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IL10$^{+/+}$ AND IL10$^{-/-}$ TROPHOBLAST CELLS PROLIFERATION, MIGRATION AND INVASION DURING HYPERGLYCEMIA AND EXPRESSION OF CENTRAL MOLECULES

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IL10$^{+/+}$ და IL10$^{-/-}$ ტროფობლასტის უჯრედების პროლიფერაცია, მიგრაცია და ინვაზია ჰიპერგლიკემიის დროს და ცენტრალური მოლეკულების ექსპრესია

თსსუ სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისი, საქართველო

რეზიუმე

ემბრიონის წარმატებული იმპლანტაციისთვის აუცილებელია ფუნქციური კომუნიკაცია ბლასტოცისტსა და მიმღებლიან ენდომეტრიუმს შორის, ხანმოკლე პერიოდში, რომელიც ცნობილია როგორც იმპლანტაციის ფანჯარა. დიაბეტის დროს იმპლანტაციის ფანჯარა ირღვევა, ხელშემშლელი პირობებია ნორმალურ იმპლანტაციისთვის, რასაც მივყავართ სპონტანურ აბორტებამდე. წინამდებარე კვლევის მიზანი იყო იმპლანტაციის პროცესში IL-10, TNF-α, LIF, MMP-9 და NO-ს როლის შეფასება და მათი ზეგავლენის დადგენა ტროფობლასტური უჯრედების ფუნქციურ აქტივობაზე ჰიპერგლიკემიის დროს. ორსული IL-10$^{+/+}$ (C57BL/6J) და IL-10$^{-/-}$ (B6.129P2-Il10tm1Cgn/J) თაგვების პლაცენტებიდან გამოყოფილ ტროფობლასტების უჯრედებზე ჩატარებულ იქნა ექსპერიმენტები. შესწავლილ იქნა გლუკოზის გავლენა მათ ფუნქციურ აქტივობაზე და ზემოაღნიშნული მარკერების ექსპრესიაზე, უჯრედების IL-10, TNF-α, LIF-ით სტიმულაციისას. ჩვენი კვლევის შედეგებიდან ჩანს, რომ ჰიპერგლიკემიის დროს, ანტიანთებითი ციტოკინი IL-10 მოქმედებს როგორც TNF-α-ს ინჰიბიტორი და ამცირებს ოქსიდაციურ სტრესს. ის არეგულირებს LIF და MMP-9-ის ექსპრესიას და გავლენას ახდენს ტროფობლასტური უჯრედების ინვაზიისა და პროლიფერაციის უნარზე. მიღებული შედეგებიდან შეიძლება დავასკვნათ, რომ ჰიპერგლიკემიის დროს IL-10 და TNF-α მნიშვნელოვან როლს თამაშობენ იმპლანტაციის პროცესებში. გარდა ამისა, IL-10 ამცირებს ჰიპერგლიკემიით გამოწვეულ ტროფობლასტური უჯრედების დისფუნქციას და შესაძლოა ამ პროცესში გადამწყვეტ როლს LIF, MMP-9 და NO ასრულებენ.

Introduction

Successful embryo implantation requires a functional communication between a blastocyst and a receptive endometrium during a brief period known as the window of implantation. During the window of implantation, the blastocyst can attach to the endometrial epithelial cells and invade the endometrial stroma and vasculature. This process can only occur when the endometrium is receptive [10]. The process of implantation in humans involves a coordinated sequence of events that are critical for the establishment of pregnancy.

On the one hand, the success of embryo implantation depends on achieving the orchestration of trophoblast proliferation, migration, and invasion into the endometrium to establish not only the anchoring to the uterine wall but also a blood supply for the conceptus [3,9]. This angiogenic process depends on spiral arteries invasion, occlusion, and endothelial remodeling by a highly invasive and migratory sub-population known as extravillous trophoblast (EVT). They invade the uterus and remodel its vasculature to establish an adequate exchange of key molecules between maternal and fetal circulation.

On the other hand, the feto-maternal interface is constituted by a complex net of cytokines, which regulates immunomodulation as well as the vascularization process [1]. They play an important role in the adhesion of the blastocyst to the luminal epithelium, facilitating the physical contact between embryo and uterus and promoting placental development [8]. Implantation can be characterized as an inflammatory response and cytokines are responsible for this response. It seems to be crucial to identify which molecules are implicated in the transition from an inflammatory process before implantation to an anti-inflammatory response necessary for placental vascularization.

Leukemia inhibitory factor (LIF) has been demonstrated to be an important factor in relation to endometrial receptivity [14]. Women with recurrent implantation failure have shown a decrease in LIF production [7]. LIF regulates trophoblast cell adhesion and might be important for embryo invasion and placental development [19]. The invasion of trophoblast cells is regulated by many factors. Matrix metalloproteinase family (MMP), mainly MMP- 9 is closely related to the invasion of trophoblast cells [16]. There are several pro- and anti-inflammatory cytokines which are required for proper implantation, but we are more interested in the cytokines, which play a role in diabetes.

