

CXCL12/CXCR4 signal involved in the regulation of trophoblasts on peripheral NK cells leading to Th2 bias at the maternal-fetal interface

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Abstract. – OBJECTIVE: In the early pregnancy, large number of decidual natural killer (dNK) cells are present in decidua, which exhibit distinctive phenotype and functions from peripheral blood NK cells (pNK)¹. Unlike the cytotoxic pNK cells, dNK cells display more pronounced characteristics of immune tolerance, which contribute the Th2 bias at the maternal-fetal interface and ensure successful pregnancy². However, the origin and the differentiation program of dNK still remain unknown.

MATERIALS AND METHODS: Our previous study has demonstrated that the CXCL12/CXCR4 signal axis is involved in the shaping of Th2 bias at the maternal-fetal interface^{3,4}.

RESULTS: In this study, we demonstrated the first-trimester human trophoblast cells secrete chemokine CXCL12 that can recruit pNK cells to the decidua. We've also found that the pNK cells differentiate locally under the influence of trophoblast cells. After co-culture with trophoblast cells, pNK cells could acquire dNK characteristics phenotypically while compared to the dNK cells; however, the blocking of CXCL12/CXCR4 signal of pNK cells has abrogated the modulation of trophoblast cells on the pNK cells. We've also found that JNK1/2/MAPK and ERK/MAPK signal pathways were required for the modulation of trophoblast cells on the pNK cells. MAPK signal pathway is involved in the functional modulation of human first-trimester trophoblast cells and decidual stromal cells on pNK and dNK cells.

CONCLUSIONS: Our study has elucidated that CXCL12/CXCR4 can recruit pNK cells to the decidua, then positively modulate pNK cells differentiation into the dNK cells, which thus results in Th2 bias and maternal-fetal immune tolerance.

Key Words:

CXCL12/CXCR4, Decidual natural killer (dNK) cells, Peripheral NK cells, Th2 bias, Maternal-fetal interface.

in which a semi-allogeneic fetus is implanted but remains spared from attack by the maternal immune system.

Despite of the factors proposed to contribute to maternal tolerance, it remains unanswered why the mother's immune system does not reject the fetus despite the proved direct interaction of maternal immune cells with cells of fetal origin at the maternal-fetal interface. During the first trimester of pregnancy, the trophoblast cells, the primary fetal cells, invade the decidual tissue of placenta of maternal origin and form the pool of so-called extravillous cytotrophoblast cells, which participate in the development of early placenta. At this stage of pregnancy, the unique population of CD56^{bright} natural killer (NK) cells has been shown of accounting for over 70% of all decidual leukocytes in early decidua⁵, but their functional significance is still unclear. Nevertheless, it has been believed that the human uterine decidual natural killer (dNK) cells play a role in implantation and pregnancy, at least in the early gestation^{6,7}.

Contrary to the progenitor NK (pNK) cells, dNK cells are characterized by their reduced cytotoxicity and their capacity to secrete Th2 bias cytokines. The dNK cells display a unique repertoire of activating and inhibitory receptors that resemble the early stage of NK cell differentiation to distinguish themselves from pNK cells^{8,9}. pNK cells express high levels of CD16, CD94-associated lectin-like NKG2 receptors and KIRs, which are granular and known to be cytotoxic.

The definite roles of dNK cells are still unknown but two hypotheses concerning the role of dNK cells have been established. First, the dNK cells regulate the angiogenesis in placenta; second, the dNK cells regulate the extravillous trophoblast invasion via the secretion of cytokines¹⁰.

Introduction

The unique immunological situation in pregnancy is known as the "immunological paradox",

The important mechanisms of the maternal dNK tolerance to the fetal trophoblast are likely the unique spectrum of major histocompatibility complex (MHC) antigens that are expressed on the surface of trophoblast cells and the repertoire of dNK receptors. It is well known that trophoblast cells do not express classical MHC class I and class II molecules, except for a low level of HLA-C¹¹, but the non-classical MHC class I molecules, such as HLA-E, HLA-G and probably HLA-F, are widely present on the surface of invasive trophoblast cells, which are conducive to protect the trophoblast from NK-mediated attack^{12,13}.

