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Decidual natural killer cells regulate vessel stability: implications for impaired spiral artery remodelling

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ABSTRACT

Decidual NK (dNK) cells are present during uterine spiral artery remodelling, an event that is crucial for successful placentation and the provision of an adequate blood supply to the developing fetus. Spiral artery remodelling is impaired in the pregnancy complication pre-eclampsia. Although dNK cells are known to play active roles at the maternal–fetal interface, little is known about their effect on endothelial integrity, an important component of vessel stability. We present a study in which we have modelled dNK–endothelium interactions, using first-trimester dNK cells isolated from both normal pregnancies and those with impaired spiral artery remodelling. dNK cells were isolated from first-trimester pregnancies, screened by uterine artery Doppler ultrasound to determine resistance indices (RI) that relate to the extent of spiral artery remodelling. dNK culture supernatant from normal-RI pregnancies (but not high-RI pregnancies) destabilised endothelial tube-like structures in Matrigel, and normal-RI dNK cells induced endothelial intercellular adhesion molecule-1 and tumour necrosis factor-α expression to a greater extent than high-RI dNK cells. We have established a functional role for dNK cells in the disruption of endothelial structures and have suggested how impairment of this process may be contributing to the reduced vessel remodelling in pregnancies with a high uterine artery resistance index. These findings have implications for our understanding of the pathology of pre-eclampsia and other pregnancy disorders where remodelling is impaired.

1. Introduction

The human uterus undergoes extensive vascular remodelling. Before implantation, angiogenic events occur in the endometrium, as part of the decidualisation process. Following implantation, specialised fetally derived cells of the placenta, the extravillous trophoblast (EVT), invade the decidua and remodel the maternal uterine arteries by removing and replacing the vascular cells that line the arteries (Pijnenborg et al., 2006). During early pregnancy, the uterine spiral arteries are remodelled into larger diameter, higher flow vessels, allowing a 10-fold increase in blood supply into the intervillous space for placental uptake. This is critical for the developing fetus to obtain sufficient oxygen and nutrients; and incomplete spiral artery remodelling can result in the dangerous hypertensive pregnancy disorder, pre-eclampsia (PE) (Brosens et al., 1972).

The mechanisms responsible for the remodelling events in normal pregnancy are beginning to be elucidated, suggesting that spiral artery remodelling might not only be reliant on the EVT, but might also be regulated by the large infiltration of maternal immune cells present in

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the decidua. Decidual natural killer (dNK) cells comprise approximately 70% of the decidual leukocyte population and, unlike peripheral blood NK cells, are a cytokine-producing cell type with limited cytotoxic capacity. dNK cells accumulate around spiral arteries, are present ahead of trophoblast cell invasion and continue to be present during the remodelling process (Smith et al., 2009). It has been suggested that dNK cells might be involved in angiogenesis during decidualisation (Hanna et al., 2006; Blois et al., 2011), regulation of trophoblast invasion (Wallace et al., 2013) and spiral artery remodelling (Fraser et al., 2012).

The evidence to date suggests that dNK cells might play an active role in the establishment of appropriately transformed spiral artery structures at the maternal–fetal interface in human pregnancy. Histological studies performed on human decidua have identified both trophoblast-dependent and independent stages of remodelling (Craven et al., 1998). The first stages of vascular remodelling are apparent in the spiral artery endothelium, with signs of endothelial cell (EC) activation and vacuolisation, and in the vascular smooth muscle layer, where there is disorganisation and the start of fibrinoid deposition. Some of these changes occur before vascular cell contact with the invading EVT (Craven et al., 1998) and occur in the presence of leukocytes, but not trophoblasts (Craven et al., 1998; Smith et al., 2009; Hazan et al., 2010). The latter stages of remodelling, where there is removal of vascular smooth muscle cells (VSMCs) and temporary replacement of ECs with trophoblast, are likely to involve both trophoblast-dependent and immune cell-dependent changes (Harris et al., 2006; Keogh et al., 2007; Harris, 2010; Hazan et al., 2010; Fraser et al., 2012). As dNK cells are the most abundant decidual leukocyte population, it is likely that these cells influence vascular structure both in the early stages of remodelling and then co-operate with invading EVT in the latter stages (Wallace et al., 2012, 2013, 2014).