Diabetes is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia), causing many complications. Among such complications, diabetes in a pregnant mother can cause reproductive abnormalities, abortion, congenital anomalies, alterations of fetal growth, and neonatal morbidity and mortality [5,12]. However, there is little information about the influences of diabetes on reproductive performance, placental morphology, and immune responses at the feto-maternal interface.

At present, most researchers accept that a hyperglycemia-induced increase in the production of reactive oxygen species (ROS) is an initial key event in the pathogenesis of diabetes-induced abnormalities. [11,15]. However, as ROS are capable of regulating numerous intracellular signal transduction pathways [2], subsequent pathological events seem to be far from completely understood. Key molecules, like interleukin-10 and tumor necrosis factor-alpha (TNFα), are involved in these processes. IL-10 inhibits TNFα production [13] and its expression is regulated by ROS and it regulates ROS production in turn [18]. The aim of our current study is to evaluate the role of IL-10, TNFα, LIF, MMP-9, and NO in implantation and to establish their possible impact on the functional ability of trophoblast cells under hyperglycemic conditions.

Materials and Methods

Experimental animals: IL-$10^{+/+}$ (C57BL/6J) and IL-$10^{-/-}$(B6.129P2-*Il10*^{tm1Cgn}/J) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed under specific pathogen-free (SPF) conditions in an animal facility with fresh food and water ad libitum, temperature (22.0°C ± 1°C), humidity (40%-60%), and light (12-hour/12-hour light/dark). 8–14 weeks old female mice were caged with males for 3 hr., from 7 to 10 am (dark period) and the presence of a vaginal plug (11 am) was determined as the first day of pregnancy. On day 11 placentas were removed and prepared for trophoblast cells purification.

Purification of trophoblast cells: Villous cytotrophoblasts were isolated by a procedure established previously [4] and used in our laboratory with slight modifications. Briefly, term placentas were first subjected to limited digestion with 0.125% trypsin (Life Technologies, Taastrup, Denmark) and 0.01% DNase I (Roche Molecular Biochemicals, Hvidovre, Denmark). The trophoblast cells were then enriched by centrifugation on a discontinuous gradient of Percoll (Amersham Pharmacia Biotech, Uppsala, Sweden) consisting of 70 and 25% concentrations, and finally purified by negative immunoselection to remove contaminating cells. This procedure involved a mouse monoclonal antibody (MAb) against the monomorphic determinant of MHC class I antigen (clone W6/32; Dako, Glostrup, Denmark) and paramagnetic beads (Dynabeads M-450 goat anti-mouse IgG; Dynal, Oslo, Norway).

Trophoblast cell culture: Isolated trophoblasts were cultured in Dulbecco's Modified Eagle's Media (DMEM) (Cell Biology media facility, Yale University) supplemented with 10% fetal calf serum and 25 mmol/L Hepes, 4 mmol/L glutamine, and 50 mg/ml penicillin, streptomycin, neomycin (Gibco BRL, Grand Island, NY). The cells were plated at a density of 1x104cells/cm2 and cultured for 18h under a humidified 5% CO2/95% air atmosphere at 37^{0}C in the respective medium supplemented with either 5,5 mmol/l (normoglycemic control) or 25mmol/l D-glucose (hyperglycaemic group). The influence of IL-10, TNFα, or LIF on cells cytokines expression ability and cells functionality were studied and the media was supplemented with each cytokine in doses of 100ng/ml, 100ng/ml, or 10ng/ml respectively. These cells and their supernatants were examined in further experiments.

Cell proliferation assay: The isolated trophoblast cells were plated onto 96-well chambers in a volume of 100µl of the medium (5X104 cells/ml). Thereafter, the cell viability was analyzed by Cell Titer 96 AQueous One Solution cell proliferation assay kit (Promega KK, Tokyo, Japan) according to the manufacturer's instructions. Three independent assays were performed from at least triplicate samples.

Invasion and migration assay: The trophoblast cells were used for the invasion or migration assays. These procedures were performed as previously reported [22]. Briefly, 24-well Matrigel invasion kits (BD Biosciences) or 24-well Transwell cell culture chambers (Costar, Ettlingen, Germany) were used for the invasion or migration assays, respectively. The incubation period of the migration assay was 20 h, whereas that of the invasion assay was 24 h. The number of migrated or invaded cells was counted at X100 magnification. Four individual experiments were performed in triplicate.

IL-10, TNF-α, LIF, and MMP-9 measurement: All these markers concentrations were quantified in cells supernatants using specific Quantikine® enzyme-linked immunosorbent sandwich assays (ELISA) (R & D Systems, Minneapolis, USA) according to manufacturer's instructions. The TNF-α assay was linear from 15 to 960 pg/mL and sensitivity was 5.0 pg/mL; and for the IL-10 assay, a standard curve was developed from 1.25 to 2000 pg/mL, with a sensitivity of 1.0 pg/mL. LIF and MMP-9 expressions were analyzed and absorbance was read at 450 nm with wavelength correction at 590 nm.

