DOI 10.1007/s10517-019-04484-2
Cell Technologies in Biology and Medicine, No. 1, May, 2019

Interaction of NK Cells, Trophoblast, and Endothelial Cells during Angiogenesis

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 1, pp. 54-62, January, 2019
Original article submitted May 11, 2018

We studied changes in angiogenesis during contact interaction of natural killer cells and endothelial cells in the presence of secretory products of trophoblast cells activated by various cytokines. Activated trophoblast regulates angiogenesis by producing soluble factors that affect endothelial cells either directly or indirectly through activation of proangiogenic activity of natural killer cells. A stimulating effect of the trophoblast supernatants activated by IL-1β and an inhibitory effect of trophoblast supernatants activated by IL-6 and TGFβ for the formation of tube-like structures by endothelial cells were revealed. During contact culturing, natural killer cells increased the length of tube-like structures formed by endothelial cells. The trophoblast activated by IL-1β affects angiogenesis both directly through the production of proangiogenic factors and indirectly through activation of the proangiogenic potential of natural killer cells. Trophoblast activated by IFNγ affects angiogenesis only by stimulating the proangiogenic potential of natural killer cells. Under conditions of contact interaction of natural killer cells and endothelial cells, soluble factors of trophoblast activated by IL-6 or TGFβ attenuated the angiogenesis-stimulating effect of natural killer cells.

Key Words: *trophoblast; natural killer cells; endothelial cells; cytokines; angiogenesis*

The interaction between cells of the mother and fetus underlies the process occurring in the uterine-placental contact area, including blastocyst implantation, trophoblast invasion, remodeling of the uterine spiral arteries, and formation of the placenta [3]. One of the most important parameters of effective trophoblast invasion and subsequent development of the placenta is the formation of a vascular tree providing fetal nutrition. Angiogenesis is regulated by balanced effect of growth factors and cytokines secreted both by the endothelial cells (EC) and microenvironment cells. The greatest contribution to the production of cytokines in the area of uteroplacental blood flow formation is made by trophoblast cells and leukocytes. Trophoblast produces a wide range of factors that ensure its invasion into the endometrium, regulate angiogenesis, and producing immunomodulating effect on maternal immune system cells. They include IL-10, proinflammatory cytokines IFNγ, IL-1β, and growth factors VEGF and TGFβ. It has been found that EC and leukocytes are also modulated by soluble form of HLA-G molecule secreted by the trophoblast [12]. Trophoblast controls the viability and functional activity, including cytotoxicity, of natural killer (NK) cells by regulating the expression of receptors for IL-15 by these cells [20]. It has been shown that syncytiotrophoblast releases microvesicles that not only considerably affect the cells of the immune system in the placenta and decidua, but also produce a systemic effect [26]; the composition of these particles is intensively studied.

NK cells constitute the most abundant population of leukocytes in the decidua. These cells are involved in all processes in the area of the uterine-placental contact during pregnancy. During implantation and

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in the first trimester of pregnancy, the interaction of trophoblast with NK cells is critical for physiological development of pregnancy, in particular, for the formation of a population of decidual NK cells (dNK) [30]. These cells have a phenotype $CD56^{bright}CD16^{dim/—}$, are characterized by reduced cytotoxic activity [11], perform predominantly regulatory function [10]. During the early stages of pregnancy, the main task of dNK is the formation of the vascular bed, in particular, maturation of blood vessels upon the formation of the so-called "tip cell" section [14]. dNK cells secrete a wide range of factors involved in the regulation of angiogenesis: IFNγ, VEGFA, VEGF-C, IL-8, TGFβ, PlGF, Ang1, Ang2 [16], uPA, uPAR, MMP MIP1a, GM-CSF, CSF1, and some other factors [30]. NK cells produce an angiogenic effect due to contact interaction of α4β7 molecules on the surface of NK cells with VCAM-1 on the surface of EC. It is believed that dNK cells contribute to the early stages of spiral artery remodeling by triggering Fas-dependent apoptosis of smooth muscle cells and EC, both from the maternal and fetal sides, so-called trophoblast-independent remodeling [22]. In co-culture of EC and NK cells, reduction of the expression of activation receptors by NK cells is observed [9].

Much attention was paid to the interaction of trophoblast and NK cells during embryo implantation, while the effect of NK cells on EC and angiogenesis is far less studied. Currently, there is practically no data on the relationship between EC, trophoblast cells, and NK cells and, in particular, on the effect of NK cells on the formation of blood vessels in the placenta.

We studied changes in the angiogenesis process caused by contact interaction of NK cells and EC in the presence of secretory products of trophoblast cells activated by different cytokines.

