ISSN 1990-7478, Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology, 2017, Vol. 11, No. 1, pp. 54–61. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © S.V. Shirshev, I.V. Nekrasova, E.G. Orlova, O.L. Gorbunova, 2017, published in Biologicheskie Membrany, 2017, Vol. 34, No. 1, pp. 47–55.

ARTICLES

Effects of Leptin and Ghrelin on the Expression of Membrane Molecules and Cytokine Production by NK Cells from the Peripheral Blood

S. V. Shirshev, I. V. Nekrasova, E. G. Orlova*, and O. L. Gorbunova

Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, ul. Goleva 13, Perm, 614081 Russia

**e-mail: orlova_katy@mail.ru*

Received June 10, 2016; in final form, July 21, 2016

Abstract—The influence of leptin and ghrelin, as well as their combined effects, on the expression of membrane molecules and cytokine production by NK cells from peripheral blood was studied in vitro. The effects of hormones were assayed at the concentrations corresponding to their peripheral blood levels in the course of physiological pregnancy. It was established that the investigated hormones exerted significant effects only at the concentrations typical of the II–III trimester of pregnancy. In particular, leptin and ghrelin and their combination increased the number of $CD56^{bright}NKp46^{+}$NK cells in the suspension of mononuclear cells and inhibited the expression of homing molecules CCR7 and inhibitor molecules LILRB in $NKp46^{+}$NK cells. Leptin and its combination with ghrelin increased the expression of L-selectin in $CD56^{bright}NKp46^{+}$NK cells but inhibited the secretion of IL-10 by $NKp46^{+}$NK cells. Leptin reduced the production of IL-4 by $NKp46^{+}$ cells, while ghrelin eliminated this effect. The hormones did not influence the expression of inhibitory molecules NKG2A in $NKp46^{+}$ cells and the production of TGF-β1, IL-17A, and IFN-γ by these cells. Thus, the investigated hormones at the concentrations typical of the II–III trimester of pregnancy effectively regulate the expression of membrane molecules and cytokine production by NK cells of the peripheral blood.

Keywords: leptin, ghrelin, NK cells, membrane molecules, cytokines, pregnancy

DOI: 10.1134/S199074781604019X

INTRODUCTION

Peptide hormones leptin and ghrelin are functional antagonists regulating energy homeostasis, the immune and reproductive systems [1]. Acting at the hypothalamic level, leptin and ghrelin oppositely control the feeling of hunger and appetite by regulating food intake, adipose tissue metabolism, energy metabolism, and the processes of growth and development [1]. Both hormones have marked immunoregulatory activities [1–3]. The immunomodulating effects of leptin and ghrelin have been studied mainly in obesity and inflammation models, when hormone concentrations considerably exceed the physiological values. It has been shown that leptin is a proinflammatory hormone contributing to a predominance of cell-mediated immune response [4], while ghrelin demonstrates an anti-inflammatory activity: it exerts an antagonistic effect on leptin by blocking leptin-induced proinflammatory reactions [5–7]. Both hormones are able to regulate the expression of each other's receptors in target cells [5–7]. The combined effect of leptin and ghrelin, both within a cell and at the level of the whole organism, results in the formation of cooperative effects that determine the energy and immune homeostasis [1, 5–7].

In pregnancy, energy metabolism, adipose tissue metabolism, and immunoreactivity of maternal organism undergo substantial changes, and the levels of leptin and ghrelin in peripheral blood significantly increase, because both hormones regulate implantation, are actively secreted by the placenta, and control fetal growth and development [1–3]. The systemic suppression of adaptive immune response during pregnancy is compensated for by the activation of innate immunity, the most important effectors of which are natural killers (NK cells). More than 80–90% of peripheral mature NK cells have a $CD16^{+}CD56^{dim}$ phenotype and demonstrate high cytotoxicity compared to $CD16^{low/neg}CD56^{bright}$, their population being predominant among the lymphoid cells localized in the decidual envelope in the period of early pregnancy [8–10]. The decidual $CD16^{low/neg}CD56^{bright}$ NK cells secrete cytokines and have low cytolytic activities, being directly involved in implantation and angiogenesis during pregnancy [11]. It is supposed that decidual NK cells maturate from the peripheral $CD16^{low/neg}CD56^{bright}$

lymphocytes migrating to the uterus [8], while the cytolytic potential of the total pool of peripheral blood NK cells decreases during pregnancy due to reduction of the percentage of $CD16^+CD56^{dim}$ lymphocytes, and the increase in their number is associated with recurrent pregnancy loss [8–12]. An important indicator of the functional activity of NK cells is the presence on their surface of inhibitory and homing receptors, the expression of which varies over the course of pregnancy [12].

It is known that the $CD56^{bright}$ NK cells are able to produce considerable amounts of various cytokines, which was a basis for distinguishing different subtypes of NK cells [10, 11]. So, NK1 produce mainly interferon (IFN)-γ and the tumor necrosis factor (TNF)-α; NK2 produce interleukine (IL)-4, IL-5, IL-6, IL-13; NK3 produce the transforming growth factor (TGF)-β1; NKr1 produce IL-10 [10, 11]. Some works have shown that leptin and ghrelin play a key role in regulation of the functional activity of NK cells expressing specific hormone receptors [13–15]. However, the mechanisms of realization of the immunoregulatory effects of leptin and ghrelin at physiological concentrations typical of pregnancy in regulation of the functional activity of NK cells are actually unstudied.