Measurement of Nitric Oxide production: NO production was analyzed by measuring nitrite/nitrate in the lysates by Griess reaction-based colorimetric assay (R & D Systems, Minneapolis, USA) according to the manufacturer's instructions. The absorbance was read at 540 nm with wavelength correction at 690 nm.

Statistical analysis: The data were expressed as the mean ± SD and the statistical analyses were performed with Student's test. The differences were considered statistically significant when P was < 0.05.

Results

Cell Proliferation

To investigate the effect of hyperglycemia on trophoblast cells proliferation activity and the influence of IL-10, TNF-α and LIF stimulation on this process, a series of experiments were conducted. For this purpose, Villous cytotrophoblasts were isolated from placentas of IL-$10^{+/+}$ (C57BL/6J) and IL-$10^{-/-}$ (B6.129P2-*Il10*^{tm1Cgn}/J) mice on day 11 of pregnancy, and cell viability was analyzed by the cell proliferation assay kit. As shown in Figure 1, in a normal environment proliferation of trophoblasts cells from wild-type and knockout mice placentas are similar.

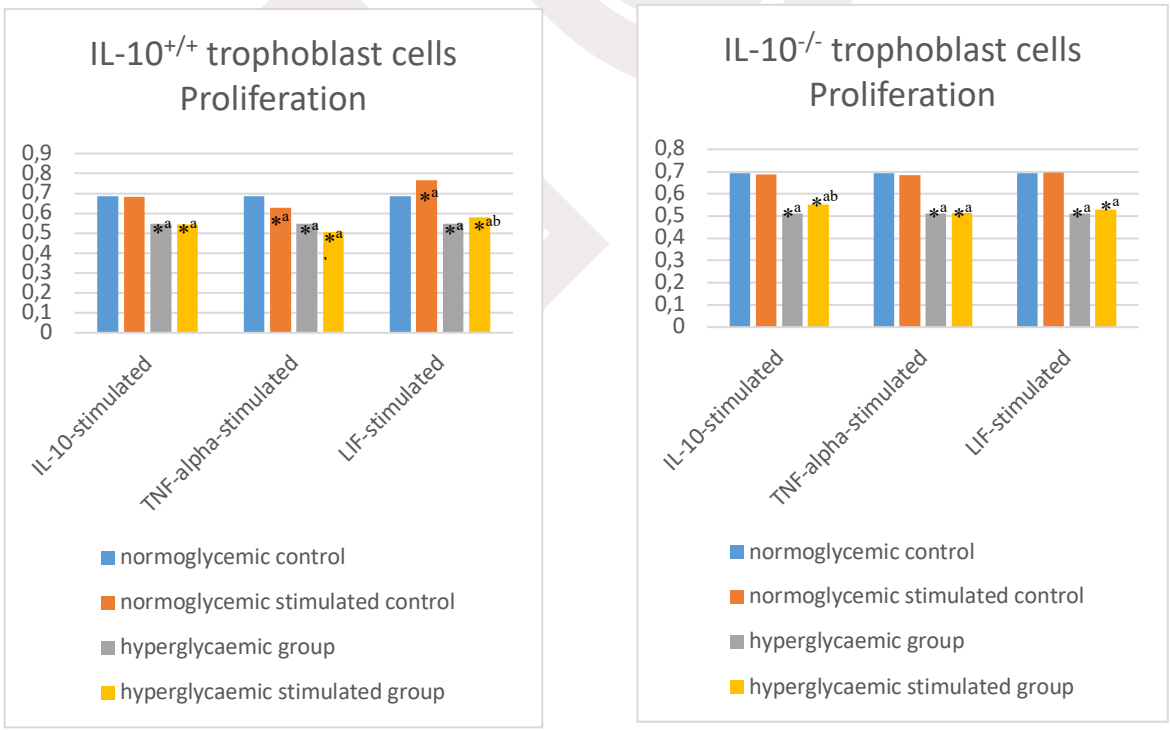

Figure 1. The effect of hyperglycemia and/or either of cytokines IL-10, TNF-α or LIF on proliferation of trophoblast cells was measured by AQueous One Solution cell proliferation assay kit. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean $*^a$ $p < 0.05$ vs. normoglycemic control group; $*^b$ $p < 0.05$ vs. hyperglycemic group.

Hyperglycemia induces retardation of trophoblast cells growth and reduces proliferation rate in both wild-type and knockout cells approximately by 20% and 26%, respectively. Wild-type cells stimulation with IL-10 didn't have any effect on proliferation capacity, whereas in a hyperglycemic environment proliferation rate of knockout cells was increased by 10%. The opposite effect was observed with TNF-α stimulation, it decreases wild cells proliferation activity in both, normoglycemic and hyperglycemic media, while doesn't affect knockout cells growth. Also, as shown in figure 1, LIF stimulation increases cells proliferation activity; there are significant differences between the growth of wild-type cells in normal media, as well as in media with a high content of D-glucose, with or without the addition of LIF. However, there is not enough significance in knockout cells.