MATERIALS AND METHODS

We used trophoblast JEG-3 cells (ATCC) that reproduce the morphological, phenotypic, and functional characteristics of the invasive trophoblast of the first trimester of pregnancy. The cells were cultured in DMEM supplemented with 10% inactivated fetal calf serum (FCS), 100 U/ml penicillin+100 μg/ml streptomycin, 0.5 mM L-glutamine, 1 ml MEM, 1 mM sodium pyruvate (Sigma-Aldrich). For subculturing, the cell monolayer was treated with Versene-trypsin 1:1 mixture (BioloT). EC of the EA.Hy926 line were kindly provided by Dr. C. J. Edgel (University of North Carolina, USA). They reproduce all the main characteristics of EC. The cells were cultured in DMEM/F-12 supplemented with 10% FCS, 100 μg/ml streptomycin, 100 U/ml penicillin (Sigma-Aldrich), 8 mmol/liter L-glutamine, and HAT (Sigma). The cells were subcultured every 3-4 days by 5-min treatment with Versene (BioloT). NK-92 cells (ATCC) reproduce the basic phenotypic and functional characteristics of activated NK cells. NK-92 cells were cultured in α-MEM containing 12.5% FCS, 12.5% inactivated equine serum, 0.2 mM myoinositol, 0.02 mM folic acid, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, 10 mM HEPES, 0.1 mM 2-mercaptoethanol (Sigma-Aldrich), and 200 U/ml recombinant IL-2 (Roncoleukin, Biotekh). All cells were cultured and experiments were performed in an incubator in humid atmosphere at 37°C and 5% CO_2. Cell viability evaluated by trypan blue exclusion was ≥96%.

The following recombinant human cytokines were used for cell activation: bFGF (1, 10, and 20 ng/ml); VEGF (1, 10, and 100 ng/ml); TGFβ (1, 5, and 10 ng/ml); IL-6 (1, 2.5, and 4 ng/m; RD); IFNγ (40, 400, and 1000 U/ml (Gammaferon, NPO Ferment, and Sanitas); IL-1β (10, 100, and 1000 U/ml, Betaleukin, Scientific Research Institute of Extremely Pure Biopreparations, St. Petersburg).

JEG-3 cells (1.7×10^5/ml medium) were cultured for 24 h in wells of 24-well plates. Cytokines in different concentrations were then added to some wells for 4 h. Then, the cells were three times washed with warm DMEM, and 1 ml complete growth medium was added to each well. In 24 h, the supernatants were collected, centrifuged at 200*g* for 10 min, frozen, and stored at -20°C until assay.

To assess the formation of tube-like structures, 1.5×10^5 EC of the EA.Hy926 line in 250 μl FCS-free medium were added to wells of a 24-well plate pretreated with Matrigel Growth Factor Reduced matrix (Becton Dickinson). In some wells, 5.5×10^4 NK-92 cells were added in 250 μl serum-free medium. Then, 500 μl supernatants obtained after culturing of trophoblast cells and 25 μl FCS were added to some wells with EC with and without NK cells. To control wells, 500 μl culture medium and 25 μl FCS were added. Formation of tube-like structures by EC in the presence of IFNγ (1000 U/ml) served as the positive control. The paltes were incubated for 24 h. The experiments were repeated twice in 3 repetitions for each position. In each well, the length of formed tube-like structures was measured 5 fields of view under an AxioObserver Z1 microscope using AxioVision image analysis system (Carl Zeiss) (Fig. 1).

The data were processed statistically using Statistica 10.0 software. For data analysis, the methods of descriptive statistics were used. The results were compared using the nonparametric Mann—Whitney *U* test. The increase in the length of tube-like structures was estimated relative to the spontaneous level (EC culturing in the absence of inducers was taken as 0).

