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Decidual stromal cell-derived IL-33 contributes to Th2 bias and inhibits decidual NK cell cytotoxicity through NF-κB signaling in human early pregnancy

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ABSTRACT

Decidual stromal cells (DSCs) are an important component of decidual tissues where they are in strict proximity with immune cells. Although previous research has indicated that DSCs participate in the regulation of immune cells during pregnancy, the crosstalk between DSCs and decidual NK cells (dNKs) has not been fully elucidated. The aim of this study was to ascertain the effect of DSC-derived IL-33 on dNK function and explore the underlying mechanism. Flow cytometry showed a considerable increase in ST2 expression on dNKs compared with peripheral NKs (pNKs). Subsequent research found that perforin production, granzyme A production, and the cytolytic activity of dNKs were impaired by DSC media. Furthermore, the addition of DSC media induced an increase in Th2 cytokine production (IL-4, IL-13, and IL-10) with a concomitant decrease in Th1 cytokine expression (TNF-α) of dNKs. However, IFN-γ, another member of the Th1 cytokine family that is thought to be necessary during early gestation increased after IL-33 stimulation. DSC media sharply inhibited the expression of major activating receptors (NKp30, NKG2D) while up-regulating the levels of inhibitory receptor (KIR2DL1) on dNKs. The biological effect of DSC media on dNKs was abrogated by the administration of sST2. Moreover, Western blot analysis suggested that the NF-κB pathway was involved in the IL-33-induced changes in the phenotype and function of dNKs, which was further confirmed by pharmacological inhibition with the NF-κB inhibitor BAY 11-7082. Our results suggest that the crosstalk between DSCs and dNKs might play a crucial role in maintaining successful pregnancy.

1. Introduction

The survival of the allogeneic conceptus has long been an immunological paradox. Under normal healthy pregnancy, effective immunity must be maintained so as to protect the mother from harmful pathogens (Dudley, 1997). Yet within the decidual environment, recognition of the fetus drives local tolerance contemporaneous with the pregnancy, which suggests that the mother is immunologically inert to her fetus (Saito et al., 2007). Therefore,

Abbreviations: DSCs, decidual stromal cells; dNKs, decidual natural killer cells; FBS, fetal bovine serum; pNKs, peripheral natural killer cells; PBMCs, peripheral blood mononuclear cells; ST2, suppression of tumorigenicity 2; sST2, soluble ST2.

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there must be a complex balance between active immunity and tolerance at the fetal–maternal interface, whereby mother and embryo achieve a symbiotic state. However, the underlying mechanisms remain unresolved.

During pregnancy, decidua represents an environmental niche where immune interactions between the mother and fetus take place (Gellersen et al., 2007). Decidual stromal cells (DSCs), accounting for 75% of decidual cells, constitute the main cellular component in decidual tissues, with which the decidual immune cells have intimate contact (Engert et al., 2007). Previous studies have indicated that the cross-talk between DSCs and immune cells is conducive to immune tolerance during early pregnancy (Vacca et al., 2011). Besides, DSCs can secrete a number of chemokines and cytokines that help to orchestrate the immunological context of the fetal–maternal interface (Segerer et al., 2012). Nevertheless, it still remains unclear whether these soluble factors participate in feto-maternal tolerance.