Cell Invasion Capacity

For the same reason, as described above, the experiments were conducted and the trophoblast cells invasion ability was investigated. In these experiments, the incubation period was 24 h. and a 24-well Matrigel invasion kit was used. The number of invaded cells was counted and presented in Figure 2. As shown, D-glucose at doses of 25 mmol/l significantly reduces the invasion capacity of trophoblasts cells from the placenta of wild-type and knockout mice, up to 87% and 80% respectively. The stimulation of wild-type cells with IL-10 affects the cells' invasion capacity in normoglycemic media and decreases it by 11%, while under conditions of hyperglycemia does not affect the ability to invade these cells, as well as the ability of knockout cells under both normoglycemic and hyperglycemic states. A strong suppressive effect was observed with TNF-α stimulation; it reduces both types of cells invasion capacity in the normoglycemic and hyperglycemic environment. In addition, as shown in figure 2, LIF stimulation increases the invasion ability of wild-type cells in normal media, while it does not affect knockout cells.

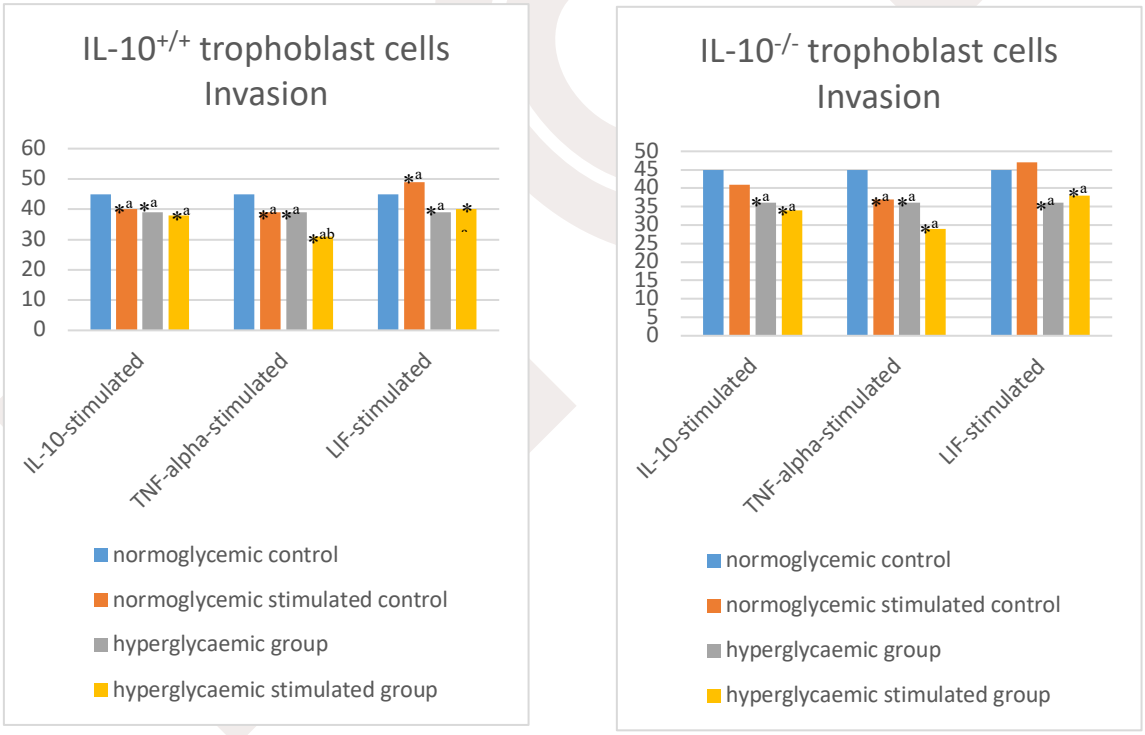

Figure 2. The effect of hyperglycemia and/or either of cytokines IL-10, TNF-α or LIF on invasion capacity of trophoblast cells was performed by 24-well Matrigel invasion kit. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean $*^{a}$ $p < 0.05$ vs. normoglycemic control group; $*^{b}$ $p < 0.05$ vs. hyperglycemic group.

Cell Migration Capacity

The effect of hyperglycemia on trophoblast cells migration ability and the influence of IL-10, TNF-α and LIF stimulation on this process were investigated. For this reason, 24-well Transwell cell culture chambers were used and migrated cells were counted after 20 h of incubation. The data show that the

concentration of D-glucose in the medium influences cells migration capacity (Figure 3.) The ability of wild-type and knockout trophoblast cells to migrate is significantly reduced by glucose (approximately 85% and 92% respectively). Stimulation of these cells by IL-10 and LIF does not affect cells migration capacity. However, TNF-alpha has a decreasing effect on migration ability and these indices of wild-type and knockout mice trophoblast cells in normal media are approximately 86% and 87% of their control levels. The cells migration in media supplemented with TNF-α and high glucose concentration are decreased up to 77% and 85% compared with hyperglycemic group functionality, without the addition of TNF-α.