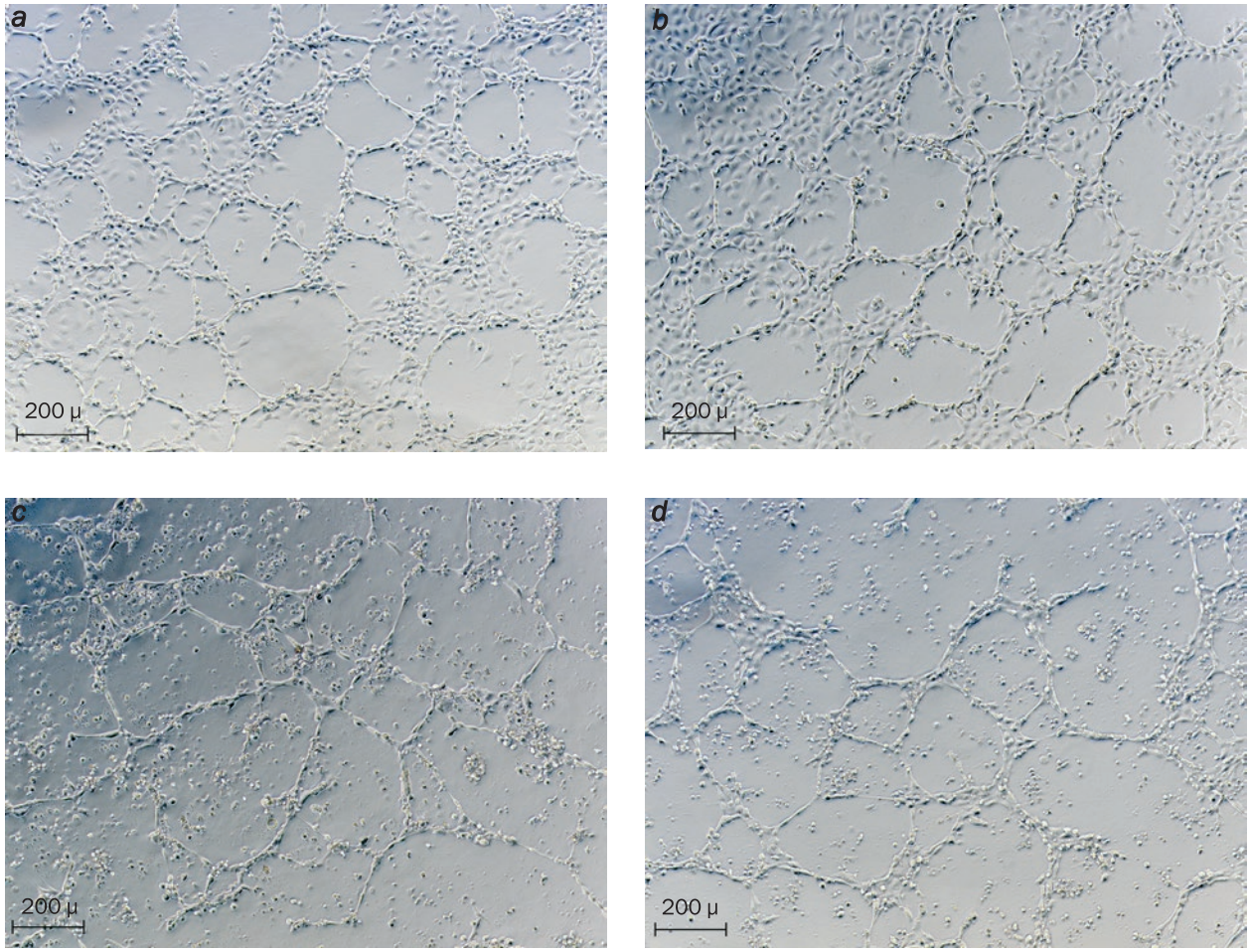

Fig. 1. Tube-like structures formed by EC of the EA.Hy926 line, ×100. *a*) EC monoculture, spontaneous level; *b*) EC monoculture in the presence of supernatants of non-stimulated trophoblast cells; *c*) co-culture of EC and NK cells; *d*) co-culture of EC and NK cells in the presence of supernatants of non-stimulated trophoblast cells.

RESULTS

Culturing of EC without NK cells, but in the presence of supernatants of non-stimulated trophoblast cells did not change the length of tube-like structures formed by EC. In co-culture of EC and NK cells, an increase in the length of tube-like structures was observed in the absence and presence of supernatants of non-stimulated trophoblast cells (Fig. 2).

Tube-like structures formed by EC with supernatants of trophoblast cells activated with 0.1 ng/ml IL-1β were longer than structures formed by EC cultured with supernatants of non-stimulated trophoblast cells. In the co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with IL-1β, an increase in the length of tube-like structures formed by EC was observed in all concentrations, compared to their spontaneous formation in monoculture of EC and during EC culturing under the same conditions in the absence of NK cells. In co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with 0.1 or 10 ng/ml IL-1β, an increase in the length of tube-like structures was observed compared to that in EC and NK cell co-culture without supernatants. In addition, an increase in the length of the tube-like structures formed by EC in their co-culture with NK cells in the presence of supernatants of trophoblast cells activated with 10 ng/ml IL-1β was observed compared to culturing of EC with NK cells in the presence of supernatants of non-stimulated trophoblast cells (Fig. 3).