The goal of this work was to investigate the role of leptin and ghrelin at concentrations characteristic of pregnancy in regulation of the expression of membrane molecules and cytokine production by peripheral blood NK cells of women in vitro.

MATERIALS AND METHODS

The work was carried out using the fractionated suspension of peripheral blood mononuclear cells (PBMC) from healthy nonpregnant women of reproductive age (from 23 to 38 years old). The PBMC suspension was obtained by centrifugation in the ficoll–verographin density gradient (1.077 g/cm^3). The cells were cultured in a complete culture medium (CCM) containing RPMI-1640 supplemented with 10% fetal calf serum (Sigma, USA), 10 mM HEPES (ICN Pharmaceuticals, Inc., USA), 2 mM *L*-glutamine (ICN Pharmaceuticals, Inc.), and 100 μg/mL gentamicin (KRKA, Slovenia). Leptin (Sigma, USA) was added to CCM at the concentrations of 10 and 35 ng/mL, corresponding to its content in peripheral blood in I and II–III trimesters of pregnancy, respectively [3]. Ghrelin (Sigma, USA) was added at concentrations 1.25 and 0.83 ng/mL that were comparable to its level in peripheral blood in I–II and III trimesters of pregnancy, respectively [2]. To study the joint effects, the hormones were added to the cultures simultaneously at the concentrations exerting statistically significant effects (leptin 35 ng/mL + ghrelin 0.83 ng/mL), which corresponds to the hormone combination in the peripheral blood in II–III trimesters of pregnancy. The control samples contained, instead of the hormones, the normal saline used for their dissolution.

For assessing the influence of the hormones on the level of CD56 expression (Anti-human CD56-PE, Beckman Coulter, USA), the PBMC suspension was incubated with the hormones for 72 h and then the phenotype of NK cells was assessed. NK cells were identified by the presence of specific marker NKp46 (Anti-human CD335-PC5, Beckman Coulter, USA) in the lymphocyte gate by the flow cytometry method. The NKp46 molecule is constitutively present in the entire population of NK cells and is one of the basic markers for their identification [9]. The $NKp46^+$ cells were used to assess the expression of the homing molecule CCR7 (Anti-human CD197-FITC, eBioscience, USA), the expression of *L*-selectin (Anti-human CD62L-FITC, Beckman Coulter, USA) and inhibitory molecules NKG2A (Anti-human CD159-PE, Beckman Coulter, USA) and LILRB (Anti-human CD85j-PE, Beckman Coulter, USA). The cells were stained according to the protocol provided by the manufacturer of monoclonal antibodies. No less than 100000 cells were counted. The respective isotype controls were used for controlling the nonspecific binding and allocation of a lymphocytic window with negative fluorescence.

At the following stage, NK cells were isolated from the PBMC suspension by the method of immunomagnetic separation using Dynabeads Untouched Human NK Cells Kit (Invitrogen, USA). The purity of isolation assessed by the NKp46 marker expression was 95%. The enriched suspension of $NKp46^+$ cells (5×10^6/mL) was incubated with the hormones in CCM in a volume of 500 μL for 72 h at 37ºC in the 5% CO_2 atmosphere. To maintain the viability of separated NK cells, cytokines (GIBCO, USA) were added to all samples (ng/mL): IL-2, 1; IL-12, 2; IL-15, 10 [18]. Upon completion of the incubation, the supernatant was collected and the levels of IL-4, IL-10, IL-17, IFN-γ, and TGF-β1 were assessed by the method of enzyme immunoassay (Cytokine, Russia). The results are given as the arithmetic mean and standard deviation of the mean (mean ± SD). Since the data distribution in all groups under study obeyed the law of normal distribution, which was ascertained by the Fischer's test, the statistical significance of differences between the groups was evaluated by the paired Student's *t*-test.

RESULTS AND DISCUSSION

The study of hormonal effects on the level of CD56 expression in $NKp46^+$ cells has shown that leptin and ghrelin at the concentrations typical of II–III trimes-

Table 1. Effects of leptin, ghrelin, and their combination on the level of CD56 expression and the expression of NKG2A, LILRB, CCR7 and *L*-selectin (CD62L) molecules by NKp46$^+$ cells