IL-10, TNF-α, LIF, MMP-9 and Nitric Oxide expression

The effect of hyperglycemia and/or IL-10, TNF-α, and LIF stimulation on the expression of several markers, mainly IL-10, TNF-α, LIF, MMP-9, and Nitric Oxide by the trophoblast cells of the placentas of wild-type and knockout mice were investigated. For this reason, the trophoblasts cells from wild-type and knockout mice were cultured for 18 h in normoglycemic (control) and hyperglycemic media and supernatants were analyzed using ELISA or Griess reaction-based colorimetric assay. As shown in Figure 4 and Figure 5, the results are presented in a fold of control. Wild-type trophoblast cells in normal media produce IL-10, TNF-α, LIF, MMP-9, and Nitric Oxide and their expression levels are changed by hyperglycemic medium; It is high glucose level reduces the expression of IL-10 and LIF up to approximately 60% and 80%, and increases the production of TNF-α, MMP-9 and NO by 2.6, 2.8 and 3.6 times, respectively (Figure 4). Whenever the medium was supplemented with cytokines (namely IL-10, TNF-alpha, or LIF), this picture was modified completely.

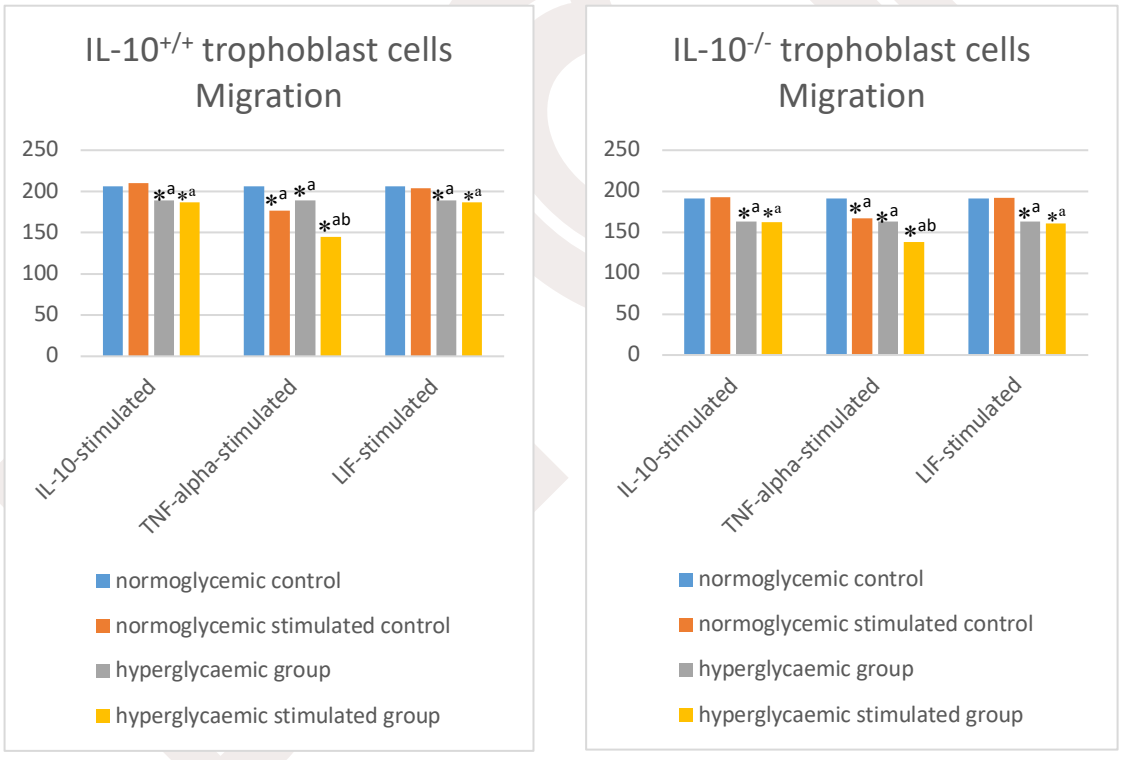

Figure 3. The effect of hyperglycemia and/or either of cytokines IL-10, TNF-α or LIF on migration capacity of trophoblast cells was performed by using 24-well Transwell cell culture chambers. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean $*^a$ $p < 0.05$ vs. normoglycemic control group; $*^b$ $p < 0.05$ vs. hyperglycemic group.