The length of tube-like structures formed by EC cultured with supernatants of trophoblast cells activated with IFNγ and non-stimulated trophoblast cells did not differ (Fig. 2). In co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with IFNγ, an increase in the length of the tube-like structures formed by EC was observed in all concentrations, compared to their spontaneous formation in monoculture of EC and during culturing of EC under the same conditions (only for IFNγ concentrations of 40 and 400 U/ml) in the absence of NK

cells (Fig. 2). In co-culture of EC and NK cells in the presence of supernatants of trophoblast cells activated with IFNγ in all concentrations, an increase in the length of tube-like structures was observed in comparison with the length of those formed in co-culture of EC and NK cells without supernatants. In addition, an increase in the length of tube-like structures formed by EC in their co-culture with NK cells in the presence of supernatants of trophoblast cells activated with IFNγ in all concentrations was observed in comparison with co-culture of EC and NK cells in the presence of supernatants of non-stimulated trophoblast cells (Fig. 2).

An increase in the length of tube-like structures formed by EC in the presence of supernatants of trophoblast cells activated with 1000 U/ml IFNγ was observed in comparison with EC monoculture. An increase in the length of tube-like structures formed by EC in the presence of supernatants of trophoblast cells activated with 400 U/ml IFNγ was also observed in comparison with EC cultured with supernatants of non-stimulated trophoblast cells. The length of tube-like structures formed by EC in co-culture with NK cells and supernatants of trophoblast cells activated with IFNγ in all concentrations (40, 400, and 1000 U/ml) was higher than in EC monoculture and in co-culture EC and NK cells in the absence and presence of supernatants of non-stimulated trophoblast cells. In the presence of 40 and 400 U/ml IFNγ, the length of tube-like structures was also increased in comparison with that formed by EC cultured under the same conditions in the absence of NK cells (Fig. 2).

The length of tube-like structures formed by EC cultured with supernatants of trophoblast cells activated with 2.5 and 4 ng/ml IL-6 was less than in EC culture in the presence of supernatants of non-stimulated trophoblast cells (Fig. 4). In co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with only 1 ng/ml IL-6, an increase in the length of tube-like structures formed by EC was observed, compared to their spontaneous formation in EC monoculture. In parallel, in the co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with IL-6, an increase in the length of tube-like structures formed by EC was observed in all concentrations in comparison with their formation when EC were cultured under the same conditions in the absence of NK cells. During the co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with IL-6 in all concentrations, the length of tube-like structures was not changed in comparison with that formed in the co-culture of EC and NK cells. In parallel, no increase in the length of tube-like structures formed by EC in their co-culture with NK cells in the presence of supernatants of trophoblast cells activated with IL-6 was observed in comparison with the culturing of EC and