Our previous study has demonstrated the first-trimester human trophoblast cells secrete chemokine (C-X-C motif) ligand 12 (CXCL12) which, in addition to inducing trophoblast proliferation and mediating crosstalk between trophoblasts and DSCs, can also recruit dNK cells into the decidua by its interaction with chemokine (C-X-C motif) receptor 4 (CXCR4). We've also found that the CXCL12/CXCR4 signal axis was actively involved in the development of Th2 bias at the maternal/fetal interface. All these observations suggest that the CXCL12/CXCR4 signal should play an important role in the cross-talk between different functional cell types at the human maternal/fetal interface.

Interestingly, the chemokine receptor profile of pNK cells as instructed by trophoblast cells closely resembles that of dNK cells. In addition to the recruitment, CXCL12 is required for NK development, suggesting that the high CXCL12 levels in the decidual environment should contribute to the unique phenotype and function of dNK cells. In this study, we've examined whether CXCL12/CXCR4 recruit pNK cells to the decidua or not, then modulated the pNK cells differentiation into the dNK cells. We propose to investigate the role of CXCL12/CXCR4 signal in the regulation of maternal-fetal functional cells on pNK cells, which lead to Th2 bias and maternal-fetal immune tolerance.

Materials and Methods

Patient Recruitment

All subjects signed in writing have informed consent forms as approved by the Human Investigation Committee in the Hospital of Obstetrics & Gynecology, Fudan University. The first-trimester human villous and decidual tis-

ues were obtained from women in healthy early pregnancy as confirmed by ultrasound (average age, 29.70 ± 4.78 years; average gestational age at sampling, 53.83 ± 6.72 days, mean $\pm$ SD), which were terminated for non-medical reasons. All the tissues were immediately collected in the ice-cold Dulbecco's modified Eagle medium (DMEM) with high D-glucose (Gibco, Grand Island, NY, USA), transported to the laboratory within 30 min after surgery and then washed in calcium- and magnesium-free Hanks balanced salt solution for trophoblast or DSC isolation.

Isolation and Primary Culture of Human First-trimester Trophoblast Cells

The villous tissue was separated carefully from the deciduas and digested by the repeated trypsin digestions following our previous method. Briefly, the placenta was digested by 0.25% trypsin (Bio Basic Inc., BBI, Ontario, Canada) and 0.02% DNase type I (Sigma Chemicals co., St Louis, MO, USA) at 37°C with gentle agitation for 5 min. Then the digested suspension was discarded and the residual tissue was subject to four cycles of digestion of 10min. The cell suspensions were pooled, carefully layered over a discontinuous Percoll gradient (65% to 20%, in 5% step) and centrifuged at 1000 g for 20 min. The cells sedimenting at densities between 1.048 and 1.062 g/ml were collected and washed with DMEM-high-glucose medium. These cells were maintained in DMEM-high-glucose complete medium (2 mM glutamine, 25 mM HEPES, 100 UI/ml penicillin and 100 mg/ml streptomycin) and supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Waltham, MA, USA). This method has yielded trophoblast cells with 95% purity, which has been described in detail in our previous publications.

Isolation and Primary Culture of Decidual Stromal Cells (DSCs) and Decidual Immune Cells (DICs)

The specimens were minced and digested in six cycles of 30 min by using IV Collagen (Sigma) in a shaking water bath at 37°C. The digested cells were, then, passed through a 38 mm gauze and purified by centrifugation through a discontinuous Percoll gradient. DSCs, which ranged in a density between 1.042 and 1.062 g/mL, were recovered and cultured in DMEM/F12 complete medium supplemented with 10% FBS, 100 U/mL penicillin, and

100lg/mL streptomycin in 5% CO₂ at 37°C. Upon the primary culture for 30 min, non-adherent lymphocytes were removed by washing, leaving DSCs with the purity above 95%. After two passages, DSCs reached a purity of over 99%. DICs, which ranged in density between 1.062 and 1.077 g/mL, were collected and cultured in RPMI 1640 complete medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin in 5% CO₂ at 37°C. After the primary culture for 30 min at 37°C in 5% CO₂, the adherent DSCs were removed by washing and leaving DICs with the purity of 98%.