Wild-type cells stimulation with IL-10 and LIF under normal conditions induces the release of higher levels of IL-10 and LIF, while the opposite effect was observed with stimulation by TNF-α. It is also important to mention that stimulation with TNF-α increases NO and MMP-9 production in the normoglycemic state. The stimulation with IL-10 in a hyperglycemic environment significantly reduces the differences between the expression levels of IL-10, TNF-α, LIF, MMP-9 and Nitric Oxide in control

and glycemic groups; trophoblast cells produce approximately 1.6, 1.6 and 1.7-times more TNF-α, MMP-9 and NO respectively, also LIF level grows up to checkpoint and IL-10 to 80% (Figure 4). Stimulation of wild-type cells with LIF doesn't affect the production of MMP-9 and NO productivity in a hyperglycemic environment, but it downregulates glucose-induced contrast for IL-10, TNF-α and LIF. The opposite effect was observed with TNF-α stimulation, it increases the contrast between control and hyperglycemic groups.

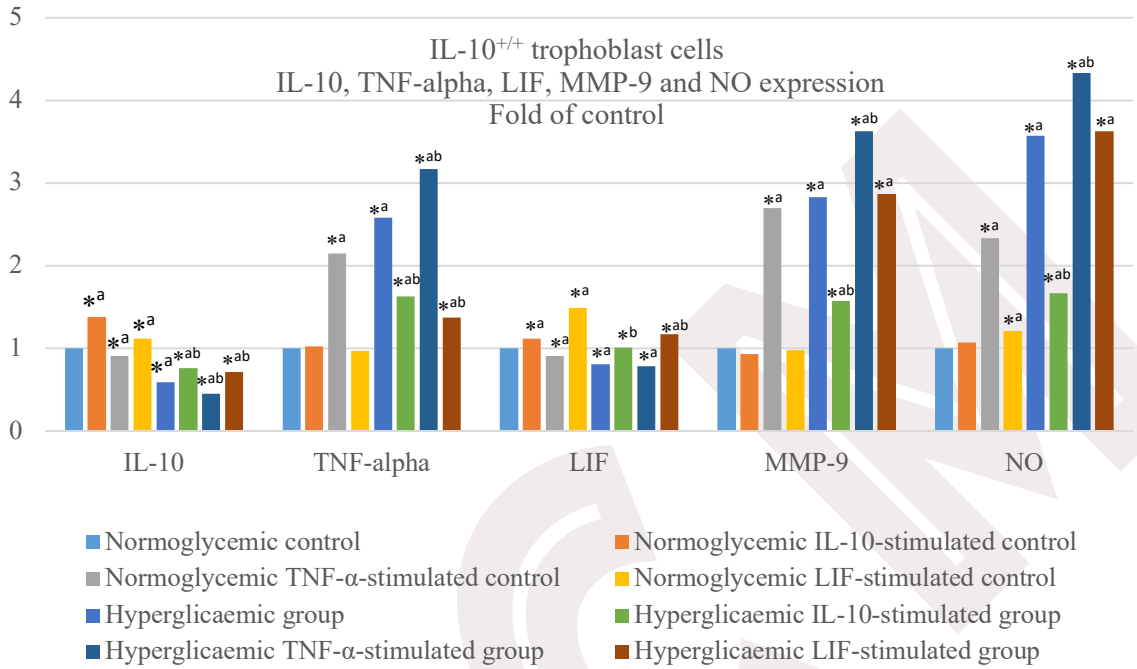

Figure 4. The effect of hyperglycemia and/or IL-10, TNF-α or LIF stimulation on expression of several markers, such as IL-10, TNF-α, LIF, MMP-9 and NO, in wild type trophoblast cells was analyzed by using "R&D systems" assay kits. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed in fold of control. $*^a$ $p < 0.05$ vs. normoglycemic control group; $*^b$ $p < 0.05$ vs. hyperglycemic group.

As shown in Figure 5, Our results verify that IL-10 null mutant mice used in experiments were authentic knockout; their trophoblast cells did not produce IL-10, and only after IL-10 stimulation was this cytokine detected. High glucose concentration alters the expression ability of IL-10-/- trophoblast cells to produce TNF-α, LIF, MMP-9 and Nitric Oxide and it seems, that knockout cells are more sensitive to hyperglycemic insult. There is a decrease in LIF expression to about 70% and an increase in the production of TNF-α, MMP-9 and NO by 3.25, 3.43 and 4.94 times, respectively. Stimulation of these cells by cytokines, such as IL-10, TNF-alpha or LIF induces this pattern modification. knockout cells production rates of investigated markers weren't changed under normal conditions after IL-10 or LIF stimulation, except for their levels. The opposite effect was observed with TNF-α stimulation under normoglycemic conditions, it increases the production of NO, MMP-9 and TNF-α approximately by 2.8, 2,7, and 2,2 respectively, and reduces LIF expression by 10%. The stimulation with IL-10 in a hyperglycemic environment significantly regulates the production of these markers, upregulates LIF expression to the checkpoint, and downregulates the expression levels of TNF-α, MMP-9 and Nitric Oxide up to approximately 2.6, 2.7 and 3.3 fold of control. As shown in Figure 5, there are no significant differences between control and hyperglycemic groups after LIF stimulation of these cells in a hyperglycemic state, other than intrinsic concentration. A strong effect was observed when TNF-α and 25mmol/l D-glucose were supplemented in the medium, knockout trophoblast cells released higher levels of TNF-α, MMP-9 and NO; these measures divided by the control level are 3.7, 3.9 and 5.7 respectively.

