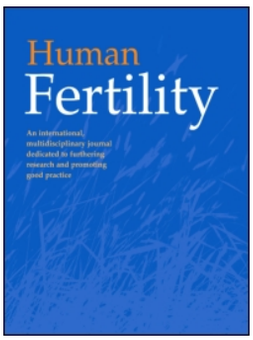

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REVIEW ARTICLE

A blessing and a curse: is high NK cell activity good for health and bad for reproduction?

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ABSTRACT

Few topics in recent reproductive medicine have been the subject of as much controversy, media attention and passionate debate as natural killer (NK) cells and their role in reproductive failure. The question of whether elevated NK cell levels are a cause of infertility and pregnancy loss, and whether they provide a potential target for therapy to improve reproductive outcomes, lacks a definitive answer. It is clear, however, that a significant number of women with reproductive failure have abnormal NK cell parameters reflecting high immunological activity. Amongst all the debate, the wider implications of NK cell overactivity – and attempts to suppress it – have not yet been considered. The literature suggests that although elevated NK cell activity may not be conducive to reproduction, it could in fact be beneficial in other areas of health and disease such as cancer and infection. Further research is needed to determine whether this hypothesis holds true in women with NK cell-related reproductive failure.

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Introduction

Reproductive failure, a broad set of conditions encompassing infertility, recurrent miscarriage and recurrent implantation failure (RIF), is common but often poorly understood. Infertility, defined as the failure to conceive after at least 12 months of regular unprotected sexual intercourse, affects 14% of couples, while recurrent miscarriage (RM) – three or more consecutive pregnancy losses before 20 weeks' gestation – occurs in 1–3% of women (Gelbaya, Potdar, Jeve, & Nardo, 2014; King, Smith, Chapman, & Sacks, 2010). RIF has various definitions but is generally accepted as the failure of three or more *in vitro* fertilization (IVF) cycles where one or two high quality embryos are transferred in each cycle (Simon & Laufer, 2012). In a large proportion of patients within these groups – including 50% of women with RM and 15–30% of infertile couples – no explanation is found for their reproductive failure from traditional fertility investigations (Gelbaya et al., 2014; Quenby & Farquharson, 2006).

For these patients, many of whom have experienced the physical, emotional and financial stress of multiple failed cycles of IVF, the diagnosis (or lack thereof) of unexplained reproductive failure is often greatly unsatisfying. Many such patients are in a position to benefit (or at least believe they are) from NK cell testing, an area of reproductive medicine with a long history but one that remains controversial. The extensive debate and politics surrounding NK cells and their relevance in reproductive failure has maligned the topic away from the mainstream (Sacks, 2015). Indeed, a recent survey of IVF clinicians revealed that 69% would recommend immunological investigations in patients with RM, although only 8% would consider a NK cell assay; similarly, 56% would recommend immunological testing in cases of RIF but only 9% would suggest a NK cell assay (Kwak-Kim et al., 2013).

Twenty years of research have provided a multitude of evidence to support the notion that NK cell overactivity is correlated with reproductive failure. One estimate suggests that elevated NK cell numbers or activity are found in 15–25% of women with unexplained RM (Sacks, 2014), but it is not yet clear what these findings mean or whether they can, so to say, explain the unexplained. No disease has yet been associated with elevated NK cell activity, but it has been suggested that such a disease does exist – one that is manifested in the failure of implantation and progression of pregnancy (Sacks, 2015). It is likely, if such a disease exists, that other manifestations reflective of high NK cell activity will be present. To look for these, it is first essential to determine the functions of

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NK cells in other areas of health and disease, including autoimmunity, infection and cancer.

