Natural killer cells and reproductive health

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Key content  
• Uterine and peripheral natural killer cells have distinct functions.  
• Uterine natural killer cells play an important role in decidualisation and implantation.  
• There is evidence to suggest that disorders in uterine natural killer cell physiology may lead to reproductive problems, such as recurrent miscarriage, recurrent implantation failure and pre-eclampsia.  
• Current evidence does not support routine testing of uterine natural killer cells and more research is needed.  

Learning objectives  
• Distinguish between peripheral and uterine natural killer cells.  
• Counsel women with recurrent pregnancy loss about natural killer cell testing.  

Ethical issues  
• Investigation and treatment of women with recurrent miscarriage or recurrent implantation failure is complex. The psychological implications of these conditions may result in a myriad of investigations and treatments of questionable benefit.  

Keywords: natural killer cells / recurrent implantation failure / recurrent miscarriage

Introduction  
A number of maternal physiological adaptations are required to facilitate continuing fetal development and survival. Advances in the field of immunology have furthered our understanding of these adaptations. For example, expression of human leucocyte antigens (HLAs) differs in the placenta and fetus. 1,2 This is a highly logical adaptation since the placenta is the first foreign tissue that comes into contact with the decidua. Additionally, specific alterations in the behaviour and repertoire of T-lymphocytes contribute to tolerance of the fetus. 3 The purpose of this review is to describe the evolution of knowledge concerning a unique subset of immune cells, uterine natural killer (uNK) cells, in physiological and pathological reproductive states. For the purposes of this review, we will describe natural killer cells found in the peripheral circulation as peripheral natural killer cells (pNK), and those found in the endometrium as uNK.

Origins of uterine natural killer cells  
Central to host defence mechanisms, natural killer cells play a vital role in the innate immune response. They are subtyped by virtue of their expression of the CD56 and CD16 cell surface antigens (Figure 1). The majority of pNK cells exhibit CD16 but have fewer CD56 surface antigens; thus they are typically described as CD56dim/CD16+. CD16 is responsible for cell lysis. 1 The primary function of pNK cells is to recognise foreign antigens that are potentially detrimental to the host organism. Recognition of foreign antigens initiates a cascade of events that result in the eventual destruction and/or removal of the foreign antigen.  

Vertebrates distinguish native and foreign proteins through the expression of a group of cell surface molecules called HLAs, which are encoded by major histocompatibility complex (MHC) genes. 4 Digestion of intracellular proteins generated by the invading organism results in peptides or amino acids that bind to specific portions of HLA. 4 This type of antigen presentation sensitises natural killer cells and killer T-lymphocytes resulting in the activation of cellular (i.e. non-antibody-related) immunity and, consequently, apoptosis and cytolysis of the presenting cell. Thus, these molecules play a critical role in transplantation genetics and are sometimes referred to as ‘transplantation antigens’.  

HLA-A, HLA-B and HLA-C are responsible for triggering this response and are often referred to as ‘classical’ MHC
molecules. They are expressed on all nucleated cells with the exception of extravillous cytotrophoblasts. Natural killer cells express killer cell immunoglobulin (Ig)-like receptors (KIRs) on their cell surface, which recognise and bind to the classical HLAs. Non-classical MHC molecules such as HLA-E, HLA-F and HLA-G present extracellular proteins that have been internalised into the cells. Both classical and non-classical HLAs are inherited in a Mendelian fashion, with the entire haplotype (i.e. combinations of classical and non-classical HLAs) being inherited from each parent.

uNK cells were first recognised as a unique cell type present in abundance during the mid-secretory phase of women with normal menses, and in the decidua of terminated pregnancies. The cells were described as large endometrial lymphocytes and were found to express the CD56bright cell surface antigen. They were eventually named uterine natural killer cells, based on a similar method used to identify pNK cells. In the context of reproductive physiology, the term 'uterine natural killer cell' is a misnomer; their cytolytic activity is in fact much reduced because of a lack of CD16. Instead, uNK cells release a wide range of cytokines and angiogenic factors that are now understood to play a critical role in decidualisation, implantation and recognition of the embryo. Additionally, binding of HLA-G by the KIR2DL4 receptor triggers release of pro-inflammatory and pro-angiogenic cytokines. Interestingly, unlike their peripheral counterparts, uNK cells lack the progesterone receptor (PR). Instead, the actions of progesterone on uNK cells are mediated via the glucocorticoid receptor (GR). Progesterone enhances the expression and activity of 11BHSD type 1 in decidualising human endometrial stromal cells, thus increasing the conversion of cortisone to cortisol. Cortisol has a direct effect on the preparation of epithelial endometrial cells for implantation. Furthermore, progesterone can also bind directly to the GR on uNK cells. Thus, differential regulation of uNK cells in the endometrium exists and contrasts with that of pNK cells, which remains unchanged in the proliferative and secretory phases of the menstrual cycle.

