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Decidual Natural Killer Cell Interactions with Trophoblasts Are Impaired in Pregnancies at Increased Risk of Preeclampsia

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Transformation of the uterine spiral arteries (SAs) during pregnancy is critical to support the developing fetus, and is impaired in some pregnancy disorders, including preeclampsia. Decidual natural killer (dNK) cells play a role in SA remodeling, although their interactions with fetal trophoblast remain unclear. A uterine artery Doppler resistance index (RI) in the first trimester of pregnancy can be used as a proxy measure of the extent of SA remodeling; we have used this technique to characterize dNK cells from pregnancies with normal (normal RI) and impaired (high RI) SA remodeling, which display least and highest risk of developing preeclampsia, respectively. We examined the impact of dNK cell secreted factors on trophoblast motility, chemoattraction, and signaling pathways to determine the contribution of dNK cells to SA transformation. We demonstrated that the chemoattraction of the trophoblast by dNK cells is impaired in pregnancies with high RI, as is the ability to induce trophoblast outgrowth from placental villous explants. These processes are dependent on activation of the extracellular signal–regulated kinase 1/2 and phosphatidylinositol 3-kinase–Akt signaling pathways, which were altered in trophoblasts incubated with secreted factors from dNK cells from high RI pregnancies. Therefore, by characterizing pregnancies using uterine artery Doppler RI before dNK cell isolation, we have identified that impaired dNK-trophoblast interactions may lead to poor placentation. These findings have implications for pregnancy pathological conditions, such as preeclampsia. *(Am J Pathol 2013, 183: 1853–1861; http://dx.doi.org/10.1016/j.ajpath.2013.08.023)*

After implantation of the blastocyst, the pregnant uterus (decidua) undergoes physiological changes to ensure a successful pregnancy. Key to this is the transformation of the uterine spiral arteries (SAs) from high-resistance, low-flow vessels to low-resistance, high-flow vessels. This establishes an increased blood flow to the intervillous space, allowing nutrient and gas exchange between the maternal blood flow and fetal placenta.[1] SA transformation is under the control of extravillous trophoblast (EVT), specialized fetal cells derived from the placenta that invade into the decidua and remodel the SA by inducing apoptosis of endothelial cells and vascular smooth muscle cells (VSMCs).[2,3] The VSMCs undergo induced hypertrophy and disruption of VSMC layers, leading to motility and dedifferentiation of vascular smooth muscle cells.[4,5] Trophoblasts then line the SA in place of the absent vascular cells. The essential presence of trophoblasts in SA transformation is inferred from pregnancy disorders, such as preeclampsia and intrauterine growth restriction, that display reduced trophoblast invasion and reduced remodeling of the arteries in the first trimester of pregnancy.[6,7] The reason for decreased EVT invasion in these pathological conditions is unknown.

It is increasingly recognized that transformation of SA begins in a trophoblast-independent manner, when only maternal cells are present surrounding the spiral arteries.[8] Several different maternal cells exist in the decidua in the first trimester of pregnancy, including leukocytes, of which 70% are decidual natural killer (dNK) cells. These differ from peripheral blood natural killer (pbNK) cells in their surface receptor expression; notably, dNK cells are predominantly $CD56^{bright}CD16^{-}$, whereas pbNK cells are predominantly $CD56^{dim}CD16^{+}$. Decidual NK cells are thought to have a cytokine-secreting role as opposed to the cytotoxic role of pbNK cells,[9] because dNK cells do not kill trophoblasts in a

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normal pregnancy,[10] despite possessing the same cytotoxic capacity as pbNK cells in terms of expression of the cytolytic proteins, perforin and granzyme.[11] Disruption of the vasculature when the SAs are surrounded by dNK cells, but the trophoblasts are absent, has been identified[8] and has implicated dNK cells in SA remodeling via both direct interaction with the vascular cells and indirect interaction with trophoblasts.