NK cells and fertility

It has long been assumed that the maternal immune system must be suppressed or redirected in some way to ensure a successful implantation and pregnancy. Investigations into the role of NK cells in infertility and miscarriage began in the mid-1990s, with the claim by Aoki et al. (1995) that RM patients with elevated peripheral NK (pNK) cell activity in the preconceptual period had an increased risk of miscarriage. Soon after, Roussev, Kaider, Price, and Coulam (1996) demonstrated that women with RM, RIF and infertility were more likely to have elevated pNK cell levels than fertile controls, while Beer, Kwak, and Ruiz (1996) claimed to be the first researchers to have shown that elevated pNK cell levels could predict reproductive failure in women with RM and RIF.

Many researchers over the past twenty years have sought to prove (or disprove) this hypothesis posed by early investigators. Significant correlations have been drawn between RM and various pNK cell parameters, including cell levels (both as a percentage of lymphocytes and as an absolute number), CD56dim levels, cytotoxicity, expression of the activation markers CD69 and CD16, and secretion of TNF-α and IFN-γ, all of which are increased in RM (Emmer et al., 2000; Fukui et al., 2008; Ghafourian, Karami, Khodadadi, & Nikbakht, 2014; Hadinedoushan, Mirahmadian, & Aflatounian, 2007; King et al., 2010; Lu et al., 2011; Ntrivalas et al., 2001; Ramos-Medina et al., 2013; Yoo et al., 2012). By contrast, RM is also associated with a decrease in certain pNK cell parameters, including CD56bright levels, CD56bright:CD56dim ratio, expression of the inhibitory marker CD94, and secretion of IL-4 and IL-10 (Emmer et al., 2000; Fukui et al., 2008; King et al., 2010; Ntrivalas et al., 2001). The majority of these observations are also true in RIF (Fukui et al., 2008; Ghafourian et al., 2014; Lu et al., 2011; Ramos-Medina et al., 2013; Sacks et al., 2012).

Decidual NK (dNK) and uterine NK (uNK) cells are more difficult to measure, but similar observations have been made: women with RM have higher uNK cell levels (Clifford, Flanagan, & Regan, 1999; Quenby, Kalumbi, Bates, Farquharson, & Vince, 2005; Tuckerman, Laird, Prakash, & Li, 2007), higher dNK cell levels (Almasry, Elmansy, Elfayomy, & Algaidi, 2015), and a lower CD56bright:CD56dim dNK ratio (Yamamoto, Takahashi, Kase, & Mori, 1999). Women with RIF have also been shown to have higher uNK cell levels than controls (Fukui et al., 2008; Santillan et al., 2015).

It is not yet understood how NK cells contribute to reproductive failure, but a number of possible mechanisms have been proposed. Firstly, it has been suggested that NK cell overactivation results in excessive production of Th1-type cytokines, primarily TNF-α and IFN-γ (Fukui et al., 2008, 2011). The maternal immune response shifts from Th1 to Th2 dominance in normal pregnancy, but Th1 activity is enhanced in reproductive failure, suggesting that it creates a hostile or unhelpful environment for implantation and pregnancy (Fukui et al., 2011; Laird et al., 2003). Furthermore, pNK production of cytokines that are known to be conducive to pregnancy, including IL-4 and IL-10, is reduced in reproductive failure (Fukui et al., 2008). Secondly, impaired epitope matching between maternal inhibitory killer-cell immunoglobulin-like receptors (KIRs) and trophoblastic HLA-C, which reduces the inhibition of uNK-mediated trophoblastic cytolysis, is thought to contribute to overactivation and excessive cytolytic activity of uNK cells (Faridi & Agrawal, 2011; Varla-Leftherioti et al., 2005). Thirdly, since uNK cells contribute to endometrial angiogenesis and spiral artery remodelling, it has been suggested that increased uNK cell density causes increased periimplantation blood flow and the early establishment of maternal circulation, resulting in excessive oxidative stress with the potential to trigger miscarriage (Quenby et al., 2009). Finally, high uNK cell levels are associated with decreased cortisol synthesis by decidualizing cells and impaired induction of local mineralocorticoid-dependent enzymes. These findings suggest that elevated uNK cell levels may be a useful marker for identifying women at risk of pregnancy loss due to uterine cortisol deficiency (Kuroda et al., 2013). As observed in the liver, NK cells also play an important role in facilitating tissue regeneration and reducing fibrosis (Krizhanovsky et al., 2008); similar processes in the endometrium could lead to reproductive failure as a result of aberrant decidualization and regeneration of endometrial tissue.

