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CELL BIOLOGY

The Effect of Kisspeptin on the Functional Characteristics of Isolated NK Cells

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Abstract—The effect of kisspeptin at concentrations typical of pregnancy on the functional activity of isolated cytokine-primed NK cells has been investigated. The hormone has been shown to promote an increase in the proportion of $CD56^{bright}$ NK cells, as well as an increase in the L-selectin expression on the cell surface. Assessment of cytokine levels has shown that kisspeptin suppresses the production of IL-4, IL-10, and IFN-γ while stimulating the production of TGF-β by isolated NK cells. The overall effect of the hormone investigated consisted in the formation of a phenotype and a cytokine spectrum characteristic of the regulatory NK3 subpopulation of NK cells in pregnancy.

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Hormones, especially those produced by the placenta [1], have a significant impact on the functioning of immune system cells. Kisspeptin, a recently discovered hormone that regulates the functioning of the gonadostatic system on the hypothalamic level [2], is produced by the placenta during pregnancy and, hence, occurs in the systemic circulation of the mother [3]. Immune cells have been shown to express specific membrane receptors for kisspeptin [4].

From the immunological point of view, pregnancy can be considered as a successful natural semiallogenic transplantation. Suppression of the adaptive immune response of the mother during pregnancy is compensated by activation of the innate immunity system. NK cells, which are the main effectors of this system, account for as much as 80% of all decidual leukocytes during early pregnancy [5]. The function of these cells is not limited to the cytotoxic action, since they play a decisive role in the normal course of pregnancy, maintaining the integrity of the decidua and ensuring modification of spiral arteries of the mother by means of secretion of interferon-γ (IFN-γ) [6]. Fulfillment of the fetotrophic function by NK cells requires specific regulatory signaling that primarily relies on hormones of the fetoplacental complex.

The goal of this study was to estimate the effect of kisspeptin on the functional characteristics of isolated NK cells.

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A fractionated suspension of peripheral blood mononuclear (PBM) cells from 10 healthy non-pregnant women of reproductive age (23 to 37 years) was used in the present work. PBM cell suspension was obtained by centrifugation in a ficoll–verographin density gradient (d = 1.077 g/cm^3). A suspension enriched with NK cells was obtained by negative selection using a Dynabeads Untouched Human NK Cells Kit (Invitrogen, United States). The content of target cells in the suspension was estimated from the expression of the NK cell marker NKp46 (anti-human CD335-PC5); it was 90–95%. The isolated cells (5×10^6/mL) were incubated with the hormone in 500 μL of complete growth medium (RPMI-1640 containing 10% fetal calf serum, 1 mM HEPES, 2 mM L-glutamine, and 200 U/mL gentamicin) for 72 h at 37°C under 5% CO_2. Kisspeptin (kisspeptin-54, Metastin, Synthetic, Calbiochem, United States) was used at physiological concentrations corresponding to the levels of this hormone in peripheral blood during pregnancy trimesters II and III (4.6 and 9.6 pM, respectively [3]). An officinal diluent was added to the control samples instead of the hormone. The stimulatory cytokines interleukin 2 (IL-2), IL-12, and IL-15 (all from Gibco, United States) were added to the cell cultures at concentrations of 1, 2, and 10 ng/mL, respectively, in order to maintain the viability and growth of the isolated NK cells [7]. The supernatants were collected after the incubation, and the cells were phenotyped by flow cytometry in a lymphocyte gate. Staining of the cells was performed according to the instructions of the manufacturer of monoclonal antibodies (Beckman Coulter, United States). At least 100 000 cells were counted. Isotype controls were used to take non-

Table 1. The effect of kisspeptin on the expression of functional molecules by isolated NK cells ($M \pm m$)

Group	n	Control	Kisspeptin (4.6 pM)	Kisspeptin (9.6 pM)
$NKp46^+$, $CD56^{bright}$	10	33.22 ± 1.441	37.12 ± 1.262*	33.51 ± 0.652
$NKp46^+$, $CD56^{dim}$	10	68.07 ± 2.688	60.30 ± 7.365	67.84 ± 6.703
$NKp46^+$, $NKG2A^+$	10	21.67 ± 1.312	27.73 ± 4.399	28.47 ± 4.938
$CD56^{bright}$, $CD62L^+$	10	8.31 ± 1.778	12.09 ± 1.673*	13.59 ± 2.911*
$CD56^{dim}$, $CD62L^+$	10	27.01 ± 4.631	26.32 ± 4.726	32.40 ± 6.430

Here and in Table 2, * $p < 0.05$ as compared with the control, n is the number of samples investigated.

specific binding into account and to delineate a non-fluorescent lymphocyte window.

The expressions of the inhibitory molecule NKG2A (anti-human CD159-PE), L-selectin (anti-human CD62L-FITC), and the marker protein CD56 (anti-human CD56-PE) in the isolated NK cells were assessed.