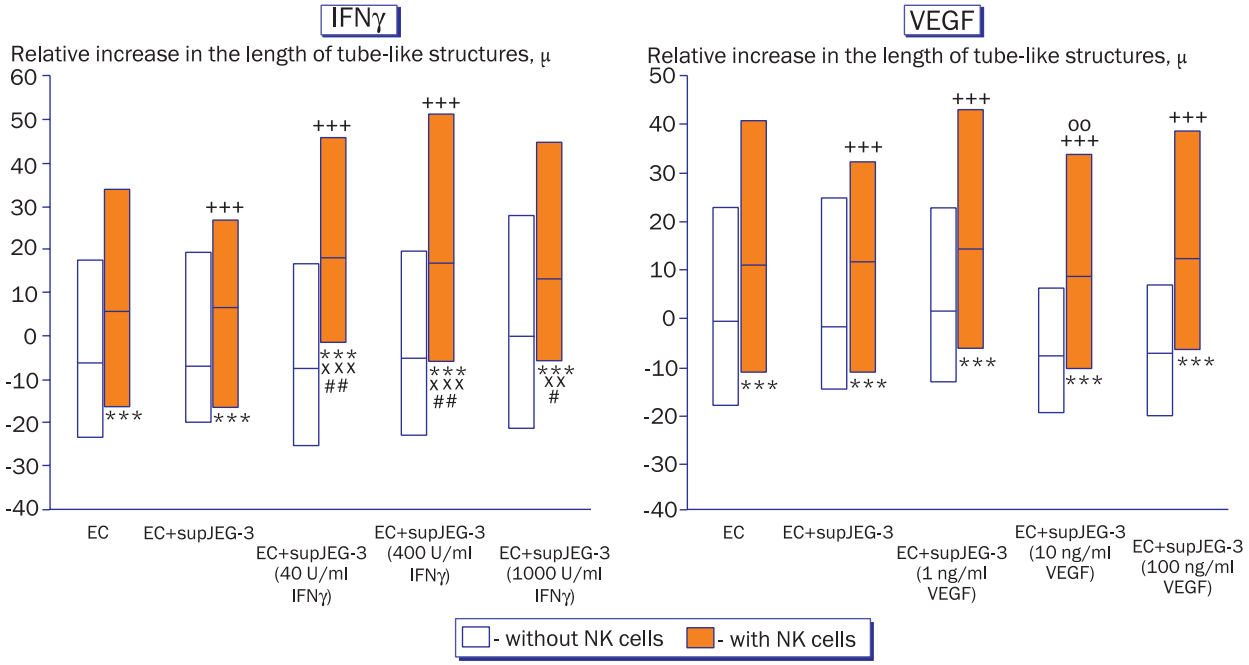

Fig. 2. Formation of tube-like structures by EC of the EA.Hy926 line in monoculture (EC; spontaneous level) and in co-culture with NK cells in the presence of supernatants of intact trophoblast JEG-3 cells (supJEG-3) or trophoblast JEG-3 cells activated with IFNγ (40, 400, and 1000 U/ml) and VEGF (1, 10, and 100 ng/ml). $p<0.001$ in comparison ***with EC, $^{+++}$with culturing without NK cells under the same conditions; $^{oo}p<0.01$ in comparison with similar culturing conditions in the presence of 1 ng/ml VEGF; $^{xx}p<0.01$, $^{xxx}p<0.001$ in comparison with co-culture of EC and NK cells; $^{\#}p<0.05$, $^{\#\#}p<0.01$ in comparison with co-culture of EC and NK cells in the presence of supernatant of trophoblast JEG-3 cells.