None of the proposed mechanisms for the role of NK cells in reproductive failure have been clearly substantiated, and it is also possible that NK cell overactivity is a marker of – rather than a cause for – reproductive failure. Yet it is clear from the evidence outlined above that a link does exist between NK cell activity and poor reproductive outcomes. Certain pNK cell parameters, particularly percentage and cytotoxicity, have been shown to be highly specific for RM (Coulam, Goodman, Roussev, Thomason, & Beaman, 1995; King et al., 2010; Lee et al., 2013) as well as

predictive of future pregnancy outcomes (Ramos-Medina et al., 2013). More importantly, though, NK cell testing facilitates the identification of patients who are likely to benefit from immunotherapy, and preliminary trials have demonstrated an improvement in rates of pregnancy and live birth in reproductive failure patients with elevated NK cell levels who are treated with intravenous immunoglobulin (IVIg), intralipid and/or prednisolone (Clark, 2008; Coulam & Acacio, 2012; Morikawa et al., 2001; Polanski et al., 2014). An additional, perhaps undervalued, benefit of NK cell testing is the reassurance it provides to patients that their doctor is seeking an explanation for (and solution to) their problem, as well as thinking beyond the unsatisfying diagnosis of 'unexplained' reproductive failure and, importantly, offering them hope (Sacks, 2014).

The practice of NK testing in the fertility setting remains controversial, however, as several groups have found no association between NK cell abnormalities and reproductive failure (Katano et al., 2013; Ozcimen, Kiyici, Uckuyu, & Yanik, 2009; Thum et al., 2005). More importantly, others have reported that no significant correlation exists between pNK and uNK cell levels (Junovich et al., 2013; Lukassen et al., 2004; Yamamoto et al., 1999), which has led some to conclude that any attempts to extrapolate information about the immune environment of the uterus from pNK cell tests are inherently flawed (Clark, 2008; Laird, Mariee, Wei, & Li, 2011).

The most likely reason for the markedly contradictory conclusions reached by different investigators is the vastly different methodologies used to measure NK cells. Not only are NK cells studied in different compartments of the body (the blood, endometrium or decidua), but the techniques used also vary between labs and even individual technicians (Laird et al., 2011; Sacks, 2015). Measuring uNK cells from an endometrial biopsy is particularly problematic since it is technically difficult and subjective (Sacks, 2015). The timing of sample collection (stage of menstrual cycle, during pregnancy or after miscarriage) is also crucial (Laird et al., 2003). Furthermore, a number of different parameters have been analysed, including percentages or absolute numbers of NK cells, cell subtypes and surface markers, with many different (often arbitrary) thresholds used to define normal and abnormal results (Laird et al., 2011). Numerous miscarriage studies have also failed to perform foetal karyotyping in every case, since chromosomally normal and abnormal miscarriages should be examined separately (Laird et al., 2003). With this many variables to account for differences in results, it is no wonder that researchers have arrived at such different conclusions.