The interest in natural killer cells in pregnancy loss was borne out of the hypothesis that rejection of the fetus occurs at the feto-maternal interface, leading to miscarriage or recurrent implantation failure. If natural killer cells have cytotoxic and cytolytic activity then it was proposed that this would have an adverse effect on the fetus.

**Physiological response during the menstrual cycle**

In humans, the endometrium undergoes a transformation during the secretory phase to become the decidua, which is capable of sustaining pregnancy. The human endometrium is unique in being able to undergo spontaneous decidualisation. Under the influence of progesterone, endometrial stromal cells proliferate and secrete cytokines and angiogenic factors essential for decidualisation (Figure 2). Angiogenic factors such as vascular endothelial growth factor-A (VEGF-A), placental growth factor (PlGF) and angiopoietin-1 (Ang-1) induce proliferation, maturation and stabilisation of the spiral arteries.
spiral arteries. Interleukin-15 (IL-15) messenger RNA (mRNA) activity and protein expression are upregulated in endometrial stromal cells. IL-15 binds to IL-15 receptors on uNK cells, which induces proliferation of the uNK cells. As a result, uNK cells are the predominant leucocyte and account for at least 30% of the endometrial stroma during the late secretory phase of the menstrual cycle, where they can be found surrounding spiral arteries (but not veins) and glands. Progesterone binding to GR on uNK cells induces interferon-gamma (IFNG), which further promotes angiogenesis and uNK cell proliferation. Whether increased numbers of uNK cells initiate or are the effect of decidualisation is unclear.

Physiological response in pregnancy

By undergoing decidualisation, the endometrium becomes receptive to the blastocyst. Implantation involves a series of cellular processes by which the embryo is embedded into the decidua. The outer cell mass layer of the blastocyst makes contact with the epithelial cells of the decidua, then attaches to and invades the endometrial stroma. If successful implantation occurs, differentiation of the outer cell mass into trophoblastic cells occurs; they subsequently secrete human chorionic gonadotrophin (hCG), which is detectable in maternal serum. As the syncytiotrophoblast progressively invades the endometrial stroma, angiogenic factors, such as IFNG, that are secreted by uNK cells activate the production of nitric oxide, a potent vasodilator and inhibitor of smooth muscle proliferation. Thus, the preferential exchange of nutrients and oxygen in favour of the embryo is facilitated.

This invasion is not detected by maternal immune cells, partly because of the lack of classical HLA molecules as mentioned previously. The extravillous trophoblast consists of cytotrophoblast cells, interstitial and endovascular trophoblast cells and placental-bed giant cells. They are unique because they express only certain types of HLAs: HLA-C, HLA-E and HLA-G (Figure 3). Binding of the classical MHC molecule HLA-C to uNK cells occurs via activating or inhibitory KIRs. Binding of the non-classical HLA-E to CD94/NKG receptors on uNK cells is thought to inhibit uNK cytotoxicity and mediate uNK–trophoblast interactions. Binding of HLA-G to KIR2DL4 on uNK cells blocks the lytic activity of uNK cells and increases pro-angiogenic cytokines. Thus, uNK cells may be regarded as regulators of the depth of syncytiotrophoblast invasion. The reader is directed to an excellent review and illustration of placentation across a selection of mammalian species by Moffett and Loke.