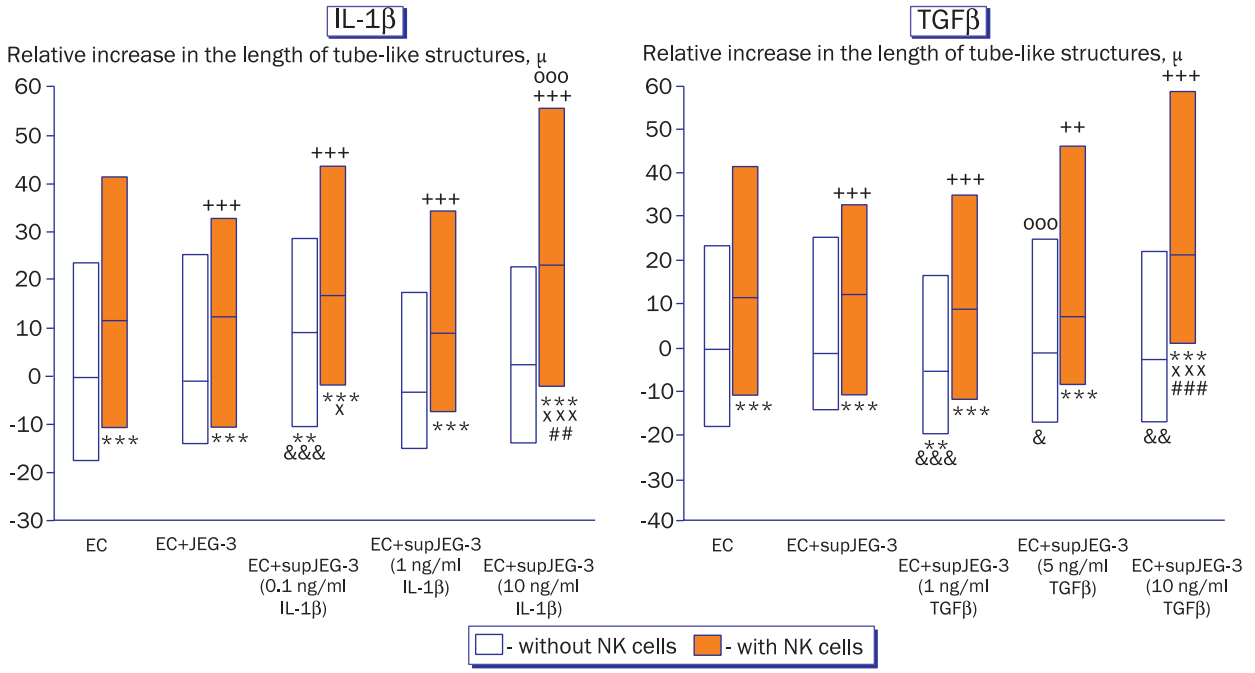

Fig. 3. Formation of tube-like structures by EC of the EA.Hy926 line in monoculture (EC; spontaneous level) and in co-culture with NK cells in the presence of supernatants of intact trophoblast JEG-3 cells (supJEG-3) or trophoblast JEG-3 cells activated with IL-1β (0.1, 1, and 10 ng/ml) or TGFβ (1, 5, and 10 ng/ml). $^{**}p<0.01$, $^{***}p<0.001$ in comparison with EC; $^{++}p<0.01$, $^{+++}p<0.001$ in comparison with culturing without NK cells under the same conditions; $^{ooo}p<0.001$ in comparison with cells activated with 1 ng/ml IL-1β or 1 ng/ml TGFβ under the same culturing conditions; $^{x}p<0.05$, $^{xxx}p<0.001$ in comparison with EC in the presence of NK cells; $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ in comparison with EC+supJEG-3 with NK cells; $^{\&}p<0.05$, $^{\&\&}p<0.01$, $^{\&\&\&}p<0.001$ in comparison with EC+supJEG-3 without NK cell.

NK cells in the presence of supernatants of non-stimulated trophoblast cells (Fig. 4).

Tube-like structures formed by the culturing of EC with supernatants of trophoblast cells activated with VEGF did not have a positive length increase compared to those formed during the culturing of EC with supernatants of non-stimulated trophoblast cells (Fig. 4). In the co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with VEGF in all concentrations, an increase in the length of tube-like structures formed by EC was observed, compared to their spontaneous formation in EC monoculture and culturing of EC under the same conditions in the absence of NK cells. In the co-culture of EC with NK cells in the presence of supernatants obtained after the incubation of trophoblast cells activated with VEGF, the length of tube-like structures did not change in comparison with the length of those formed in the co-culture of EC and NK cells. In parallel, there was no increase observed in the length of tube-like structures formed by EC in their co-culture with NK cells in the presence of supernatants of trophoblast cells activated with VEGF in comparison with the culturing of EC and NK cells in the presence of supernatants of non-stimulated trophoblast cells (Fig. 2).

The length of tube-like structures formed by the culturing of EC with supernatants of trophoblast cells activated with TGFβ in all concentrations was less than that of tube-like structures formed by the culturing of EC with supernatants of non-stimulated trophoblast cells (Fig. 3). In the co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with TGFβ an increase in the length of tube-like structures formed by EC was observed in all concentrations, compared to their spontaneous formation in EC monoculture and during culturing of EC under the same conditions in the absence of NK cells. In the co-culture of EC with NK cells in the presence of supernatants of trophoblast cells activated with 10 ng/ml TGFβ, an increase in the length of tube-like structures was observed compared to the length of those formed in the co-culture of EC and NK cells. In addition, an increase in the length of tube-like structures formed by EC in their co-culture with NK cells in the presence of supernatants of trophoblast cells activated with 10 ng/ml TGFβ was observed in comparison with the culturing of EC and NK cells in the presence of supernatants of non-stimulated trophoblast cells (Fig. 3).

In order to simulate the interaction of trophoblast, NK cells, and EC in the process of angiogenesis, we studied the formation of tube-like structures by EC of the EA.Hy926 line in the presence of supernatants of JEG-3 trophoblast cells both in EC monoculture and co-culture with NK-92 cells. It was found that super-