ELISA

Supernatants of trophoblasts, trophoblast-DSC and DSCs cultures were harvested after 48 hours. Each supernatant was centrifuged at 2000 × g and stored at -80°C. Enzyme-linked immunosorbent assays were performed with human SDF-1a kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Five individual samples were tested.

Isolation of pNK Cells and dNK Cells

The peripheral blood mononuclear cells (PBMC) from human early pregnancy were isolated by using Ficoll-Haque methods. Briefly, they were centrifuged at 2000 × g for 15 min at the room temperature. pNK and dNK cells were purified by using a magnetic activated cell sorting (MACS) human NK cell negative selection isolation kit (Miltenyi Biotec Inc., Auburn, CA, USA) according to the manufacturer's instructions. The purity of pNK and dNK cells was above 95%, as analyzed by flow cytometry with CD56-FITC, CD3-PE, and CD16-APC antibodies.

In vitro Cell Migration Assay

3 × 10⁵ pNK cells of in 100ml DMEM were loaded into each Transwell filter (5 µm pore filter Transwell, 24-well cell clusters; Corning, NY, USA). The filters were then plated in each well containing the supernatant medium of trophoblast cell cultures or differing concentrations of recombinant human SDF-1 (rhSDF-1, R&D Systems). Upon incubation at 37°C in 5% CO₂ for 3h, the upper chambers were removed and suspension cells in the bottom chamber were collected, counted and analyzed by flow cytometry. Results are expressed as per the percentage of migrating cells (number of migrated cells/total number of input cells ×100). The experiments were repeated by four times.

Co-culture of Trophoblast-pNK Cells

Trophoblast cells were cultured in 12-well plates at a density of 3 × 10⁵ cells/well. In this co-culture system, pNK cells were subsequently added in the wells directly in the same density. Some wells were pretreated with CXCR4 antagonist AM3100 (50 µmol/L) or the isotype control to block the CXCR4 of pNK. Meanwhile, pNK cells of 3 × 10⁵ cells/well were cultured alone as controls. The total volume of medium (DMEM/F-12 with 10% FBS) in each well was 1 ml. After 48 hour, the floating pNK cells were collected for flow cytometry immediately.

Flow Cytometry

pNK cells were harvested and re-suspended in PBS. The cell suspension was placed in Falcon 2054 polystyrene round-bottom tubes (Becton Dickinson, USA) in 100-µL aliquots for the purpose of immunolabeling. The floating pNK cells were washed twice. The expression of surface molecule on pNK cells was detected by means of labeling them with CD56, CD3, CD16, CXCR4, CCR2, NKp44, and KIR2DL1. For the sake of intracellular molecule detection, pNK cells were gated for the expression of perforin, TNF-α, IL-4, and IL-10.

Some wells were pretreated with JNK1/2/MAPK signal antagonist (SP600125, 30 µmol/L), P38/MAPK signal antagonist (SB202190, 30 µmol/L) and MAPK/ERK signal antagonist (U0126, 30 µmol/L) to block the MAPK signal of pNK cells. Samples were analyzed in a FACS Calibur flow cytometer (BD Biosciences) by using CellQuest software (Becton Dickinson, Franklin Lakes, NJ, USA). Statistical analysis was performed by using the isotype-matched control as the reference.

CytoTox 96® Non-Radioactive Cytotoxicity Assay of pNK Cells

96 well assay plates were set up for the following group: pNK cell spontaneous LDH release, pNK and K562 LDH release, K562 cell spontaneous LDH release, K562 cell maximum LDH release, volume correction control and culture medium background. The experimental and control reactions were carried out in triplicates. The assay plates were centrifuged at 250 × g for 4 min so as to guarantee the contact of pNK and K562 cells. The Cytotoxicity assay plates were incubated for 4h in a humidified chamber at 37°C with 5% CO₂. The incubation for at least 4h is needed for sufficient contact between the target and the effector