The role of uNK in reproductive disorders

Recurrent miscarriage

The Royal College of Obstetricians and Gynaecologists (RCOG) defines recurrent miscarriage (RM) as having lost three or more consecutive pregnancies before 24 weeks of

Figure 3. Extravillous cytotrophoblast cells and uNK cell interaction. Extravillous cytotrophoblast (EVT) cells express non-classical human leucocyte antigens (HLAs), which interact with killer cell immunoglobulin-like receptors (KIRs) found on uterine natural killer (uNK) cells. Consequently uNK cell cytotoxicity is inhibited and angiogenic cytokine production increases. For example, nitric oxide production is mediated via interferon gamma (black arrow) produced by uNK cells, resulting in vasodilation and inhibition of smooth muscle proliferation. Thus the depth of syncytiotrophoblast invasion is regulated. B = blastocyst cavity, CT = cytotrophoblast, GR = glucocorticoid receptor, P4 = progesterone receptor.
The incidence of RM is estimated to be between 1% and 3%, depending on the definition used. The causes of RM are varied and can be categorised into maternal and fetal causes. Maternal causes include pre-existing medical conditions such as anti-phospholipid syndrome, polycystic ovarian syndrome, and uterine causes such as malformations of the uterus and fibroids. Fetal causes include chromosomal abnormalities that may be congenital or arise de novo.

The interest in natural killer cells as a cause of RM stemmed from a growing body of evidence demonstrating natural killer cells as a biosensor of foreign antigens. The emergence of uNK cell testing was the result of evidence demonstrating the crucial role of uNK cells in decidualisation and implantation. Alterations in the ‘form, fit and function’ of uNK cells were proposed as a mechanism to explain miscarriage in women without pre-existing medical causes, as well as those for whom a fetal cause could not be found.

In a study of the endometria of women with RM versus controls, Lachapelle et al. identified a decrease in the abundance of CD56bright/CD16+ (i.e. pro-cytolytic) cell type. Women with RM had higher uNK cell counts than controls. Although a systematic review investigating the role of pNK and uNK cells in RM failed to identify any differences in RM patients versus controls, a key finding of this review was the lack of standardisation for uNK cell estimation in the endometrium. Studies included in the systematic review measured pNK by virtue of a total proportion of natural killer cell activity, as determined by flow cytometry or a specialised assay. The normal range varied from one study to another. uNK cells were measured using immunohistochemistry or flow cytometry. Again, the normal range varied from one study to another. Thus, this review was limited by the significant heterogeneity of included trials and highlighted a lack of good quality primary studies. In contrast to uNK cells, pNK cells have no role in decidualisation or implantation, thus testing for pNK cells in RM would appear to be of little benefit.

**Recurrent implantation failure**
Implantation failure is typically diagnosed when there is evidence that implantation has not taken place. It is nearly impossible to quantify the incidence of this in spontaneous conceptions; however, it is a diagnosis that is commonly made following assisted reproductive techniques (ART). There is a lack of standardisation in the definition of recurrent implantation failure (RIF). The most commonly used definition is “three or more failed treatment cycles” followed by “two or more failed cycles”. An impaired decidualised response prohibiting implantation is a proposed cause.

Some researchers have drawn similarities between patients with RIF and RM, although their definitions would indicate distinct entities. However, given the role of uNK cells in decidualisation and implantation, it would seem biologically plausible that uNK cells might have a role in RIF. Nevertheless, a systematic review comparing women with RIF versus controls did not identify a difference in pNK or uNK cells in the endometrium. Again, study heterogeneity prohibited any meaningful meta-analysis of the study results. Thus, testing for pNK or uNK cells in the context of RIF requires further research in the form of an adequately powered, prospective, blinded observational study.

**Impaired placentation**
The concept of maternal rejection of paternal antigens is not new, and in fact this purported ‘incompatibility’ sparked trials investigating paternal leucocyte immunisation. There is now clear evidence that this treatment does not increase the pregnancy rate. Some studies have now demonstrated a plausible link between pre-eclampsia, the paternal HLA-C molecule and uNK cells. The MHC molecules are coded for by hundreds of alleles, which allow innumerable conformational changes in the peptide-binding portion. These polygenic and polymorphic changes are critical for recognition of the myriad foreign peptides that challenge the host immune system. Recent evidence suggests that certain combinations of the paternally derived HLA-C molecule may be associated with pre-eclampsia. The absence of activating KIRs on maternal uNK cells and the HLA-C2 allotype in the fetus have been demonstrated to increase the incidence of pre-eclampsia. It was proposed that the absence of this activating KIR resulted in impaired trophoblast invasion and spiral artery remodelling; hallmarks of pre-eclampsia.