Experimental conditions	CD56^{bright}, % (n = 12)	CD56^{bright} CD62L$^+$, % (n = 7)	CD56^{dim} CD62L$^+$, % (n = 7)	LILRB$^+$, % (n = 6)	CCR7$^+$ (n = 7)	NKG2A$^+$, % (n = 7)
Control	1.67 ± 0.52	1.26 ± 0.13	15.56 ± 2.59	4.04 ± 0.69	0.81 ± 0.11	5.01 ± 0.56
Leptin, 10 ng/mL	3.45 ± 0.99	2.88 ± 0.89	15.05 ± 3.17	4.05 ± 0.78	0.73 ± 0.19	4.11 ± 0.78
Leptin, 35 ng/mL	3.38 ± 0.09*	2.10 ± 0.32*	18.65 ± 3.02	2.93 ± 0.46*	0.53 ± 0.06*	4.77 ± 0.76
Ghrelin, 1.25 ng/mL	2.06 ± 0.98	2.74 ± 0.89	14.95 ± 3.19	4.87 ± 0.83	0.81 ± 0.20	4.86 ± 0.09
Ghrelin, 0.83 ng/mL	3.05 ± 0.74*	2.18 ± 1.01	15.40 ± 2.03	2.28 ± 0.53*	0.49 ± 0.08*	5.36 ± 0.95
Leptin, 35 ng/mL + Ghrelin, 0.83 ng/mL	3.08 ± 0.81*	2.40 ± 0.25*	13.07 ± 2.45	2.72 ± 0.41*	0.41 ± 0.06*	4.23 ± 0.83

Here and in Table 2: * The difference from the respective control is significant at $p < 0.05$ by the paired Student's t-test. The data in column 1 are given as percentage of CD56^{bright} of the total number of CD56$^+$NKp46$^+$ cells; the data in columns 2 and 3 are given as percentage of CD56^{bright}CD62L$^+$ and CD56^{dim}CD62L$^+$ of the total number of NKp46$^+$ cells; the data in columns 4–6 are given as percentage of NKp46$^+$ cells positive for a given marker of the total cell number in the lymphocyte gate.

ters of pregnancy, acting both separately and together, increase the number of CD56^{bright}NKp46$^+$ cells in the PBMC suspension (Table 1, Fig. 1). The hormones have no significant effects at the concentrations corresponding to their levels in peripheral blood in I–II trimesters of pregnancy. Given that decidual CD56^{bright} NK cells mature from peripheral cells via the transformation of CD56^{dim}CD16$^+$-NK [8], it may be supposed that the hormones at the concentrations under study promote an acquisition of the regulatory phenotype by NK cells. Such conversion was observed also during cultivation in vitro of CD56$^+$CD16$^+$ NK cells isolated from peripheral blood with TGF-β1 or with the cells of decidual stroma [16].

The regulatory NK cells express inhibitory receptors, such as NKG2A and LILRB, which allows them to implement the immunotrophic function instead of the cytolytic one [11, 17, 20]. The inhibitory molecule LILRB belongs to the family of immunoglobulin-like receptors limiting the transduction of activation signal into the cell when it binds to the ligand [11, 17, 20]. NKG2A is a high-affinity inhibitory receptor, the ligand of which is a "nonclassical" major histocompatibility complex (MHC) class I molecule – HLA-E, which does not allow the NK cells to lyse the trophoblast cells recognized by them [20]. It was established that leptin and ghrelin have no effect on the expression of the NKG2A inhibitory molecule but, at the concentration corresponding to II–III trimesters of pregnancy, reduce the expression of LILRB molecule in NKp46$^+$ cells, acting both independently and jointly (Table 1, Fig. 2), which suggests the involvement of these hormones in the regulation of the cytotoxic potential of NK cells.

At the following stage, the hormonal effects on the expression of homing molecules CCR7 and *L*-selectin (CD62L) in the population of NKp46$^+$ cells were assessed. It was shown that leptin and ghrelin at the concentrations corresponding to the II–III trimesters of pregnancy, as well as the hormonal combination, suppress the expression of chemokine receptor CCR7 in the NKp46$^+$ cells (Table 1, Fig. 3). Leptin at the concentration of the II–III trimesters of pregnancy and the leptin/ghrelin combination increase the number of CD56^{bright} NK cells bearing *L*-selectin (Table 1, Fig. 4), whereas the expression of *L*-selectin in CD56dim NK cells is not influenced by the hormones. Considering that a high level of expression of CCR7 molecules in CD56^{bright} NK cells is associated with their homing to lymph nodes [21] and a heightened expression of *L*-selectin is associated with their enhanced level in the placenta [22], we can suppose that these hormones will promote the migration of CD56^{bright} NK cells to the placenta in the course of physiological pregnancy. It should be noted that the hormonal effects manifest themselves only in the subpopulation of CD56^{bright} NK cells with a statistically significant increase in their level. This fact is important as it reflects the possibility of hormone-mediated late migration of the CD56^{bright} NK cells to the decidual envelope.