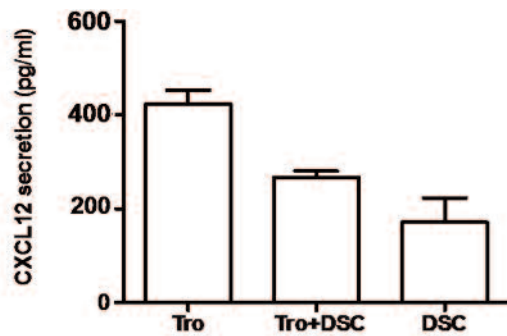


Figure 1. The expression of CXCL12 at the maternal-fetal interface.

cells; then 10 μ l Lysis Solution (10X) was added for every 100 μ l of target cells to the wells containing the Target Cell Maximum LDH Release Control 45 min before harvesting the supernatants. Upon the incubation for 4h, the plates were centrifuged at $250 \times g$ for 4 min. 50 μ l aliquots from all wells were transferred by using the multi-channel pipets to a fresh 96 well flat-bottom (enzymatic assay) plate. 12 ml Assay Buffer was thawed and removed and the unused portion was promptly stored at -20°C . Warm the Assay Buffer of 12 ml to the room temperature (protected from light); then the Assay Buffer was added to one bottle of Substrate Mix, which was inverted and shaken gently to be dissolved. One bottle of substrate is sufficient for two 96 well plates. Once re-suspended, the substrate should be protected from light and used immediately; then 50 μ l reconstituted substrate mix was added to each well of the enzymatic assay plate containing samples transferred from the cytotoxicity assay plate. The plate was covered with foil or an opaque box to get protected from light and be incubated for 30 min at room temperature. Afterwards, 50 μ l stop solution was added to each well. Large bubbles were popped by a syringe needle and the absorbance was recorded at 490 nm or 492 nm within 1h after the stop solution was added. Finally, the results were calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{(\text{The experimental} - \text{the effector spontaneousness} - \text{target spontaneousness})}{(\text{Target maximum} - \text{target spontaneousness})} \times 100$$

Statistical Analysis

One-way or two-way analysis of the variance was used for the statistical comparisons of cytokine production. The post hoc Dunnett *t*-test was

used to compare the significance between the control and various treatments. All error bars in the figures indicate standard errors (SE) and $p < 0.05$ is considered as statistically significant.

Results

Expression of CXCL12 at the Maternal-fetal Interface

In order to validate the expression of CXCL12 at the maternal-fetal interface, we isolated and cultured human first-trimester trophoblast cells and DSCs for 48h. ELISA was performed to determine the secretion of CXCL12 by these cells. We've found that the dominant CXCL12 expression on the maternal-fetal functional cells is trophoblasts, the accumulated concentration of CXCL12 by trophoblast is about 412 ng/ml after culture for 48h, whereas that by DSCs is about 178 ng/ml (Figure 1).

Migration of pNK Cells to CXCL12

Because it has been shown that the first-trimester human trophoblast cells produce CXCL12 spontaneously, we assessed the chemotaxis of CXCL12 to the pNK cells, which highly expressed CXCR4, the receptor of CXCL12. The results showed that rhSDF-1 indeed induced the chemotaxis of decidual pNK cells within a suitable concentration range in a dose-dependent manner while compared with cells pre-incubated with isotype-matched irrelevant IgG. A total of $15.4 \pm 2.16\%$ and $18.80 \pm 4.43\%$ of the input cells migrated to the bottom chamber containing 10 ng/ml and 100 ng/ml rhSDF-1 respectively ($p < 0.05$ while compared with cells migrating toward DMEM only). When the cells were pre-incubated with CXCR4 antagonist, they failed to migrate to the bottom chamber containing rhSDF-1, which clearly demonstrated that the interaction between the CXCL12 secreted by human trophoblast cells and the CXCR4 expressed by the pNK cells directs the migration of the pNK cells (Figure 2).

