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Natural killer cells: Role in fertility and pregnancy

Ashok K Gupta[1], US Sharma[2]

1 *Assistant Professor, Division of Pharmacology, Sir Madanlal Institute of Pharmacy (SMIP), SMGI, Etawah, Uttar Pradesh- 206001, India*

2 *Director and Professor, Division of Pharmacology, Sir Madanlal Institute of Pharmacy (SMIP), SMGI, Etawah, Uttar Pradesh- 206001, India*

Abstract

The immune system plays a major role in pregnancy. The immune system adapts itself to accommodate the growing foetus and makes the environment conducive for its growth while reducing the chances of infection at the same time. Immune system, particularly natural killer cells have been identified as one among the most important factors in the 'implantation window'. Implantation window is the mid secretory phase of the menstrual cycle. The endometrium undergoes significant changes during this period called 'decidualisation'.

Keywords: NK cell, Pregnancy.

***Corresponding author:**
Ashok K Gupta
Assistant Professor, Division of Pharmacology, Sir Madanlal Institute of Pharmacy (SMIP), SMGI, Etawah, Uttar Pradesh-206001, India

INTRODUCTION

The immune system plays a major role in pregnancy. The immune system adapts itself to accommodate the growing foetus and makes the environment conducive for its growth while reducing the chances of infection at the same time. [1-4] Immune system, particularly natural killer cells have been identified as one among the most important factors in the 'implantation window'. Implantation window is the mid secretory phase of the menstrual cycle. The endometrium undergoes significant changes during this period called 'decidualisation' [5]

Natural killer (NK) cells are innate lymphocytes that are very important in defense against virally-infected cells and in tumor surveillance. They are part of the innate immune system as they are not required to a have prior exposure to the antigen. NK cells are the original members of a larger family of "innate lymphoid cells" (ILCs). [6] The importance of NK cells in human health and disease is demonstrated by a group of human diseases in which NK cells are absent or defective. These conditions are primarily characterized by severe and atypical viral infections. The infections can be recurrent and sometimes life threatening.[7]

NK cells belong to the group of lymphocytes. They form about 10-20% mononuclear fraction in peripheral blood. They lack T Cell receptor (TCR) complex and are CD3-. The marker expressed on natural killer cells that helps in identifying them is CD56, or NCAM (neural cell adhesion molecule) which was first identified first in brain tissue. There is no particular function identified for CD56.[6]Another marker identified on NK cells is CD16.

There are two main classes of peripheral blood (PB) NK cells described: the majority (more than 90%) express CD56 at low density and CD16, and are referred to as CD56dim CD16+ cells, while the remaining 10% of PB NK cells have high surface expression of CD56, but do not express CD16, and are called CD56bright CD16– cells. PB NK cells produce abundant cytokines. The CD56dim CD16+ cells can lyse target cells.[8] NK cells also express CD2 or receptor for adhesion molecule lymphocyte function associated antigen (LFA)-3[9]

Functions of NK cells

NK cells play a vital role in the innate immune system by cellular killing or cytotoxicity and in immune surveillance. NK cells kill virally-infected and malignant cells. They are constantly in circulation and keep a lookout for markers of healthy cell like MHC Class I. Multiple families of inhibitory receptors on the surface of NK cells, including the killer cell Ig-like receptors (KIRs) and killer cell lectin like receptors (KLRs), which inhibit the cytotoxicity of NK cells on recognising MHC Class I molecules (opposing signals model) [6,9].