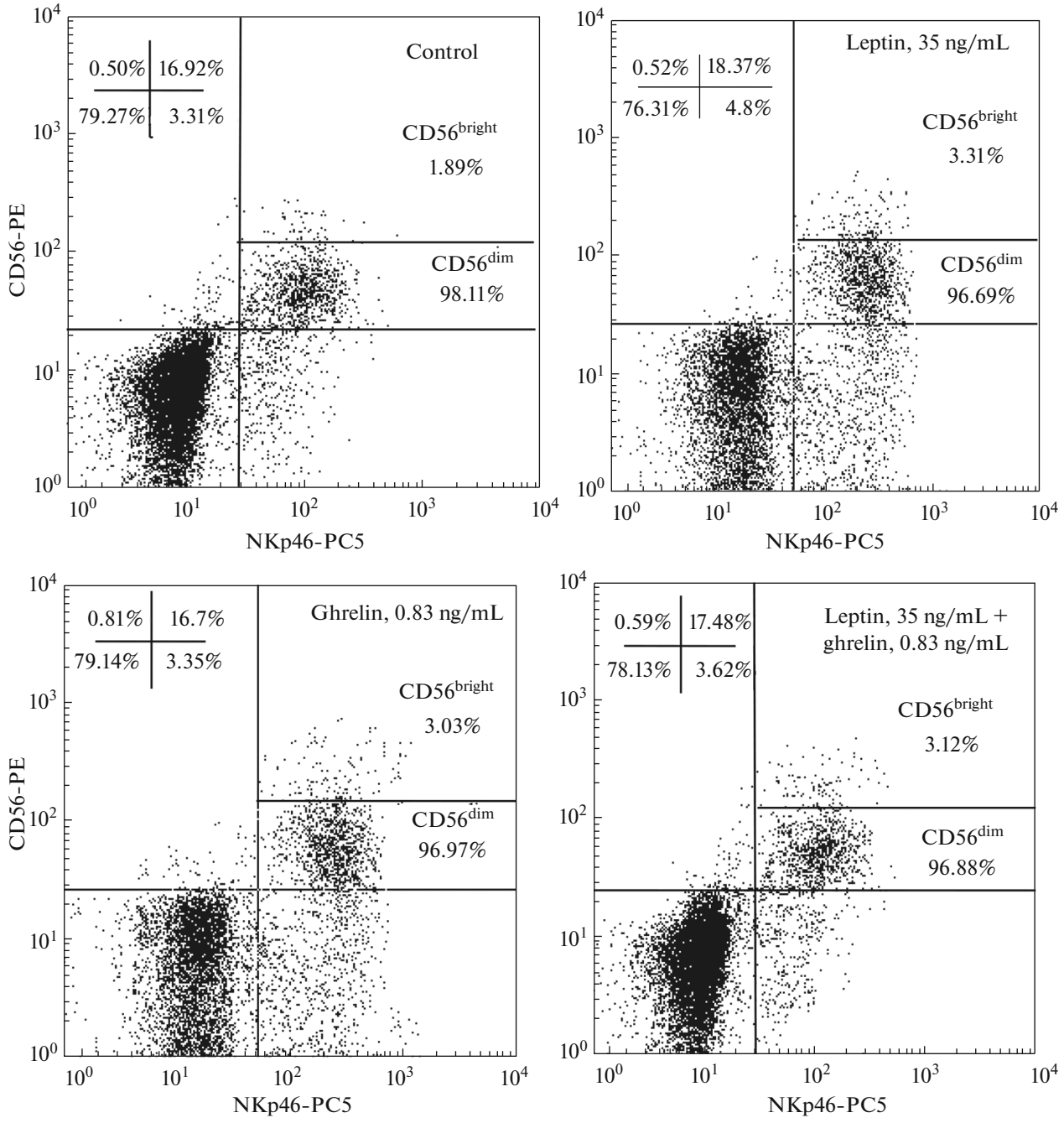

Fig. 1. Histograms demonstrating the hormonal effects on the level of CD56 expression in NKp46$^+$ cells in the 72-h PBMC culture (by the example of one experiment). *Abscissa,* fluorescence intensity on channel FL3 (PC5 staining); *ordinate,* fluorescence intensity on channel FL2 (PE staining).

It is known that CD56^{bright} NK cells are able to produce considerable amounts of cytokines. The IFN-γ-producing NK1 cells are predominant in the peripheral blood of nonpregnant women [19]. The number of NKr1 cells secreting IL-10 increases over the course of pregnancy. Decidual NK cells are represented mainly by NK3 lymphocytes producing TGF-β1, which determines their immunosuppressive effect on many lymphocyte populations [19]. Analysis of changes in the cytokine spectrum of NK cells under the influence of leptin and ghrelin indicated that both hormones, irrespective of their concentrations, have no effect on the production of IFN-γ, TGF-β1, and IL-17A but suppress the secretion of IL-10 by NKp46$^+$ cells. In addition, leptin at the concentration corresponding to the II–III trimester of pregnancy also reduces IL-4