CXCL12/CXCR4 is Involved in the Functional Modulation of Human First-Trimester Trophoblast Cells on pNK Cells

Our previous study has demonstrated that the CXCL12/CXCR4 axis is playing an important role to contribute the Th2 bias at the maternal-fetal interface. As more than 70% of decidual

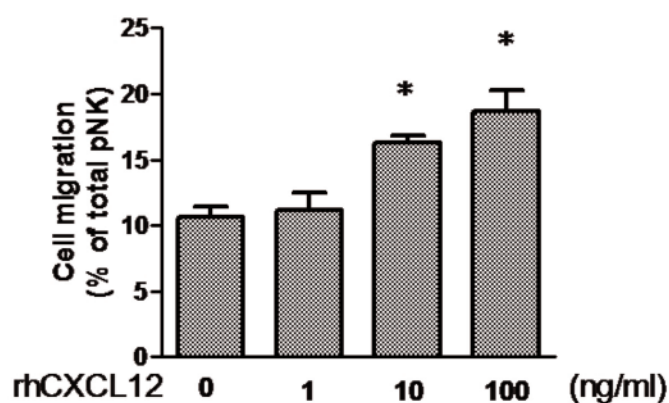


Figure 2. Migration of pNK cells to CXCL12.

immune cells are NK cells, in this study, we investigated whether the CXCL12/CXCR4 axis was involved in the functional modulation of trophoblast cells on pNK cells or not. pNK cells were pre-treated with CXCR4 antagonist, then co-cultured with trophoblasts. pNK cells were harvested and their phenotype, Th1/Th2 cytokine expression and cytotoxicity of NK cells were examined by flow cytometric (FCM) and lactate dehydrogenase (LDH) release assay. We've found that blocking of the CXCL12/CXCR4 signal increased the expression of activated receptor CD16 and NKp44 on pNK cells co-cultured with trophoblasts, whereas the inhibitory receptor KIR2DL1 on pNK cells co-cultured with trophoblasts was decreased (Figure 3); furthermore, the blocking CXCL12/CXCR4 signal significantly increased the expression of Th1 cytokine TNF- α , and inhibited Th2 cytokine expression, such as IL-4, IL-10 on NK cells co-cultured with trophoblasts. The increased perforin expression and the cytotoxicity of pNK cells were also observed upon treatment (Figure 3). It can be concluded that human recombinant CXCL12 could amplify and CXCR4 blockage could abrogate the modulation of trophoblast cells on pNK cells. The influence of trophoblast cells on pNK cells is via the CXCL12/CXCR4 signal pathway.

MAPK Signal Pathway is Involved in the Functional Modulation of Human First-trimester Trophoblast Cells on pNK cells

pNK cells were pre-treated with JNK1/2/MAPK signal pathway inhibitor SP600125, P38/MAPK signal pathway inhibitor SB202190 and ERK/MAPK signal pathway inhibitor U0126. And then the phenotype, Th1/Th2 cytokine ex-

pression and cytotoxicity of NK cells were determined. We've found that blocking of JNK1/2/MAPK and ERK/MAPK signal pathway can abrogate the modulation of trophoblast cells on the pNK cells (Figure 4). Our results indicate that mitogen-activated protein kinase (MAPK) signal pathway is involved in the functional modulation of human first-trimester trophoblast cells on pNK cells.

Discussion

NK cells represent a distinct population of circulating and tissue-resident lymphocytes that can eliminate their targets by different mechanisms, including direct cell cytotoxicity or cytokine production¹⁴. The net balance between activating and inhibitory signals would determine the outcome of NK cell responses against various threats^{15,16}. During pregnancy, one prominent feature of the human decidua is the striking abundance of NK cells, which constitute 70% of resident lymphocytes, forming one of the maternal-fetal interfaces. These dNK cells play a pivotal role in the tissue homeostasis and endometrial vasculature remodeling, which are necessitated by successful pregnancy¹⁷. Evidence suggests that reduced number of dNK cells has been demonstrated in patients with pre-eclampsia and intrauterine growth restriction (IUGR), which are the conditions associated with the poor SA remodeling and the reduced trophoblast invasion in the decidua¹⁸.

dNK cells are generally considered having a cytokine-secreting role rather than having the predominantly cytotoxic defensive role of pNK cells because of their unique expression profile^{19,20}. Although dNK cells express cytotoxic