NK cells and autoimmunity

NK cells have been implicated in multiple autoimmune diseases in both protective and pathogenic roles. The most consistent finding amongst patients with autoimmune disease is a low pNK cell count, which has been described in multiple sclerosis (MS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type I diabetes mellitus (TIDM), Sjögren's disease, psoriasis and autoimmune thyroid disease. Rather than a true NK cell deficiency, however, this most likely reflects the sequestration of NK cells to target tissues, since NK cells accumulate in the affected tissues of RA (synovium), psoriasis (skin lesions), TIDM (pancreatic islets), alopecia areata (hair follicles), and dematomyositis (muscle). Certain activating KIR alleles have also been identified as predisposing factors to a number of autoimmune diseases, while a lack of certain inhibitory KIRs predisposes to others. It is unclear whether changes in NK cell location and function in autoimmunity are a primary pathogenic defect, or whether they simply reflect a secondary response to the disease. Certain patterns in NK cell abnormalities, however – that they are often present in newly onset but not chronic disease and active but not quiescent disease – suggest that NK cells contribute to the initiation of autoimmune processes rather than being the result of them (Fogel, Yokoyama, & French, 2013).

Yet NK cells also appear to play a protective role in autoimmune disease. The production of IL-10 by NK cells inhibits dendritic cells (DCs), reducing antigen presentation and therefore T cell proliferation which might otherwise result in an autoimmune response. NK cells are also capable of lysing activated macrophages, thereby downregulating inappropriate phagocytic activity (Folci, 2014). The most convincing evidence for the protective role of NK cells in a specific disease is MS, since pNK cell numbers are diminished in active disease but are restored in remission and upon effective treatment with the monoclonal antibody dacluzimab (Flodstrom-Tullberg, Bryceson, Shi, Hoglund, & Ljunggren, 2009; Folci, 2014). Nevertheless, much like the area of reproductive failure, the evidence for NK cell involvement in autoimmunity is, as described by Fogel et al. (2013), 'tantalising but incomplete'.

NK cells and cancer

NK cells play an important role in cancer immunosurveillance, primarily through their ability to kill tumour cells directly without prior sensitisation. This occurs via four mechanisms: (1) perforin- or granzyme-mediated

cytotoxicity; (2) death receptor-mediated apoptosis; (3) release of effector molecules such as IFN-γ; and (4) antibody-dependent cell-mediated cytotoxicity (Cheng, Chen, Xiao, Sun, & Tian, 2013). Furthermore, NK cells and their products interact with other components of the immune system to initiate and maintain a powerful system-wide anti-tumour response. By communicating with DCs, NK cells improve antigen presentation and thereby facilitate the generation of a tumour-specific cytotoxic T cell response. This effect is compounded by the ability of NK cells to promote CD4 + and CD8 + T cell differentiation as well as induce T cell-mediated cytotoxicity through IFN-γ (Cheng et al., 2013; Langers, Renoux, Thiry, Delvenne, & Jacobs, 2012). Chemokines produced by NK cells also induce the migration of DCs and T cells to malignant tissue (Terunuma, Deng, Dewan, Fujimoto, & Yamamoto, 2008).

A landmark study by Imai, Matsuyama, Miyake, Suga, and Nakachi (2000) measured the baseline natural cytotoxicity levels of 3500 participants using a chromium-51 release assay of the peripheral blood, dividing the participants by tertiles into groups of low, medium and high cytotoxicity levels. The participants were followed up after an 11-year period to assess cancer incidence. Participants with low levels of cytotoxic activity had a significantly increased risk of cancer, while those in the medium and high cytotoxicity groups had a relative risk of 0.63 compared to the low-level group. This trend was especially marked in the female population; women with high cytotoxic activity had approximately half the risk of cancer as compared to women with low cytotoxicity.

The function of NK cells in cancer has been thoroughly reviewed by Levy, Roberti, and Mordoh (2011) and Cheng et al. (2013). The presence of NK cells in malignant tissue is a positive prognostic marker: high levels of tumour-infiltrating NK cells are associated with favourable outcomes in various carcinomas, while lower intraneoplastic NK cell counts are associated with a higher risk of metastasis (Cheng et al., 2013; Levy et al., 2011). Cancer patients also appear to have an altered NK cell phenotype, marked by overexpression of inhibitory receptors, poor expression of activating receptors, increased CD56bright:CD56dim ratio, decreased cytotoxic activity, and reduced cytokine production (Levy et al., 2011). These abnormalities reflect a suppression of NK cell activity; indeed, many immunotherapeutic approaches to cancer management are targeted at expanding or stimulating the NK cell population (Cheng et al., 2013; Levy et al., 2011). Incidentally, these abnormalities are the opposite of those seen in the pNK cell population of many women with reproductive failure.