The concentrations of IL-4, IL-10, IL-17, IFN-γ, and transforming growth factor (TGF) β in cell culture supernatants were evaluated by ELISA using reagents from Tsitokin (Russia).

Pairwise Student's t test was used for statistical processing of the results.

The effect of kisspeptin at concentrations typical of pregnancy on the functional activity of isolated cytokine-primed NK cells has been investigated. An increase in the proportion of $CD56^{bright}$ NK cells and elevation of L-selectin expression at the cell surface were identified as effects of the hormone. Quantification of cytokine levels showed that kisspeptin suppressed the production of IL-4, IL-10, and IFN-γ while stimulating the production of TGF-β by the isolated NK cells. On the whole, the hormone investigated contributed to the formation of a phenotype and a cytokine spectrum characteristic of the regulatory NK3 subpopulation of NK cells during pregnancy.

A low level of the expression of CD56 ($CD56^{dim}$) is known to be a marker of a high cytotoxicity of NK cells; the level of such cells decreases during pregnancy. In contrast, the proportion of NK cells expressing CD56 at a high level ($CD56^{bright}$) increases, with exactly these cells infiltrating the decidua [8]. NK cells of this type show weak cytotoxicity and fulfill regulatory functions [9, 10].

Assessment of the effect of kisspeptin on the amounts of NK cells with different levels of CD56 expression ($CD56^{bright}$/$CD56^{dim}$) revealed a significant increase (as compared to control) of the proportion of $CD56^{bright}$ cells in cultures treated with kisspeptin at a concentration corresponding to trimester II of pregnancy. The hormone treatment also evoked an increase in the proportion of $CD56^{bright}$ NK cells expressing L-selectin, although it did not affect the expression of the inhibitory molecule NKG2A (Table 1). The NK cell subpopulation with high expression levels of CD56, L-selectin, and NKG2A is currently believed to fulfill regulatory functions [9, 10]. Thus, kisspeptin may be one of the factors modulating the functional activity of NK cells to increase their regulatory potential and reduce the cytotoxic action during pregnancy. Moreover, a significant increase in the relative abundance of $CD56^{bright}$, $CD62L^+$ cells in hormone-treated samples is indicative of the ability of the hormone to enhance the migration of NK cells of this type into the placenta during the physiological course of pregnancy [11].

In contrast to $CD56^{dim}$ cells, $CD56^{bright}$ NK cells are capable of producing considerable amounts of different cytokines [12], which allows the discrimination between different subtypes of peripheral and decidual NK cells. For example, IFN-γ and tumor necrosis factor (TNF) α predominate among the cytokines produced by NK1 cells, while NK2 cells mostly produce IL-4, IL-5, IL-6, and IL-13; NK3 cells produce (TGF)-β; and NKr1 cells produce IL-10 [10, 13, 14]. NK1 cells producing IFN-γ and (TNF)-α (in smaller amounts) have been shown to predominate in the peripheral blood of non-pregnant women [13]. Assessment of cytokine production by isolated NK cells from the peripheral blood of non-pregnant women revealed a significant predominance of IFN-γ, this being in accordance with the published data.

Treatment of isolated NK cells with kisspeptin at either concentration used in our study had no effect on the production of IL-17, while it significantly suppressed the secretion of IL-4, IL-10, and IFN-γ (Table 2). The hormone at the higher concentration significantly stimulated the secretion of TGF-β, the major immunosuppressive cytokine.

Thus, kisspeptin is a physiological inducer of the differentiation of NK1 cells into NK3 cells. The documented role of TGF-β as a facilitator of the transformation of peripheral NK cells into decidual NK cells [15] shows that the role of kisspeptin as a differentiation factor is considerably more important than it was believed previously.

Table 2. The effect of kisspeptin on the secretion of different types of cytokines by isolated NK cells, pg/mL ($M \pm m$)

Treatment	n	Control	Kisspeptin (4.6 pM)	Kisspeptin (9.6 pM)
IL-4	10	8.69 ± 0.188	7.46 ± 0.449*	7.72 ± 0.370*
IL-10	10	25.62 ± 1.247	22.97 ± 1.056*	22.24 ± 1.049*
TGF-β	10	263.08 ± 7.843	247.26 ± 10.622	278.89 ± 14.257*
IL-17	10	239.36 ± 4.736	243.82 ± 14.965	241.96 ± 14.501
IFN-γ	10	506.45 ± 26.403	345.82 ± 34.631*	366.27 ± 42.325*

In conclusion, the essential role of kisspeptin synthesized in the placenta in the modulation of the functional activity of NK cells should be emphasized. The hormone evokes an increase in the proportion of $CD56^{bright}$ regulatory lymphocytes of the NK3 subtype due to activation of TGF-β production and reduction of the concentration of the fetotoxic IFN-γ. Moreover, kisspeptin can induce targeted migration of these cells into the placenta during late pregnancy.

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