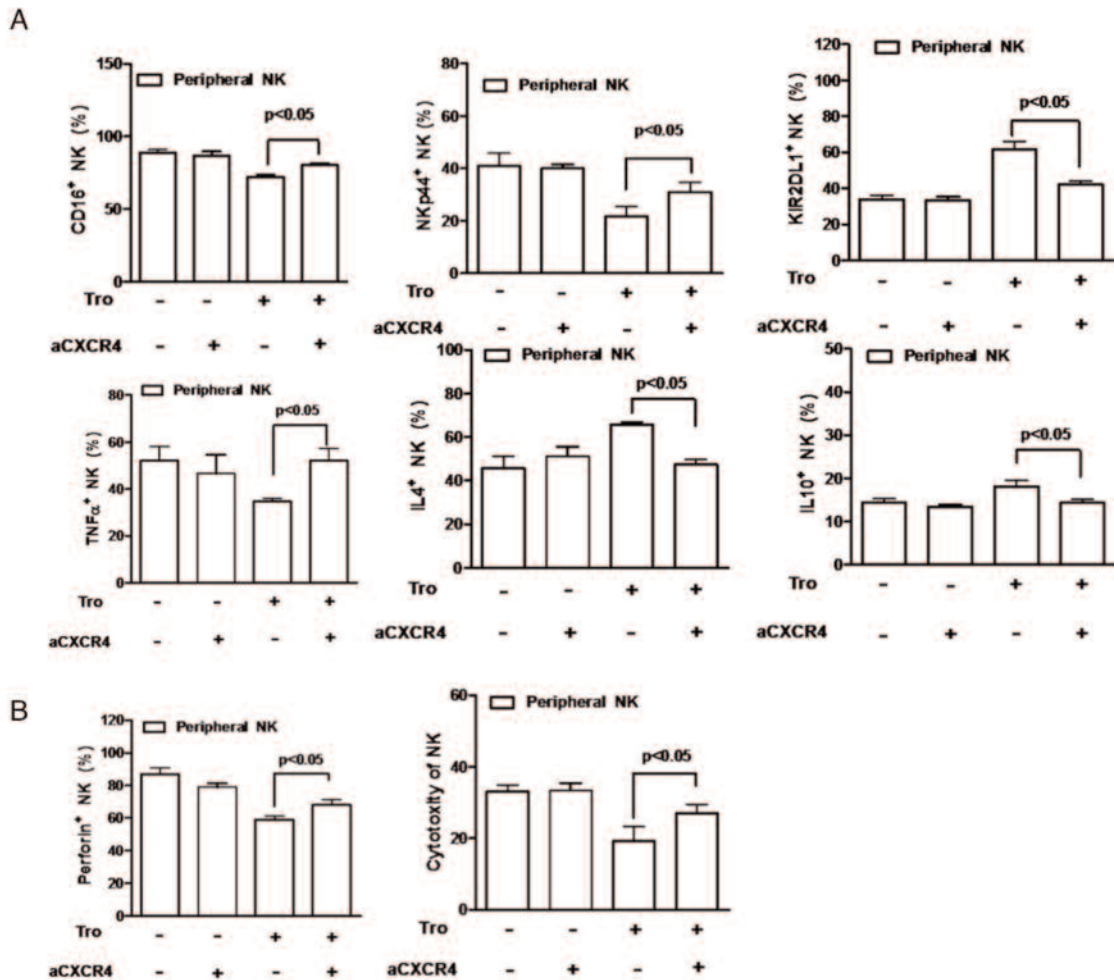


Figure 3. CXCL12/CXCR4 is involved in the functional modulation of human first-trimester trophoblast cells on pNK cells.

proteins, such as perforin, granzymes A and B, and granulysin and, thus, have cytolytic capacity; this cytotoxic machinery does not cause death of the invading trophoblasts. But in response to the infection, they show the cytotoxicity. The pattern of inhibitory and activating receptors expressed on the surface of dNK cells may contribute to their dynamic cytotoxicity^{21,22}.

