with the reduction in RANTES secretion upon TGF-β1 treatment. On the contrary, it has been observed that RANTES serves as a stimulant for the proliferation and migration of NK cells [14], this is a piece of evidence that supports the hypothesis of fostering tolerance towards fetal cells. Nonetheless, it should be noted that cytokine also serves as a stimulant for the migration and infiltration of trophoblast cells [4]. Consequently, a reduction in the production of NK cells hinders the development of fetal cells. This observation may suggest the existence of a regulatory mechanism employed by trophoblast cells to prevent their own over-invasion.

Conclusion

In the presence of TGF-β1, NK cells decrease the production of IFNγ, IL-10 and RANTES. This finding may indicate that trophoblast cells and other cells in the microenvironment, producing TGF-β1, can alter NK cell function, creating an optimal environment for fetal development. The data obtained reflect processes occurring between cells of the maternal and fetal immune systems during pregnancy and could serve as a basis for developing tests to diagnose causes of reproductive pathologies resulting from disruptions in intercellular communication.

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Авторы:

Гребенкина П.В. *— младший научный сотрудник ФГБНУ «Институт акушерства, гинекологии и репродуктологии имени Д.О. Отта», Санкт-Петербург, Россия*

Михайлова В.А. *— д.б.н., старший научный сотрудник лаборатории межклеточных взаимодействий ФГБНУ «Институт акушерства, гинекологии и репродуктологии имени Д.О. Отта», Санкт-Петербург, Россия*

Authors:

Grebenkina P.V., *Junior Research Associate, D. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation*

Mikhailova V.A., *PhD, MD (Biology), Senior Research Associate, Laboratory of Intercellular Interactions, D. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation*

Беспалова О.Н. *– д.м.н., заместитель директора по научной работе ФГБНУ «Институт акушерства, гинекологии и репродуктологии имени Д.О. Отта», Санкт-Петербург, Россия*

Bespalova O.N., *PhD, MD (Medicine), Deputy Director for Research, D. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation*

Селькov С.А. *– д.м.н., профессор, заслуженный деятель науки РФ, заведующий отделом иммунологии и межклеточных взаимодействий ФГБНУ «Научно-исследовательский институт акушерства, гинекологии и репродуктологии имени Д.О. Отта»; профессор кафедры иммунологии ФГБОУ ВО «Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова» Министерства здравоохранения РФ, Санкт-Петербург, Россия*

Selkov S.A., *PhD, MD (Medicine), Professor, Honored Scientist of the Russian Federation, Head, Department of Immunology and Intercellular Communications, D. Ott Research Institute of Obstetrics, Gynecology and Reproductology; Professor, Department of Immunology, First St. Petersburg State I. Pavlov Medical University, St. Petersburg, Russian Federation*

Соколов Д.И. *– д.б.н., доцент, заведующий лабораторией межклеточных взаимодействий ФГБНУ «Научно-исследовательский институт акушерства, гинекологии и репродуктологии имени Д.О. Отта»; профессор кафедры иммунологии ФГБОУ ВО «Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова» Министерства здравоохранения РФ, Санкт-Петербург, Россия*

Sokolov D.I., *PhD, MD (Biology), Associate Professor, Head, Laboratory of Intercellular Communications, D. Ott Research Institute of Obstetrics, Gynecology and Reproductology; Professor, Department of Immunology, First St. Petersburg State I. Pavlov Medical University, St. Petersburg, Russian Federation*

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