Decidual NK cells are proposed to contribute directly to SA remodeling by expression of secreted factors that disrupt vascular cell interactions,[12] including matrix metalloproteinases that disrupt vascular extracellular matrix connections, therefore enabling migration of VSMCs from the vessel.[8,13] Some apoptosis of VSMCs and endothelial cells during remodeling has also been attributed to dNK cells via an Fas-ligand pathway.[14] Decidual NK cells may also have indirect effects on remodeling by controlling EVT invasion, by both promotion and inhibition. EVT invasion is dependent on motility and chemotaxis. dNK cells have been demonstrated to increase EVT motility via hepatocyte growth factor secretion,[14] and to play a role in chemoattraction of the EVT to the sites of remodeling, in particular through expression of the chemokines IL-8 and CXCL10.[15] They have also been reported to decrease trophoblast invasion via an interferon-γ–secreted mechanism.[16] However, many chemokines have been identified as secreted by dNK cells, and these may also play a role in the promotion of invasion of EVT through the decidua.[17–19]

Although there is strong evidence for the role of dNK cells in mouse SA remodeling,[20] and human dNK cells have been linked to pregnancy disorders associated with poor SA remodeling,[21,22] the key role of dNK cells in human pregnancy is proposed to occur during the first trimester of pregnancy, hampering studies because of a lack of access to tissue. When first-trimester termination of pregnancy samples is used for isolation of dNK cells, the outcome of that pregnancy, if brought to term, and the extent of remodeling are unknown. Uterine artery Doppler resistance index (RI) in the first trimester can be used as a proxy measure of the extent to which remodeling of the spiral arteries has occurred,[23,24] and can, therefore, be used as a technique to separate pregnancies of a normal and high RI, which are at least risk (<1%) and most risk (21%) of developing preeclampsia, respectively.[14,25] We can, therefore, examine the role of dNK cells isolated from these pregnancies. We have previously demonstrated that dNK cells isolated from high RI pregnancies are less able to induce vascular cell apoptosis.[14] In this study, we examined the interactions of dNK cells from normal and high RI pregnancies with trophoblasts, to determine the contribution of dNK to trophoblast-induced SA remodeling.

Materials and Methods

Doppler Ultrasound Characterization

Determination of uterine artery RI was performed in women attending a clinic for termination of pregnancy in the first trimester, as previously described,[14,26] at the Fetal Medicine Unit, St. George's Hospital (London, UK). Ethics committee approval and full written consent were obtained. Inclusion criteria were singleton pregnancy, gestational age of 8 to 14 weeks, normal fetal anatomical features, and nuchal translucency thickness with no known maternal medical condition or history of recurrent miscarriage. High-resistance cases were defined as those with bilateral uterine diastolic notches and a mean RI >95th percentile. The presence of diastolic notches reduces the possible effect of an extremely lateral placental insertion, causing a unilateral high RI reading.[27] Normal-resistance cases had a mean RI of <95th percentile. These resistance groups represent cases most (21%) and least (<1%) likely to have developed preeclampsia, respectively, had the pregnancy progressed.[24,28]

dNK Cell Isolation

Decidual tissue was minced and digested in serum-free M199 media containing 2 mg/mL collagenase and 0.1 mg/mL DNase overnight with constant agitation at room temperature. The supernatant was filtered, centrifuged, and resuspended in PBS (2% fetal calf serum) and layered onto Ficoll-Paque (GE Healthcare Life Sciences, Buckinghamshire, UK) to collect the buffy layer. Cells were resuspended in 10 mL dNK cell culture media [phenol red–free RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), containing 2 mmol/L L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL amphotericin] and plated in a 37°C incubator for 15 minutes. Nonadherent cells containing the dNK cell fraction were counted by light microscopy, and dNK cells were purified using negative selection using a MagCellect Human NK Cell Isolation Kit (R&D Systems, Abingdon, UK), according to the manufacturer's instructions. Viability was assessed as measured by the Annexin V Apoptosis Detection Kit and propidium iodide (eBioscience, San Diego, CA) (Supplemental Figure S1). Purity, as measured by $CD56^+$ cells, was, on average, 95.7% ± 0.92% and viability after 6 hours of culture was, on average, 84.6% ± 2.8%.

Cell Culture and Generation of Conditioned Media

Decidual natural killer cells were cultured in dNK culture media supplemented with 5 ng/mL IL-15 and 250 ng/mL stem cell factor (Peprotech, London, UK). The mean gestational ages of dNK cells used in these experiments are listed within the text and were not significantly different between normal and high RI groups. The human EVT cell line, SGHPL-4, was cultured in Hams F10 media supplemented with 10% (v/v) FBS, containing 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin. These cells are well characterized and share many characteristics with isolated primary cells, including the expression of cytokeratin-7 and human leukocyte antigen-G.[29,30] All cells were incubated with 95% air and 5% CO_2 at 37°C in a humidified incubator.

Conditioned media (CM) were obtained after a 6-hour incubation of dNK cells at a density of 1×10^6 cells/mL in