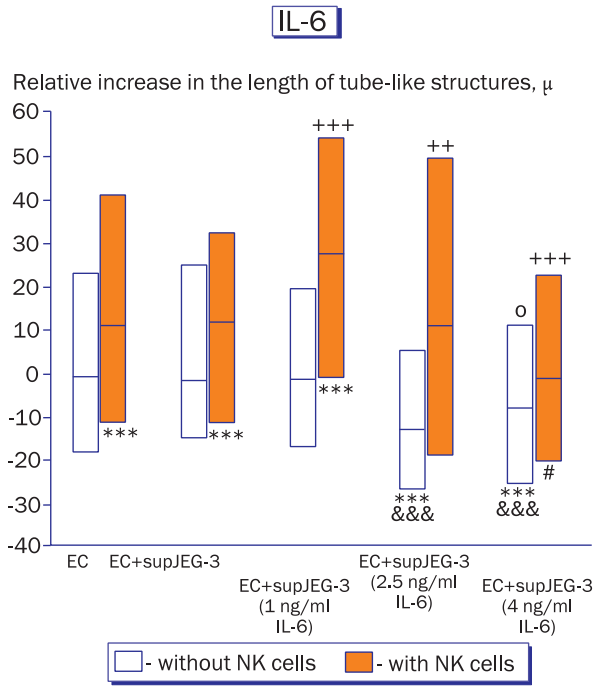

Fig. 4. Formation of tube-like structures by EC of the EA.Hy926 line in monoculture (EC; spontaneous level) and in co-culture with NK cells in the presence of supernatants of intact trophoblast JEG-3 cells (supJEG-3) or trophoblast JEG-3 cells activated with IL-6 (1, 2.5, and 4 ng/ml). ***p<0.001 in comparison with EC; ^{++}p<0.01, ^{+++}p<0.001 in comparison with culturing without NK cells under the same conditions; p<0.05 in comparison ^{o}with similar culturing conditions, but in the presence of 2.5 ng/ml IL-6; $^{\#}$with EC+supJEG-3 with NK cells; $^{\&\&\&}p$<0.001 in comparison with EC+supJEG-3 without NK cells.

natants of non-stimulated JEG-3 trophoblast cells did not change the length of tube-like structures formed by EC. The stimulating effect of supernatants of trophoblast activated with IL-1β and the inhibitory effect of supernatants of trophoblast activated with IL-6 and TGFβ on the formation of tube-like EC structures were revealed. This confirms distant cytokine regulation of angiogenesis by activated trophoblast. It was also found that NK cells increase the length of tube-like structures formed by EC during their contact co-culturing. In this case, supernatants of non-stimulated trophoblast cells did not affect the stimulating effect of NK cells. It was previously demonstrated that both decidual and peripheral NK cells are the source of VEGF and other angiogenic factors and are able to stimulate the formation of blood vessels [22], which coincides with our findings.

Supernatants of trophoblast activated with VEGF did not change the length of the capillary tubes in EC monoculture. They also did not modulate the angiogenesis-stimulating effect of NK cells. This can be associated with another function of VEGF: this directly cytokine also stimulates proliferation and increases viability of trophoblast and endothelium [2].

Supernatants of trophoblast activated with IFNγ did not change the length of the capillary tubes in EC monoculture. In contrast, supernatants of trophoblast activated with IFNγ increased the effect of NK cells stimulating the angiogenesis. Therefore, the trophoblast activated with IFNγ does not affect the angiogenesis directly, but is likely to activate the proangiogenic potential of NK cells. The main source of IFNγ in the placenta is represented by dNK [28], macrophages, and trophoblast. According to the literature, the addition of IFNγ to the cells of choriocarcinoma of the BeWo line inhibited the production of IL-6 and TNFα by trophoblast of this line [1]. Previously, it was established that IFNγ inhibits the invasion and migration of trophoblast, inhibits the production of MMP-2 by trophoblast, stimulates its proliferation [25], and its production of IL-10 [8]. It is likely that IFNγ activates the secretion of cytokines by trophoblast. These cytokines can activate the production of proangiogenic molecules by NK cells. Thus, the ability of NK cells to secrete IL-8, PlGF, angiopoietins 1 and 2 (Ang-1, Ang-2), and VEGF [28] — molecules with a proangiogenic effect — has been proved.

Supernatants of trophoblast activated with 0.1 ng/ml IL-1β increased the length of capillary tubes in EC monoculture. In this case, supernatants of trophoblast activated with 0.1 or 10 ng/ml IL-1β increased the effect of NK cells stimulating the angiogenesis. Therefore, trophoblast activated with IL-1β is able to influence angiogenesis both directly through the production of proangiogenic factors, and indirectly through the activation of the proangiogenic potential of NK cells. The main source of IL-1β in the placenta is represented by trophoblast, macrophages and endometrium [21]. It was demonstrated that IL-1β influences the invasion and migration of trophoblast cells, improving it due to the secretion of MMP-9 [24] by trophoblast cells, which causes the destruction of the matrix and the release of angiogenic factors of VEGF, bFGF, which can determine the stimulation of angiogenesis in case of EC culturing on the Matrigel matrix. The effect of IL-1β on trophoblast (on the example of BeWo choriocarcinoma) dose-dependently stimulates the secretion of IL-6 by these cells, which provides the autocrine stimulation of the production of human chorionic gonadotropin (hCG). The hCG molecule has an angiogenic effect by binding to its receptor on the surface of EC [19]. Given the data on angiogenesis-inhibiting properties of trophoblast cells activated with IL-6 obtained by us, this effect on hCG can be characteristic of either trophoblast preactivated with IL-1β or exclusively of cells of the BeWo line.