NK cells and infection

Another major function of NK cells is the control of intracellular pathogens including viruses, bacteria, fungi and protozoa. In infection, NK cells are activated either directly or indirectly. They are able to directly recognize and respond to infected cells since viruses tend to downregulate MHC-I molecules and upregulate stress-regulated surface molecules, marking the infected cells as abnormal. Through the indirect pathway, myeloid accessory cells – monocytes, macrophages and DCs – respond to pathogens by releasing cytokines and upregulating costimulatory receptors, which overrides the inhibitory signals emitted by infected but MHC-I-competent cells that would otherwise not be recognized by NK cells. This pathway of indirect activation is especially important in bacterial and protozoal infections and in viruses that have developed strategies to evade direct detection by NK cells (Horowitz, Stegmann, & Riley, 2011). Once activated, NK cells contribute to containing the infection either through cytotoxicity of infected cells or the production of cytokines that control viral replication (Orange & Ballas, 2006). Although pNK cell activity is highest within the first two days of infection, NK cells remain active as the innate immune response transitions to an adaptive one, playing an immunoregulatory role in the later stages of infection (Jost & Altfeld, 2013; Whiteside & Herberman, 1994).

As Orange (2002) notes, one way to appreciate the importance of NK cells in infection is to examine the effects of their absence. Although NK cell deficiencies – numerical or functional – are rare, they are associated with a clear phenotype characterized by increased frequency and severity of infections, especially viral (Whiteside & Herberman, 1994). Susceptibility is increased most significantly to herpes simplex virus (HSV), varicella zoster virus, Epstein–Barr virus (EBV), cytomegalovirus and human papillomavirus, as well as mycobacteria and other bacteria. In genetic disorders resulting in secondary impairment of NK cell cytotoxicity or cytotoxic signalling, patients are most susceptible to infection with HSV and EBV (Orange, 2013). In patients with a complete loss of NK cytotoxicity, often due to perforin deficiency, viral infection can result in overwhelming and potentially fatal disease (Orange & Ballas, 2006). Patients with normal NK cell levels but an overexpression of inhibitory KIRs with strong binding affinity to HLA-C1, such as KIR2DL2, tend to clear acute viruses less quickly and

respond less well to treatment (Horowitz et al., 2011). It follows, therefore, that women with NK cell-related reproductive failure would be less susceptible to viral infections than their immunologically normal or deficient counterparts.

Conclusion

After twenty years of research and debate, it is clear that a significant number of patients with reproductive failure have high NK cell activity. Little is understood, however, of what this finding actually means, and it is not associated with any currently defined disease. Attempts to define a clinical phenotype among these patients should consider the roles of NK cells in other areas of health beyond fertility and the implications of high NK cell activity in these domains. Given what we know about the role of NK cells in non-reproductive disease, it seems likely that NK cell overactivity in the peripheral blood of women with reproductive failure would contribute to health and disease in other respects. Perhaps these women are at higher risk of autoimmune disease, and lower risk of cancer and infection.

Although high NK cell activity appears not to be conducive to reproduction, it also appears to provide protection against other disease. Many reproductive-aged women who are told they have high NK cell activity view their immune system as a curse, when in fact the literature suggests it may be a blessing in other respects. In light of this, it is important to consider the potential ramifications of immunosuppressive therapy in women with high NK cell levels and whether the harm done by such treatments could outweigh the benefits. After years of research and debate focused on reproduction, perhaps it is time to look at the bigger picture of what it means to have elevated NK cell activity.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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