**Management of patients**

**Recurrent miscarriage**
Women with RM are a diverse population. As such, treatment should be tailored to the cause, if found. For the majority of women with unexplained RM, supportive treatment early in pregnancy will result in a successful pregnancy, although the precise mechanism underpinning this is unclear. There is no evidence to support measurement of pNK cells in women with RM. Current evidence does not recommend routine measurement of uNK cells in women with RM. This is partly because of a lack of good quality studies describing a consistent technique for measuring uNK cells, and a lack of evidence surrounding effective treatments. Quenby et al. demonstrated that treatment with prednisolone reduced the uNK cell count in women with RM. This randomised controlled trial (RCT) randomised women with RM (where RM was defined as having three or more lost pregnancies) and a uNK cell count greater than 5% to either treatment with prednisolone or a placebo. However,
the effects of treatment with prednisolone were not significant; this could be explained by the sample size. Further RCTs involving a larger cohort are required to confirm this. That 57% of women with three or more consecutive miscarriages involved in the study did not have an elevated uNK cell count demonstrates the complex nature of assessing the role of uNK cells in patients with RM. Kuroda et al. demonstrated a difference in glucocorticoid signalling in patients with RM, and a higher proportion of uNK cells close to the luminal epithelium. uNK cells are normally found in surrounding perivascular cells and glands in the endometrial stroma. Thus, it was proposed that elevated uNK cells in patients with RM might be a reflection of relative glucocorticoid deficiency in the endometrial stroma, rather than a cause. Conversely, steroid deficiency could be a reflection of impaired decidualisation, in which uNK cells may be directly involved. Treatment with corticosteroids aims to correct this deficiency and therefore distribution of uNK cells in the endometrial stroma.

**Recurrent implantation failure**

A 2012 systematic review demonstrated a beneficial effect of endometrial injury inflicted either by hysteroscopy (and/or curettage) or by pipelle biopsy in the cycle preceding embryo transfer. The meta-analysis of four RCTs demonstrated a higher cumulative pregnancy rate (risk ratio 1.71 [95% CI 1.40–2.09]). The precise mechanism behind an increase in the cumulative pregnancy rate is unclear, especially when not all women in the treatment group actually underwent curettage or biopsy. However, the meta-analysis of a total of 411 patients in the treatment groups with 572 controls did not demonstrate significant statistical heterogeneity, indicating that the results are unlikely to be influenced by differences between the trials. Three of the RCTs included women with two or more unsuccessful cycles. One of the RCTs included women with one or more unsuccessful cycles. The proinflammatory response caused simply by instrumentation of the uterus could result in recruitment of immune regulatory cells, either transiently or permanently. It is also unclear how long this effect is expected to last, given the lack of studies comparing endometrium harvested from one menstrual cycle to the next, and beyond. We are unaware of studies investigating the biochemical or clinical pregnancy rates after delaying embryo transfer by more than one cycle following instrumentation of the uterus.

**Pre-eclampsia**

A recently published large observational study demonstrated an increased risk of pre-eclampsia in women who had three or more recurrent miscarriages. Given the role of uNK cells in spiral artery remodelling, treatment aimed at reducing oxidative stress could reduce the risk of pre-eclampsia. Certainly, in vitro experiments demonstrated a beneficial effect of antioxidants on syncytiotrophoblasts and spiral artery remodelling. However, this failed to translate into clinical benefit as a large RCT investigating treatment with high-dose vitamin C and E did not demonstrate a reduction in pre-eclampsia, and was associated with a reduction in birthweight. A systematic review of nine trials confirmed that high-dose vitamin C and E supplementation did not reduce the risk of pre-eclampsia. This is, in part, unsurprising as supplementation with these antioxidants were only begun from the 20th week of pregnancy, well past the period of spiral artery remodelling.