Supernatants of trophoblast activated with 2.5 and 4 ng/ml IL-6 reduced the length of capillary tubes in EC monoculture. In addition, supernatants of tropho-

blast activated with IL-6 attenuated the angiogenesis-stimulating effect of NK cells; the latter persisted but was insufficient to increase the length of tube-like structures in comparison that observed in with EC monoculture. Hence, trophoblast activated with IL-6 can inhibit angiogenesis directly through the production of anti-angiogenic factors. In this case, NK cells retain their proangiogenic potential, but due to the "low base" effect after exposure of trophoblast factors to EC, they cannot stimulate angiogenesis in full. The main source of IL-6 in the placenta are cytotrophoblast, endometrium, decidual macrophages [4], placental macrophages, and decidual $CD8^+$ T cells [23]. It was demonstrated that IL-6 stimulates trophoblast invasion and migration [25], which is accompanied by stimulation of endothelial apoptosis under conditions of spiral artery remodeling. Trophoblast cells via secretion of sHLA-G can stimulate the production of IFNγ, G-CSF, IL-1, IL-6, and IL-8 cytokines by dNK and inhibit angiogenesis by triggering EC apoptosis [12], which can be the main mechanism angiogenesis inhibition in our experiments. However, we found no published reports on the effect of IL-6 on the production of sHLA-G by trophoblasts. Identification mediators released by trophoblast activated by IL-6 and other cytokines in our model can become the subject of future research.

The main source of TGFβ in the placenta are dNK [17] and T lymphocytes and trophoblast. TGFβ stimulates differentiation of trophoblast [6], inhibits its proliferation, migration, and invasion [13], and inhibits the secretion of IL-6 and IL-17A by trophoblast cells [1]. We found out that supernatants of TGFβ-activated trophoblast reduced the length of capillary tubes formed by EC in monoculture. Previous studied demonstrated that TGFβ induced the production of the soluble form of FasL (sFasL) surface molecule by trophoblast cells, which induces the apoptosis of Fas-bearing cells [27]. TGFβ inhibits the production of MMP-9 by trophoblast and simultaneously increases activity of TIMP [7], which may underlie the observed effect. It should be noted that, according to other researchers, TGFβ stimulates the invasion of JEG-3 trophoblast cells by stimulating production of MMP-2 and MMP-9 by these cells [15]. We found that supernatants of trophoblast activated with 1 and 5 ng/ml TGFβ attenuated the angiogenesis-stimulating effect of NK cells (the latter was insufficient to increase the length of tube-like structures in comparison with that observed in EC monoculture without inducers). However, NK cells overcame the inhibitory effect of supernatants of trophoblast activated with 10 ng/ml TGFβ. In this case, we observed an increase in the length of tube-like structures formed by EC in co-culture with NK cells in comparison with that observed in the presence of supernatants of non-stimulated trophoblast cells. It can be hypothesized that the pattern of trophoblast activation depends on the concentration of TGFβ. Dose dependence of TGFβ effects on endothelial cells is described in many reports [29]. Both pro- and anti-angiogenic effect of TGFβ were described. The stimulating effect of trophoblast on differentiation of NK cells and modification of their functional and phenotypic properties also cannot be excluded [18]. We found no published reports confirming that NK cells acquire the phenotype and properties of dNK during culturing with trophoblast cells and/or EC on Matrigel. However, recent studies in this area showed that TGFβ and methylating agents modulate the phenotype of NK cells towards dNK [5].

Activated trophoblast regulates angiogenesis via production of soluble factors affecting EC directly or indirectly through stimulation of proangiogenic activity of NK cells. Thus, the stimulating effect of supernatants of trophoblast activated with IL-1β, as well as the inhibitory effect of supernatants of trophoblast activated with IL-6 and TGFβ, on the formation of tube-like EC structures have been established. During the contact culturing, NK cells of the NK-92 line increase the length of tube-like structures formed by EC of the EA.Hy926 line. Trophoblast activated with IL-1β is able to influence the angiogenesis both directly through the production of proangiogenic factors, and indirectly through the activation of the proangiogenic potential of NK cells. Trophoblast activated with IFNγ affects the angiogenesis only by stimulating the proangiogenic potential of NK cells. Under conditions of the contact interaction of EC with NK cells, soluble factors of trophoblast activated with IL-6 or TGFβ inhibit the effect of NK cells stimulating the angiogenesis.

This study was supported by the Russian Science Foundation (grant No. 17-15-01230; EC culturing, evaluation of the tube-like structures formation) and partially supported by the State Program No. AAAA-A18-118011020016-9 (culturing of NK cell and trophoblast cells).

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