It is well known that chemokines play well-established role as attractants of naive and effector T cells. Different chemokine receptors are preferentially expressed on these cells^{23,24}. Our previous study has demonstrated that the fetal trophoblast cells and maternal DSCs and DICs produce various cytokines. *In vitro*, the co-culture of these cell types results in the production of both Th1-type and Th2-type cytokines in the supernatant, but Th2-type cytokines predominate over Th1-type cytokines. rhCXCL12 can promote the production of Th2 and inhibit Th1 cytokine pro-

duction from DICs, and blockade of the CXCL12/CXCR4 signal switches the Th2 to a Th1 bias in the co-culture. These findings shed light on the complicated interactions among functional cells through the CXCL12/CXCR4 signal, which is involved in the Th1/2-type cytokine production and the development of the Th2 bias at the maternal/fetal interface. The decreased Th2 cytokine production and CXCL12/CXCR4 expression are observed in the decidua and villi from miscarriage, which has deepened our understanding of the mechanisms of Th2 bias at the maternal/fetal interface.

CXCR4 is the chemokine receptor of CXCL12. In addition to its important effect on HIV-1 infection²⁵, CXCR4 and its specific ligand CXCL12 play a key role in lymphocyte trafficking and recruitment at sites of inflammation and in hematopoiesis and development processes, such as organogenesis, vascularization, and embryoge-

nesis²⁶⁻³². Recently, it has been reported that CXCL12 can cause CXCR4+ Treg cells to migrate into the pregnant mouse uterus and establish a beneficial microenvironment for the fetus³³.

It has also been demonstrated that CXCL12 induces both the chemotaxis and calcium mobilization in NK cells. CXCR4 binds to the heterotrimeric G proteins and is coupled to Go, Gq, and Gs but not to Gi, Gz, G12, or G13. Both Go and Gq are involved in CXCL12-induced chemotaxis and calcium mobilization in NK cells. Abs to these G protein subfamilies inhibit both activities³⁴⁻³⁵.

The mechanism of dNK cell recruitment is still controversial. One possibility is that this pNK cells might move into the uterus to proliferate, differentiate and proliferate in the decidual microenvironment. It has been suggested that a possible differentiation route for dNK cell origin. CD16+ pNK cells may migrate into uterine tissue, then differentiate locally under the influence of TGF- β ¹⁵, and thereby acquire the dNK phenotype. This hypothesis is attractive because CD16+ pNK cells represent the overwhelming majority of pNK cells and these cells share the presence of extensive cytotoxic granules with dNK cells. More recently, it has been reported that CXCL12/CXCR4 signaling could regulate the biology and development of natural killer cells³⁶.

Since CXCL12 is a chemoattractant for NK cells and in our experiments, CXCR4 was inter-

mediately expressed on dNK cells and highly expressed on pNK cells. We speculated that the interaction between CXCL12 and CXCR4 at the maternal-fetal interface on the pNK cells' surface might contribute to the recruitment of these cells into decidua.

In the current study, we've found that CXCL12 was mainly from trophoblasts and less from decidual stromal cells (DSCs) at the maternal-fetal interface. The first trimester trophoblast cells constitutively express CXCL12. In addition, the supernatant of trophoblast cell cultures has also showed chemotactic activity on the pNK cells. When the cells were pre-incubated with CXCR4 antagonist, the pNK did not specifically migrate to the culture supernatant of trophoblast cells, which clearly indicates that the chemotactic property of the trophoblast supernatant is attributable to the presence of CXCL12.

Our study has also demonstrated the first-trimester human trophoblast cells could modulate the phenotype, Th1/Th2 cytokine expression and cytotoxicity of pNK cells, which can cause pNK cells to acquire some characteristics of dNK cells, contributing to maternal-fetal immune tolerance. The blocking of the CXCL12/CXCR4 signal would abrogate the modulation of trophoblast cells on the pNK cells, which suggest that the effect of trophoblast cells on pNK cells should be via the CXCL12/CXCR4 signal pathway.