Wong et al. investigated the correlation between uNK cell count and pre-eclampsia in 71 women with a history of RM (three or more lost pregnancies) who subsequently had a live birth. There was no significant difference in the incidence of pre-eclampsia between women with a high uNK cell count (defined as 13.9% of stromal cells staining positive for CD56). Thus, on balance, screening for gestational hypertension in women with a history of unexplained recurrent miscarriage alone is unjustified on the basis of an absence of preventative strategies.

**Current controversies and avenues for further research**

A key gap in the current knowledge of uNK cells relates to their origin. A number of hypotheses have been proposed. As a small cohort of pNK cells are CD56brightCD16+, it was proposed that these pNK cells migrate into the endometrium. It has also been suggested that uNK cells are the result of cellular differentiation directly from bone marrow stem cells. However, it has not been possible to demonstrate the migration of bone marrow stem cells to the endometrium. In fact, bone marrow stem cells are not known to leave their site of origin. A more likely explanation is that uNK cells are derived from a cohort of stem cells residing in the endometrium itself. However, this is as yet unproven.

An area of controversy relates to the method by which uNK cells are measured. Commonly described methods include cell counting using formalin-fixed tissue stained for CD56 cells and fluorescent activated cell-sorting. The reader will note that a key limitation of both these techniques lies with the availability of a specific antibody for uNK cells. Furthermore, the threshold for normality differs, as does the way in which uNK cell numbers are estimated by cell counting in histological sections. Efforts are underway to ascertain the most consistent method.

Because of the complex interactions between the blastocyst and the epithelial layer of the endometrium, it is also unclear
if recurrent miscarriage or implantation failure is the result of aberrant uNK cell migration into the endometrium, or signalling from the abnormal embryo. Evidence in favour of the latter comes from a study of uNK cells using flow cytometry in missed miscarriages. The decidual of karyotypically abnormal pregnancies demonstrated a higher uNK cell population than in karyotypically normal miscarriages. However, given that the endometrium in one cycle is dissimilar to the next, and no two embryos are identical, testing this hypothesis would be difficult. In this respect, the use of animal models may be useful.

Whilst uterine instrumentation appears to improve cumulative pregnancy rates, it is unclear if this procedure results in regulation of the uNK cell population. To our knowledge, no studies have investigated this directly, since embryo transfer is undertaken in the cycle immediately after uterine instrumentation. In this respect, research on the impact of uterine instrumentation or ‘endometrial scratch therapy’ on the uNK cell population would be useful. If endometrial trauma increases the cumulative pregnancy rate, then hypothetically, primary endometrial scratch could be beneficial for all patients about to embark on ART and embryo transfer and not just those experiencing RIF. The benefits of a successful first cycle cannot be emphasised, given the psychological, emotional and financial cost of assisted reproduction. Furthermore, where the first cycle is offered on the National Health Service (NHS), this could represent a significant cost saving to the NHS. However, further research is needed since the procedure can cause discomfort and is not risk free.

Conclusion

Advances in molecular biology techniques have furthered our knowledge and understanding of uNK cells in the context of reproductive physiology and pathology. The causes of RM and RIF comprise a spectrum of disorders in which uNK cells may be partly involved. Future research in the field will benefit women with a history of recurrent miscarriage or recurrent implantation failure. Until such a time, uNK cell testing should remain within the remit of research trials.

Disclosure of interests

Professor Quenby is an advisor on several international and national committees: the European Society for Human Reproduction and Endocrinology Early Pregnancy Special Interest Group, the Medicines and Healthcare Products Regulatory Agency (MHRA) Expert Advisory Panel for Women’s Health, the Scientific Advisory Committee of the Royal College of Obstetricians and Gynaecologists (RCOG), the RCOG Preterm Labour Clinical Study Group, and the RCOG Early Pregnancy Clinical Study Group. She is currently an Associate Editor for *BMC Pregnancy and Childbirth* and has served as an Associate Editor for *Human Reproduction*.

**Contribution to authorship**

HPC wrote the manuscript and CPD questions. SMQ edited the manuscript and CPD questions for content accuracy. Both authors read and approved of the final version of the manuscript.

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**Supporting Information**

Single Best Answer questions are available for this article at [https://stratog.rcog.org.uk/tutorial/tog-online-sba-resource](https://stratog.rcog.org.uk/tutorial/tog-online-sba-resource)

**References**