The hallmark of early gestation is the accumulation of a unique subset of NK cells referred to as decidual NK cells (dNKs, 50–70% of leukocytes present locally) in the maternal decidua, and they are necessary for the success of pregnancy by educating the tolerance of the maternal immune system against the allogeneic embryo (Male et al., 2010). The major dNK subset is $CD56^{bright}CD16^{neg}$, which contrasts with peripheral NKs (pNKs) whose major subpopulation is $CD56^{dim}CD16^{pos}$. Unlike pNKs, which possess a high content of lytic granules and are highly cytotoxic, dNKs produce a large number of cytokines and perform immune modulation using the secretory dichotomy of Th1 (TNF-α, IFN-γ) and Th2 cytokines (IL-4, IL-13 and IL-10) upon interaction with DSCs (Croxatto et al., 2014; Fu et al., 2014; Jabrane-Ferrat and Siewiera, 2014). It has been suggested that in a successful pregnancy, a Th2 bias is present at the maternal–fetal interface, whereas a Th1 bias at the maternal–fetal interface is associated with pregnancy wastage (Wilczynski, 2005). Although dNKs express high levels of perforin and granzyme A in addition to a number of activating receptors, including NKp46, NKp30, NKp44 (collectively termed natural cytotoxicity receptors, NCRs), NKG2D, and CD16, dNKs express various inhibitory receptors (*e.g.*, killer Ig-like receptors, KIRs, and CD94/NKG2A) simultaneously. NK cell activation is finely tuned by these activating and inhibitory receptors (Lanier, 2005; Moretta and Moretta, 2004). An emerging body of evidence suggests that DSCs might operate on many levels to suppress dNK effector functions; for example, DSC-derived TGF-β can promote the conversion of $CD16^{+}$ NK cells in the peripheral blood into decidual $CD16^{-}$ NK cells, rendering these potential killers unresponsive to targets that express the corresponding ligands (Hu et al., 2014; Keskin et al., 2007). However, to date, the effects of DSCs on the cytotoxicity and the cytokine production pattern of dNKs have not been fully clarified.

Interleukin-33 (IL-33), a member of the IL-1 family, has been shown to play an important role in initiating and perpetuating Th2-driven responses *via* its orphan receptor, suppression of tumorigenicity 2 (ST2) (Komai-Koma et al., 2007). ST2 exists in several forms, the best characterized of which are transmembrane-bound (ST2L) and soluble variants (sST2). ST2L is considered to be a functional component to induce IL-33 bioactivity, while sST2 acts as a negative regulator of IL-33 action by binding and neutralizing IL-33 (Palmer and Gabay, 2011). Substantial evidence has indicated that IL-33 drives the production of Th2-associated cytokines and is linked to important Th2 effector functions, while blocking IL-33/ST2 signaling leads to an enhancement of Th1 responses and has an inhibitory effect on Th2-associated pathological conditions (Ohno et al., 2012). In addition to the action on leukocytes, IL-33 exerts different effects on other types of cells, and regulates disparate biological functions (Miller, 2011; Yin et al., 2013). Our previous studies demonstrated that DSCs secreted IL-33 in an autocrine manner and IL-33 enhanced the proliferation and invasiveness of DSCs through up-regulation of CCL2/CCR2 production *via* NF-κB and ERK1/2 signaling pathways (Hu et al., 2014). However, the regulatory mechanism underlying the effect of IL-33 on dNKs at the maternal–fetal interface remains poorly understood.

In the present study, we measured ST2 expression on dNKs, examined the effects of rhIL-33 and DSC-derived supernatant on cytotoxicity, phenotype profile, and Th1/Th2-type cytokine production of dNKs. Collectively, we demonstrated that DSC-derived IL-33 contributed to Th2 bias and inhibited dNK cytotoxicity *via* the NF-κB signaling pathway in human early pregnancy.

2. Materials and methods

2.1. Human tissue collection

This study was approved by the Human Investigation Committee in the Hospital of Obstetrics and Gynecology, Fudan University. Informed consent was obtained from all donors. Decidual tissues were obtained from 30 healthy women (age, 29.70 ± 4.78 years, mean ± SD) undergoing elective terminations of their pregnancy (53.83 ± 6.72 days, mean ± SD), washed repeatedly with antibiotic-containing PBS to remove blood clots, and kept on ice until subsequent processing. To compare the difference in ST2 expression between peripheral NKs (pNKs) and decidual NKs (dNKs), matched venous blood and decidual samples were obtained from donors at the time of surgery ($n = 6$).

2.2. Isolation and culture of DSCs

The DSCs were isolated by trypsin–DNase digestion and discontinuous Percoll gradient centrifugation, according to our previous procedures (Wu et al., 2004). DSCs, which ranged in density between 1.042 and 1.062 g/mL, were recovered and cultured in complete RPMI 1640 medium (Gibco, Langley, OK, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin in 5% CO_2 at 37 °C. After primary culture for 30 min at 37 °C in 5% CO_2, non-adherent lymphocytes were removed by washing, leaving DSCs that were 98% pure. Our previous publication described the characterization of DSCs that were cultured *in vitro* (Hu et al., 2014).

The DSCs (8×10^5/well) were incubated in 24-well plates with complete RPMI 1640 medium. After 24 h of