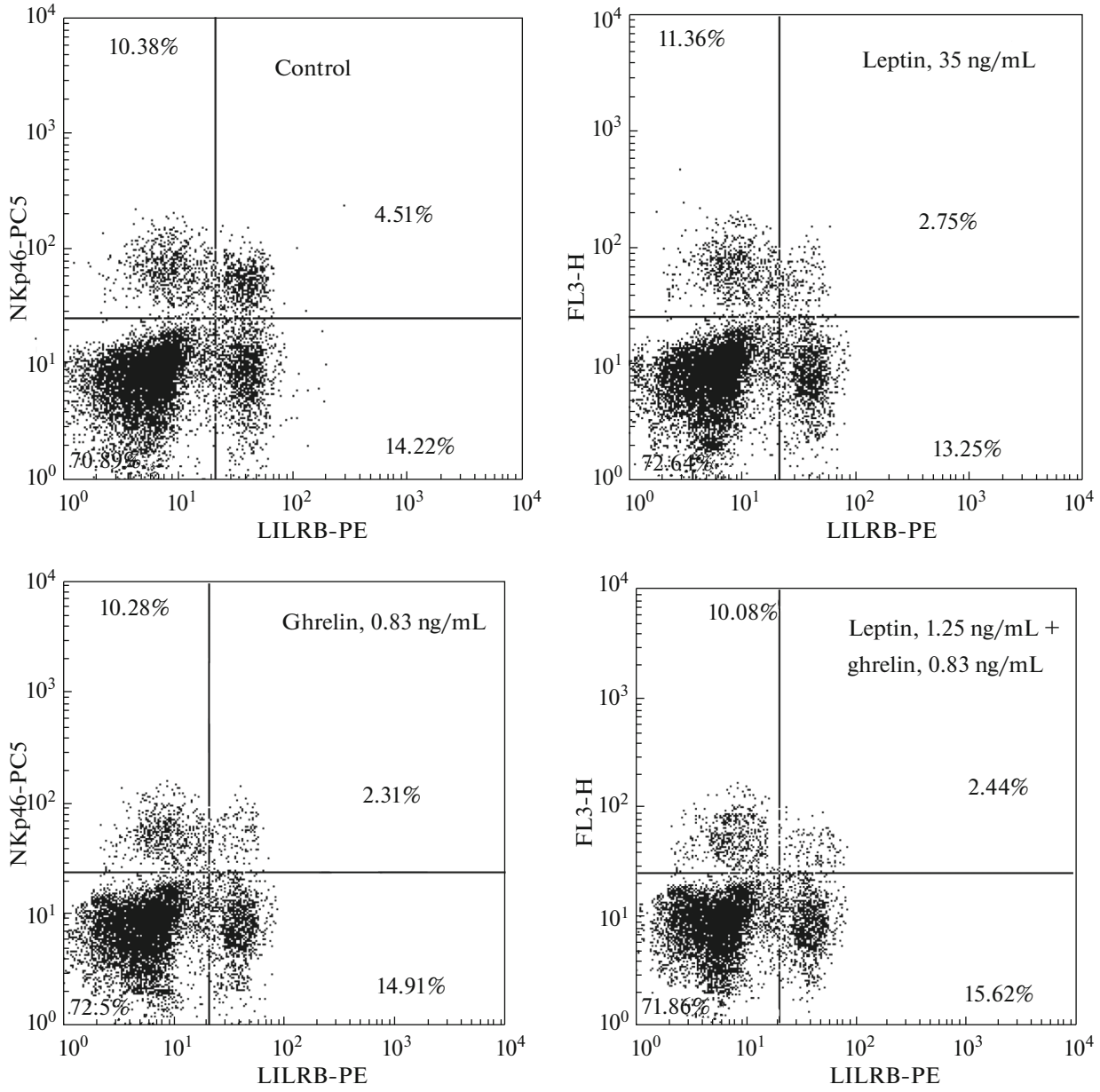

Fig. 2. Histograms demonstrating the hormonal effects on the inhibitory molecule LILRB expression in NKp46$^+$ cells in the 72-h PBMC culture (by the example of one experiment). *Abscissa,* fluorescence intensity on channel FL2 (PE staining); *ordinate,* fluorescence intensity on channel FL3 (PC5 staining).

production by the separated NK cells. The hormonal combination of the second half of pregnancy has no effect on the IL-17A, IFN-γ and TGF-β1 production either but significantly reduces the IL-10 production by NKp46$^+$ cells. However, the IL-4-suppressing effect of leptin is lost when it is used together with ghrelin, i.e., ghrelin neutralizes the effect of leptin (Table 2). Thus, the hormones do not influence the production of cytokines determining the type of regulatory NK cells, such as NK1 and NK3, but reduce the possibility of formation of NK2 and NKr1. It seems likely that the effects of the hormones are possible only in the II–III trimesters of pregnancy, but this matter needs further investigation.

Thus, leptin and ghrelin at the above concentrations actively participate in the control of surface molecule expression and cytokine production by NK cells, which is associated with the regulation of their functional activity. The hormones exert their basic modulatory effects at the concentrations typical of the second half of pregnancy. In spite of the fact that leptin and ghrelin are functional antagonists in the regula-