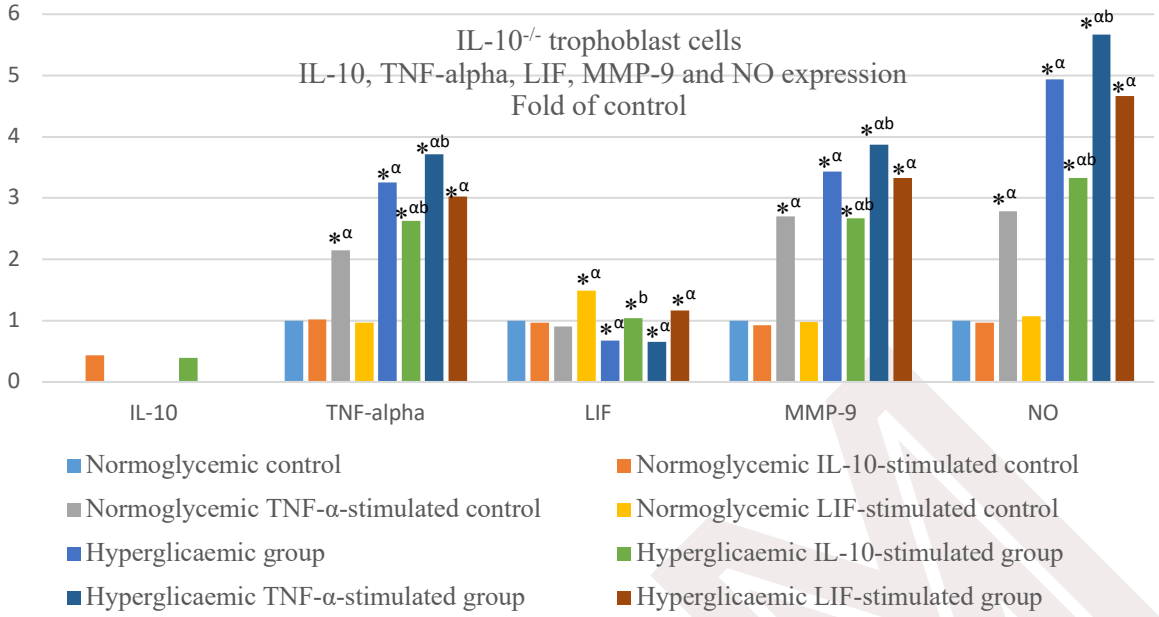

Figure 5. The effect of hyperglycemia and/or stimulation of any cytokines IL-10, TNF-α or LIF on expression of several markers, such as IL-10, TNF-α, LIF, MMP-9 and NO, by knockout (IL-$10^{-/-}$) trophoblast cells was investigated. The production of these markers was analyzed by using "R&D systems" assay kits. Cells were stimulated with IL-10 (100ng/ml), TNF-alpha (100ng/ml) or LIF (10ng/ml) and/or exposed to 25mmol/l of D-glucose for 18 h. The data were expressed as mean. $*^{a}$ $p < 0.05$ vs. normoglycemic control group; $*^{b}$ $p < 0.05$ vs. hyperglycemic group.

Discussion

Successful implantation requires a receptive endometrium, a functional embryo and a synchronized dialogue between them. Under certain inflammatory conditions, such as diabetes, the window of implantation can be affected preventing normal implantation which could lead to pregnancy loss. The results of our previous study suggest that diabetes-induced pregnancy loss resulted from the death of peri-implantation stage embryos [6, 20]. During diabetes mellitus blastocysts develop normally and the cause of pregnancy loss is unsuccessful implantation in the uterus. Embryo–maternal communication is principally mediated via the action of several cytokines and chemokines and especially via interleukins (ILs), which constitute an essential part of the uterine microenvironment. Interleukins significantly affect the process of embryo implantation, from decidua formation and embryo acceptance to trophoblast invasion and placenta formation [17, 20]. However, abnormal IL production, as it happens during diabetes, may detrimentally affect implantation, despite embryos being of good quality and high developmental dynamic, subsequently leading to pregnancy failure. However, the exact role of ILs on the pathophysiology of diabetes is poorly understood and still debatable. On the assumption of literature data, we hypothesized, that IL-10 has an important role in the implantation processes during diabetes. To improve our hypothesis experiments were conducted on trophoblast cells isolated from placentas of IL-$10^{+/+}$ (C57BL/6J) and IL-$10^{-/-}$(B6.129P2-*Il10*^{tm1Cgn}/J) pregnant mice. The cells were stimulated by various cytokines and the effect of glucose concentration on their functional activity and expression ability of several markers were determined.