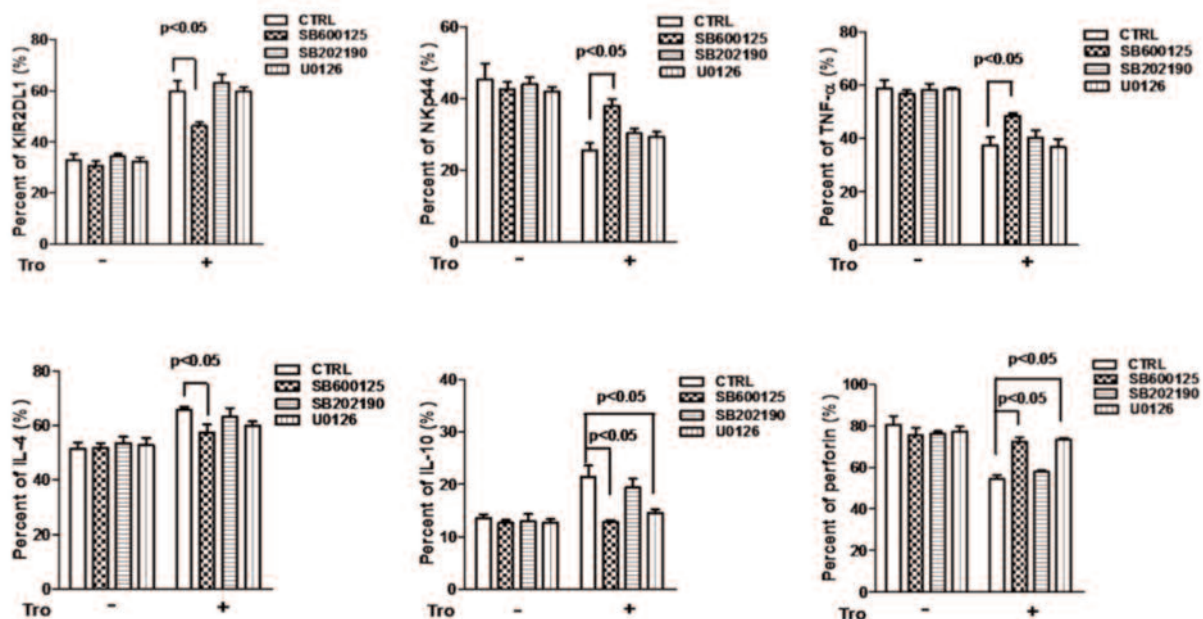


Figure 4. MAPK signal pathway is involved in the functional modulation of human first-trimester trophoblast cells on pNK cells.

As the signaling by the MAPK family is a major mechanism through which the cells respond to a variety of stimuli from the extracellular environment. Molecules that comprise the core of MAPK signaling are conserved from yeasts to mammals. Several subfamilies of MAPK cascades coexist in mammalian cells: the growth promoting extracellular signal-regulated kinase (ERK) family and namely the c-jun N-terminal kinase (JNK) and p38 families^{37,38}.

Among these MAPK family cascades, the ERK family cascade is the best studied. In the ERK cascade, a cell surface receptor is firstly stimulated by a growth factor (i.e., a mitogen). The JNK and p38 MAPK family cascades are initiated by physiological mediators such as transforming growth factor- β (TGF- β), tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) as well as by environmental stresses such as ultraviolet light, gamma rays, translation inhibitors, hyperosmotic stress and oxidative stress³⁹.

In this study, by means of JNK1/2/MAPK signal pathway inhibitor SP600125, P38/MAPK signal pathway inhibitor SB202190 and ERK/MAPK signal pathway inhibitor U0126, we've found that blocking JNK1/2/MAPK and ERK/MAPK signal pathway can abrogate the modulation of trophoblast cells on the pNK cells. It indicates that MAPK signal pathway is involved in the functional modulation of human first-trimester trophoblast cells on pNK cells.

Conclusions

We've provided indirect evidences that dNK cells originate from the pNK. We've showed that chemokine ligand CXCL12, the ligand of chemokine receptors CXCR4 expressed by trophoblasts has a role in the migration of pNK cells to the decidua. We've also showed that CXCL12/CXCR4 favors conversion of pNK cells to less cytotoxicity NK cells resembling dNK cells on the JNK1/2/MAPK and ERK/MAPK signal pathway. Although it is still not possible to draw conclusions on the nature of the *in vivo* precursor cells of dNK, this study suggests that CXCL12/CXCR4 may play an important role in influencing the trophoblast regulation of pNK cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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