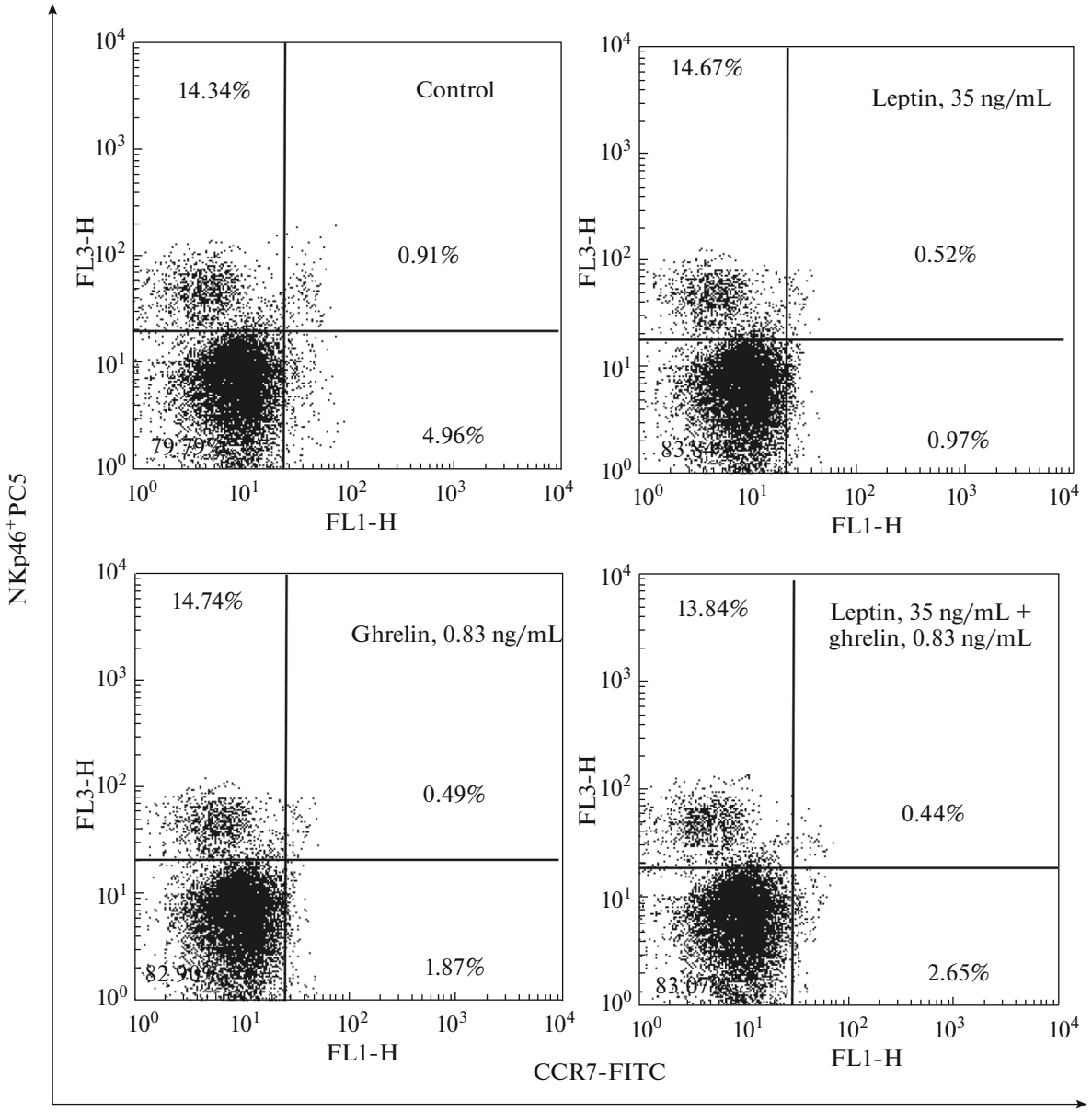

Fig. 3. Histograms characterizing the hormonal effects on the CCR7$^+$ expression by NKp46$^+$ cells in the 72-h PBMC culture (the data of one experiment). *Abscissa,* fluorescence intensity on channel FL1 (FITC staining); *ordinate,* fluorescence intensity on channel FL3 (PC5 staining).

Table 2. Effects of the hormones on the cytokine secretion (pg/mL) by separated NKp46$^+$ cells ($n = 9$)

Experimental conditions	IL-4	IL-10	TGF-β1	IL-17A	IFN-γ
Control	8.69 ± 0.19	25.62 ± 1.25	263.08 ± 7.84	239.36 ± 4.74	506.45 ± 26.40
Leptin, 10 ng/mL	7.5 ± 0.98	24.96 ± 1.69	270.49 ± 20.37	225.39 ± 12.38	520.45 ± 45.29
Leptin, 35 ng/mL	7.63 ± 0.31*	23.61 ± 0.97*	275.88 ± 19.99	221.63 ± 13.63	555.91 ± 36.44
Ghrelin, 1.25 ng/mL	8.87 ± 0.89	23.89 ± 1.95	271.98 ± 10.39	253.35 ± 14.39	450.36 ± 45.69
Ghrelin, 0.83 ng/mL	8.26 ± 0.58	22.04 ± 1.33*	267.50 ± 8.00	244.58 ± 15.85	439.89 ± 55.32
Leptin, 35 ng/mL + Ghrelin, 0.83 ng/mL	8.29 ± 0.83	22.20 ± 1.30*	271.07 ± 8.94	213.00 ± 11.12	444.15 ± 52.47