The results of our investigation showed that trophoblasts cells from knockout mice incubated in media with high glucose concentration released higher levels of pro-inflammatory cytokine TNF-α and NO than cells from wild-type mice. During hyperglycemia anti-inflammatory cytokine IL-10 occurs as an inhibitor of TNFα releases and thereby decreases oxidative stress which is an initial key event in the pathogenesis of diabetes-induced pregnancy loss. As known, TNFα induces activation of the transcription

factor NF-κB, which has the potential both to stimulate the expression of TNFα and inducible Nitric Oxide Synthase, which in turn produced NO overexpression. Diabetes mellitus induces alterations of NO production in tissue. NO is able to modulate the activation of MMPs in the fetoplacental unit and provides supportive evidence that increases NOS activity. During diabetes, MMP-9 and Leukemia Inhibitory Factor (LIF) production in the uterus are fully disturbed, and because of that no uterine receptivity for implantation. As our results showed, hyperglycemia reduces LIF production in wild-type and knockout cells; also, under hyperglycemic conditions, there is an overexpression of MMP-9, but its effect is higher on knockout cells. IL-10 stimulation improves LIF expression up to the control level and drops MMP overexpression. As regards LIF stimulation, it also reduces the imbalance of anti and pro-inflammatory markers productivity between normoglycemic and hyperglycemic environments.

Proper invasion of trophoblast into the endometrium is necessary for normal placentation. The results of our investigation establish that IL-10 evolved in the control of invasion and proliferation of trophoblast cells during diabetes. In a normal environment proliferation, invasion and migration of trophoblasts cells from wild-type and knockout mice are similar. D-glucose in doses of 25 mmol/ml significantly decreases cells proliferation, invasion and migration capacity. From our results, we can conclude, that IL-10 has no influence on cell proliferation and invasion, but it's important in a stressful environment. Unclear is the role of IL-10 in cells migration capacity. TNF-α reduces proliferation, invasion and migration of trophoblasts cells from wild-type and knockout mice, but it is necessary to mention, that IL-10 deficient cells are much sensible to this pro-inflammatory cytokine and diabetic environment. From our data we can also conclude, that TNF-α fully downregulates trophoblast functionality, it reduces cell proliferation, invasion and migration capacity. Also, our results carried out on knockout and wild-type cells show that LIF improves the ability of cells to proliferate and invade and IL-10 participates in this process because LIF stimulation works out just in wild-type cells. There is no significant data to understand the role of this cytokine in the migration capacity of trophoblast cells. Also, the results of our investigation have shown, that the stimulation of the cells with anti-inflammatory cytokine IL-10 in the non-diabetic environment affects cells functionality and suggests, that IL-10 is an important cytokine in hyperglycemic, but not in a normal environment; high concentration of this cytokine leads to the imbalance of production of crucial mediators and mediates unsuccessful implantation by this mechanism.

In conclusion, the results of our study suggest that IL-10 and TNF-alpha are crucial cytokines in implantation processes during hyperglycemia. TNFα act as a mediator of diabetes-induced embryotoxic stimuli leading to the death of peri-implantation stage embryos and IL-10 as a suppressor of diabetes-induced abortion in this stage. In addition, they suggest that molecules such as NO, LIF and MMP-9 may be critical players in the mechanisms determining the outcome of diabetes-induced embryopathic stress.

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IL10$^{+/+}$ AND IL10$^{-/-}$ TROPHOBLAST CELLS PROLIFERATION, MIGRATION AND INVASION DURING HYPERGLYCEMIA AND EXPRESSION OF CENTRAL MOLECULES

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SUMMARY

Successful embryo implantation requires a functional communication between a blastocyst and a receptive endometrium during a brief period known as the window of implantation. Under certain inflammatory conditions, such as diabetes, the window of implantation can be affected preventing normal implantation which could lead to pregnancy loss. The aim of our current study was to evaluate the role of IL-10, TNFα, LIF, MMP-9, and NO in implantation and to establish their possible impact on the functional ability of trophoblast cells under hyperglycemic conditions. For this reason, the experiments were conducted on trophoblast cells isolated from placentas of IL-10$^{+/+}$ (C57BL/6J) and IL-10 $^{-/-}$(B6.129P2-

Il10^{tm1Cgn}/J) pregnant mice. The cells were stimulated by IL-10, TNF-alpha and LIF, and the effect of glucose concentration on their functional activity and expression ability of several markers were determined. The results of our investigation showed that during hyperglycemia anti-inflammatory cytokine IL-10 occurs as an inhibitor of TNFα production and decreases oxidative stress. IL-10 regulates LIF and MMP expressions and evolves in the control of invasion and proliferation processes of trophoblast cells. In conclusion, IL-10 and TNF-alpha play a central role in the processes associated with implantation during hyperglycemia. IL-10 is a suppressor of hyperglycemia-induced trophoblast cell dysfunction and LIF, MMP-9, and NO may be critical players in it.

Keywords: Hyperglycemia, IL-10, Trophoblast cell, TNF-alpha, LIF, MMP-9, NO