Additional NK cell functions include cytokine and chemokine production and co- stimulation of other immune cells. [10]

Uterine NK cells (uNK cells)

NK cells are distributed in several tissues and their distribution varies according the anatomical site in mammals [10]. The human endometrium has considerable number of NK cells which vary through the menstrual cycle. uNK cells increase significantly during the mid-secretory phase. The uNK cells continue to increase in late secretory phase and first trimester of pregnancy. [8] They form almost 70% of the lymphocyte population at their peak [6]. They play an important role in implantation and placental development. [6,8].It is postulated to be one of the major causes for recurrent miscarriage(RM) and recurrent implantation failure(RIF) in the first trimester. uNK cells have cytoplasmic granules containing 'perforin' and 'granzyme'.[8] There is no consensus regarding the origin of uNK. Some experts suggest that these are mature PB NK cells that circulate and reach the endometrium. This is in response to the chemokines which are secreted by endometrium at various stages of menstrual cycle as well as during pregnancy like CXCL-12 [11] Regardless of the origin of the uNK cells, certain studies have described that uNK cells and PB cells are distinct.[12]

NK cells in fertility and pregnancy

The PB NK cells after reaching the endometrium under the influence of TGF-a1 get converted to uNK cells. IL-15 also seems to have a chemoattractant property to these cells.[8] The uNK cells have weak cytotoxicity , and presently postulated to have an important role in trophoblast invasion and normal placentation. [13] The uNK cells that are located in close proximity to the site of implantation, under the influence of progesterone and IL-15 produce endothelial cell modulating factors like vascular endothelial growth factor (VEGF). Thus uNK cells effect cytokine secretion in the uterus and trophoblast remodelling. [14] Many studies have evaluated the role of NK cells in implantation by studying the NK cell deficient mice which showed 64% loss of foetus and abnormal development of decidua and reduced placental size among others. [15,16,17].

Unlike PB NK cells uNK cells exhibit lesser cytotoxicity. They show cytotoxicity against K562 cells in early pregnancy decidua. The cytokine milieu of the endometrium changes significantly during pregnancy. There are studies that have suggested that a tilt in the balance towards type 1 cytokines would be detrimental to the pregnancy as against a tilt towards type 2 cytokines which is in favour of a successful outcome. [8] uNK cells produce different type of cytokines at different intervals of gestation, at 8-10 weeks it is predominantly angiogenic factors while at 12-14 weeks it is mainly cytokines. [18] They also secrete metalloproteinases which help in the breakdown of extracellular matrix which is essential for the remodelling of vasculature and trophoblast invasion. [5,8]

Results of several studies have evaluated the role of natural killer cells in both reccurent implantation failure and recurrent miscarriage. Recurrent implantation failure has been linked to a defective endometrium. [8]. The uNK cells secrete VEGF resulting in vascular remodelling, control of trophoblast remodelling as well as cytokine secretion

in the uterus [14]. Recent evidence has also identified that placental vascular diseases likes abnormal spiral artery remodelling and absence of vascular smooth muscle cell apoptosis impairs the spiral artery remodelling. uNK cells secrete TGF 1which regulates the maternally expressed gene-3(MEG-3). MEG-3 mediates vascular smooth muscle cells apoptosis and migration. Liu et al hence demonstrated the importance of uNK cells in vascular remodelling of the uterus in early pregnancy. [19]

There are several studies that have studied the number and the function of uNK cells prior to implantation and in early pregnancy. However these studies have had conflicting results.

Beer et al concluded in their study that PB NK cells were higher in number in women with recurrent spontaneous abortions compared to multiparous women who were controls used in the study[20]. It was supported by another study conducted by Tuckerman et al [21] which demonstrated an increase in the number of NK cells in endometrium in women who had repeated implantation failure after IVF. This was in contrast to the study conducted by Thum et al which did not find any difference in the number of PB NK cells. [22]

Despite all the conflicting results currently uNK cells are considered to be one of the prime factors in women with recurrent implantation failure and recurrent miscarriage. [23] As uNK cells also influence the spiral artery remodelling, it is also postulated to contribute in preeclampsia. [24]

Measurement of NK cells:

Several studies have evaluated the number of PB NK cells and uNK cells in women with recurrent miscarriages. However there is no consensus on the method of analysis and the timing of the menstrual cycle during which the testing has to be done. There is also conflicting reports on whether endometrial biopsy and uNK cells would be essential or measuring the PB NK cells is essential. uNK cells were traditional analysed with immunohistochemistry. Marron et al. employed flow cytometry for the analysis of uNK cells in endometrial tissue and demonstrated that it can provide detailed information on the subsets of the constituent lymphocytes. The study identified a higher concentration of PB NK cells in subjects with repeated implantation failure.[25] Other methods used to assess the NK cells function are measurement of ability of NK cells to lyse K562 cells and expression of NK cell marker CD69.[8] There is no clear evidence if assessing the PB NK cells or uNK cells will reflect the function and number of the other. The analysis of uNK cells or PB cells expressed as numbers or percentage is common practice currently and the need of the hour is to form a consensus on the method of analysis and units for expression as well as the timing of analysis. There is also a need to identify the population in whom such a testing would be warranted.

Treatment modalities for defect in uNK cells

Treatment modalities similar to the testing of NK cells has been studied extensively and the following modalities are currently in practice. IV immunoglobulin(IVIg) has been tried prior to IVF and in recurrent implantation failure and recurrent miscarriage. Ahmadi et al demonstrated successful outcomes in pregnancy following IVIg treatment in women with recurrent miscarriage due to immunological etiology. IVIg was identified to modulate the cytotoxic activity of NK cells. [26] Nine randomised studies evaluating the role of IVIG in RPL was analysed in 2013 by Li et al, the study in demonstrated that in 2415 women, live birth occurred in 406 of 816 (49.8%) in the IVIG treatment group compared to 506 of 1599 (31.6%), this was statistically significant (P = .003).[27] However there are conflicting reports noted in a cochrane review conducted in 2014 which included 8 randomised control trials that showed no improvement with IVIg treatement. [28]

Steroid have been extensively used in clinical practice in NK cell defect with significant benefit. Alhalabi et al studied 112 women who were divided into two groups randomly, one group received prednisolone 20mg per day starting on the first day of ovulation stimulation and the other group received placebo. The prednisolone group had a clinical pregnancy rate of 48.48% as compared to control group which was 29.63% [29] However certain studies have observed that the beneficial effect of corticosteroids were observed more when they were used in combination with aspirin, unfractionated heparin, or with progesterone

and enoxaparin.[14,30,31] When corticosteroids was used in combination with progesterone and enoxaparin resulted in a success rate of 85%, while with enoxaparin alone was 80% and the control group had 48%.[31] The pro inflammatory mediators as well as the anti-inflammatory mediators together function in order to bring about the immunomodulatory effects of corticosteroids.[32]. In the context of NK cells corticosteroid treatments have shown conflicting results, Gomaa et al showed no differences in the number of PB NK cells following treatment with corticosteroids,[30] whereas Quenby et al demonstrated that uterine endometrium in women with recurrent pregnancy loss were high and administration of prednisolone at 20mg from day 1 to day 21 of the menstrual cycle resulted in reduction of uNK cells from a median of 14% to 9%. [33]

Other treatment modalities that have been tried with conflicting results include TNF blockade, Tacrolimus, Intravenous intralipid therapy which consists of a sterile fat emulsion with soya oil, egg lecithin, glycerol, sodium hydroxide and water. Presently a multicentre, randomised, placebo controlled double blind study is underway to investigate the effects of hydroxychloroquine in bringing about a successful pregnancy outcome with women with history of recurrent miscarriage irrespective of the mother's thrombophilia status.[34]

CONCLUSION

Several researchers have reiterated the fact that NK cells play an important role in early pregnancy and prior to implantation. This is an area that has been investigated time and again with conflicting reports. However, presently even though there is no specific guidelines for the assessment and method of analysis, NK cells have to be considered in women with history of recurrent pregnancy loss. The treatment modalities have also identified that raise inflammatory parameters should be addressed prior to planning pregnancy in such subjects.. The projects under progress could throw light on usage of hydroxychloroquine as a potential immunomodulator to enhance the chance of implantation in women with repeated implantation failure. Presently evidence is in favour of usage corticosteroids in combination with aspirin, or if warranted enoxaparin along with other supportive measures to bring about a successful outcome. However the need of the hour is to develop a consensus on who should be tested and method and timing of testing NK cells and guidelines on treatment.

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