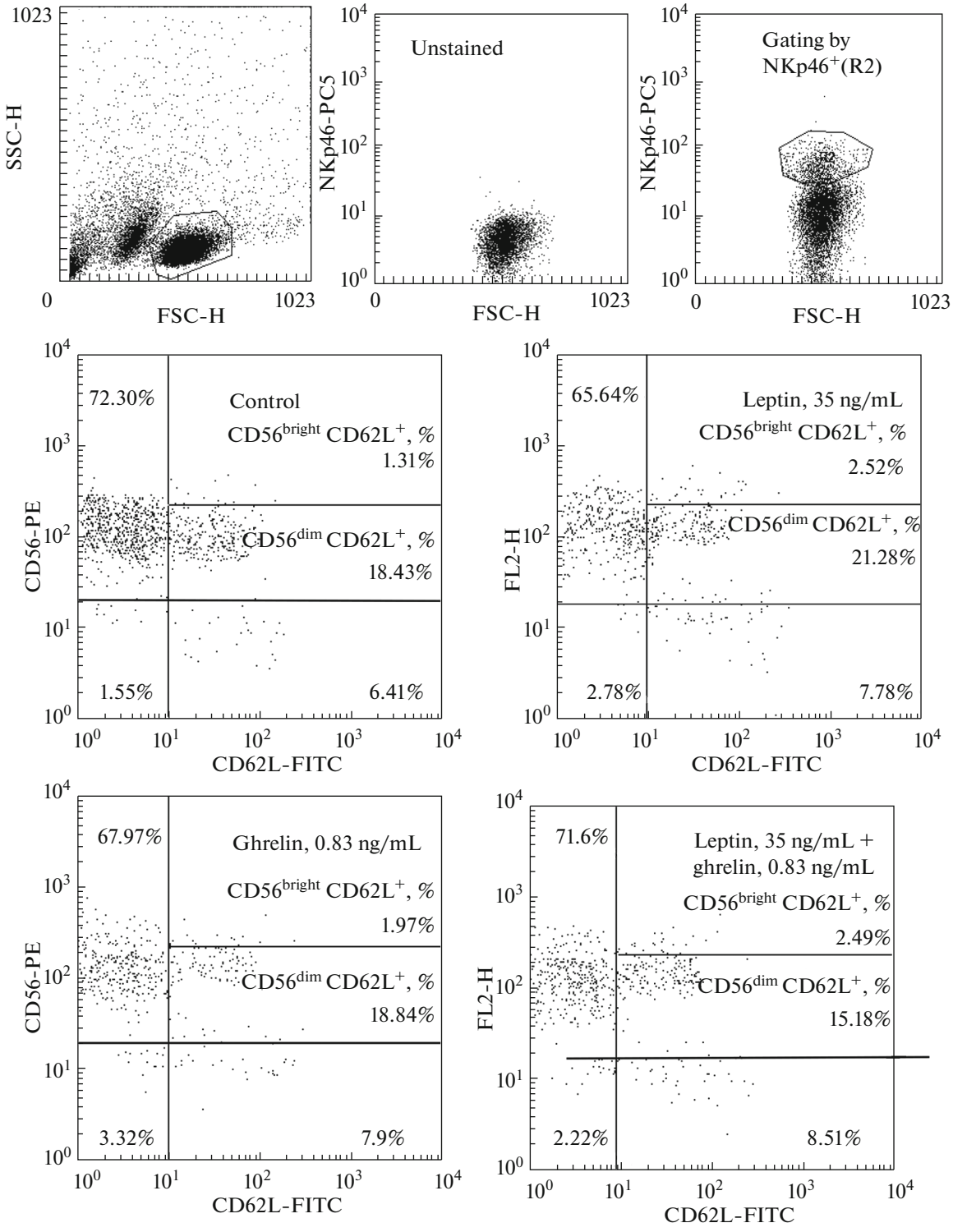

Fig. 4. Histograms demonstrating effects of hormones on the expression of CD62L molecules in $CD56^+NKp46^+$ cells in the 72-h PBMC culture (by the example of one experiment). *Abscissa,* fluorescence intensity on channel FL1 (FITC staining); *ordinate*, fluorescence intensity on channel FL2 (PE staining).

tion of appetite, metabolism and functions of most cells of the immune system [1, 6, 23], their effect on NK cells is mainly unidirectional and maintained in the case of their joint application. In the second half of pregnancy, the hormones and their combination increase the number of $CD56^{bright}$ NK cells bearing

L-selectin but reduce the content of the chemokine receptor CCR7 and the inhibitory molecule LILRB in NK cells without affecting the level of NKG2A. The hormones do not influence the production of cytokines determining the types of regulatory NK cells but prevent the formation of NK2 and NKr1 subtypes. Generally, it may be supposed that the joint effect of leptin and ghrelin during pregnancy promotes the formation of the regulatory phenotype of NK cells for their subsequent migration to the endometrium.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (project no. 13-04-96035-r_ural_a).