The alterations in vessel wall architecture that occur during vascular remodelling are likely to be regulated by interactions between the cell types that form the vessel structure itself, as well as those present in the vicinity of the vessel (Bennett et al., 2012). ECs are able to sense stimuli that induce remodelling, both from the lumen of the vessel (such as haemodynamic stress) and within the vessel wall (such as cytokine signalling from VSMCs), or via immune cells located in their microenvironment. Although it is known that the endothelium changes considerably in its activation and stability, the regulation of this process by decidual leukocytes has not been investigated.

We have used dNK cells isolated from women undergoing elective termination of pregnancy at 9–14 weeks' gestation to investigate their role in both the establishment of a healthy pregnancy and the pathogenesis of complications where remodelling is impaired. These pregnancies have been classified by Doppler ultrasound scanning of the uterine arteries, a proxy measure of the extent of spiral artery remodelling/successful placentation. We have modelled dNK–endothelium interactions at the maternal–fetal interface, using dNK cells isolated from both normal and aberrantly remodelled early human pregnancies.

2. Materials and methods

2.1. Doppler ultrasound of uterine artery resistance

Maternal uterine artery Doppler ultrasound scans were conducted on women attending clinic for elective termination of pregnancy at 9–14 weeks of gestation as previously described (Melchiorre et al., 2008). Wandsworth Local Research Ethics Committee approval was in place for both the Doppler ultrasound and donation of tissue after surgical termination (ethical committee references: 01.96.8 and 01.78.5), and all women gave informed written consent. Gestational age was calculated by crown–rump length measurement. All were singleton pregnancies, with no pre-existing medical conditions. High resistance index (high-RI) cases were defined as those presenting with bilateral uterine diastolic notches and a mean RI above the 95th percentile. Normal-RI cases were defined as presenting with no diastolic notches and a mean RI below the 95th percentile. Abnormal uterine artery Doppler in the first trimester is associated with deficient trophoblast invasion of spiral arteries (Prefumo et al., 2004). The normal-RI cases represent the least (<1%), while the high-RI cases represent the most (21%) likely to have developed pre-eclampsia, had the pregnancy progressed (Prefumo et al., 2004; Whitley et al., 2007; Melchiorre et al., 2008; Fraser et al., 2012).

2.2. Positive selection of dNK cells

Decidual tissue was isolated, washed with HBSS, and dNK cells were isolated using the methods described previously (Fraser et al., 2012). Isolated $CD56^+$ dNK cells were cultured at 6×10^5 cells/ml in RPMI 1640 medium (Invitrogen, UK) with 10% (v/v) fetal calf serum (FCS), containing 2.5 μg/ml amphotericin B, 2 mM L-glutamine, 50 μg/ml penicillin, 50 μg/ml streptomycin, 50 ng/ml stem cell factor (SCF) and 5 ng/ml IL-15 (Peprotech, UK) at 37 °C in a 5% CO_2 humidified incubator. No T cells or macrophages were detected and dNK cell purity was as previously determined (Fraser et al., 2012). Cells were cultured for 24 h after which they were pelleted and lysed for 15 min on ice, in RIPA buffer (50 mM TRIS, pH8, 150 mM NaCl, 1% [v/v] Nonidet P-40, 0.5% [w/v] sodium deoxycholate, 0.1% [w/v] sodium dodecyl sulphate, 1 nM sodium orthovanadate, 1 nM phenylmethyl sulfonyl fluoride, and 10 μg/ml aprotinin). Protein concentration was determined by Bradford assay (BioRad). Conditioned medium was centrifuged for 10 min at $700 \times g$ at 4 °C to remove debris. Lysates and culture supernatants were stored at −80 °C. Consistent with our previous studies (Fraser et al., 2012), conditioned medium was pooled from normal-RI dNK and high-RI cultures, matched for the protein concentration in the cell lysates, and used for experiments. There was no significant difference in the gestational ages of the patient samples used in each group.

2.3. Culture of the endothelial cell line SGHEC-7

SV40 transfected human umbilical vein endothelial cells (SGHEC-7) (Fickling et al., 1992) were cultured in a 1:1 ratio of Medium 199 supplemented with Earle's