REFERENCES

1. Tena-Sempere M. 2013. Interaction between energy homeostasis and reproduction: Central effects of leptin and ghrelin on the reproductive axis. *Horm. Metab. Res.* **45**, 919–927.
2. Fuglsang J., Skjærbæk U., Espelund J., Frystyk J., Fisker S., Flyvbjerg A., Ovesen P. 2005. Ghrelin and its relationship to growth hormones during normal pregnancy. *Clin. Endocrinol. (Oxf).* **62**, 554–559.
3. Hardie L., Trayhurn P. 1997. Circulating leptin in women: Longitudinal study in menstrual cycle and during pregnancy. *Clin. Endocrinol.* **47**, 101–106.
4. Faggioni R., Fantuzzi G., Fuller J., Dinarello C.A., Feingold K.R., Grunfeld C. 1998. IL-1 beta mediates leptin induction during inflammation *Am. J. Physiol.* **274**, 204–208.
5. Dixit V.D., Taub D.D. 2005. Ghrelin and immunity: A young player in an old field. *Exp. Gerontol.* **40**, 900–910.
6. Dixit V.D., Schaffer E.M., Pyle R.S. Collins G.D., Sakthivel S.K., Palaniappan R., Lillard J.W.Jr., Taub D.D. 2004. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J. Clin. Invest.* **1**, 57–66.
7. Dixit V.D., Yang H., Cooper-Jenkins A. Giri B.B., Patel K., Taub D.D. 2009. Reduction of T cell-derived ghrelin enhances proinflammatory cytokine expression: Implications for age-associated increases in inflammation. *Blood.* **113,** 5202–5205.
8. Koopman L.A., Kopcow H.D., Rybalov B., Boyson J.E., Orange J.S., Schatz F., Masch R., Lockwood C.J., Schachter A.D., Park P.J., Strominger J.L. 2003. Human decidual natural killer cells are a unique NK subset with immunomodulatory potential. *J. Exp. Med.* **198**, 1201–1212.
9. Tomasello E., Yessaad N., Gregoire E., Hudspeth K., Luci C., Mavilio D., Hardwigsen J., Vivier E. 2012. Mapping of NKp46(+) cells in healthy human lymphoid and non-lymphoid. *Tissues Front. Immunol.* **3**, 344–358.
10. Higuma-Myojo S., Sasaki Y., Miyazaki S., Sakai M., Siozaki A., Miwa N., Saito S. 2005. Cytokine profile of natural killer cells in early human pregnancy. *Am. J. Reprod. Immunol.* **54**, 21–29.
11. Saito S., Nakashima A., Myojo-Higuma S., Shiozaki A. 2008. The balance between cytotoxic NK cells and regulatory NK cells in human pregnancy. *J. Reprod. Immunol.* **77**, 14–22.
12. Dosiou C., Giudice L.C. 2005. Natural killer cells in pregnancy and recurrent pregnancy loss: Endocrine and immunologic perspectives. *Endocr. Rev.* **26**, 44–62.
13. Orlova E.G., Shirshev S.V. 2010. Leptin and ghrelin regulation of the expression of membrane molecules and the apoptosis of human lymphocytes during pregnancy. *Problemy endokrinologii* (Rus.) **56** (3), 26–30.
14. Hattori N., Saito T., Yagyu T. Jiang B.H., Kitagawa K., Inagaki C. 2001. GH, GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. *J. Clin. Endocrinol. Metab.* **86**, 4284–4291.
15. Zhao Y., Sun R., You L., Gao C., Tian Z. 2003. Expression of leptin receptors and response to leptin stimulation of human natural killer cell lines. *Biochem. Biophys. Res. Commun.* **300**, 247–252.
16. Keskin D.B., Allan D.S., Rybalov B., Andzelm M.M., Stern J.N., Kopcow H.D., Koopman L.A., Strominger J.L. 2007. TGFbeta promotes conversion of CD16+ peripheral blood NK cells into CD16- NK cells with similarities to decidual NK cells. *Proc. Natl. Acad. Sci. USA.* **104**, 3378–3383.
17. Hong H.S., Ahmad F., Eberhard J.M. 2012. Loss of CCR7 expression on CD56(bright) NK cells is associated with a CD56(dim)$CD16^+$ NK cell-like phenotype and correlates with HIV viral load. *PLoS One.* **7**, e44820.
18. Vitale M., Bassini A., Secchiero P. Mirandola P., Ponti C., Zamai L., Mariani A.R., Falconi M., Azzali G. 2002. NK-active cytokines IL-2, IL-12, and IL-15 selectively modulate specific protein kinase C (PKC) isoforms in primary human NK cells. *Anat. Record.* **266** (2), 87–92.
19. Levine R.J., Lam C., Qian C., Yu K.F. Maynard S.E., Sachs B.P., Sibai B.M., Epstein F.H., Romero R., Thadhani R., Karumanchi S.A. 2006. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N. Engl. J. Med.* **355**, 992 – 1005.
20. King A., Allan D.S., Bowen M., Powis S.J., Joseph S., Verma S., Hiby S.E., McMichael A.J., Loke Y.W., Braud V.M. 2000. HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. *Eur. J. Immunol.* **30**, 1623–1631.
21. Mailliard, R.B., Alber S.M., Shen H., Watkins S.C., Kirkwood J.M., Herberman R.B., Kalinski P. 2005. IL-18-induced $CD83^+CCR7^+$ NK helper cells. *J. Exp. Med.* **202**, 941–953.
22. Heuvel M., Peralta C., Bashar S., Taylor S., Horrocks J., Croy B.A. 2005. Trafficking of peripheral blood CD56(bright) cells to the decidualizing uterus – new tricks for old dogmas? *J. Reprod. Immunol.* **67** (1–2), 21–34.
23. Orlova E.G., Shirshev S.V. 2013. The role of leptin and ghrelin in the induction of differentiation of IL17-producing and T-regulatory lymphocytes. *Byull. Exp. Biol. Med.* (Rus.). **156** (12), 786–791.

Translated by E. Makeeva