Role of ILC3-produced IL-22 as Modulator of Angiogenesis in Early Pregnancy

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Introduction

Insufficient vascular remodeling has been associated with preeclampsia, IUGR and miscarriage. Angiogenesis is one of the important processes in the adaption of vascular system during placentation. Although the process has not been fully elucidated, decidual immune cells has been shown to support placentation. IL-22 stimulates endometrial stroma cells (ESC) to secrete VEGF, which consequently enhances angiogenesis in endothelial cells. However, the link between IL-22, decidual stroma cells (DSC) and uterine microvascular cells has not been demonstrated yet. Recently described uterine ILC3 produce IL-22, IL-17A, IL-8 and GM-CSF which are described as regulators of tissue remodeling and angiogenesis in other tissues. We hypothesize that ILC3-derived IL-22 can either directly stimulate angiogenesis of human uterine microvascular cells (HUtMECs) or acts indirectly through the stimulation of VEGF produced by DSC.

Methods

HUtMECs were used to study direct effects of IL-22. To investigate functional effects, 2D tube formation assay and scratch assay were performed. The expression of VEGF, MMP-2 and TIMP-1 were assessed by ELISA. VEGF was also confirmed by qPCR. The effect of IL-22 on cell viability was measured with Cell Titer Blue assay. Further experiments were performed under 1 % O_2 and 21 % O_2. Detection of IL-22RA1 was performed by In-Cell Western. In order to obtain conditioned media (CM) of ILC3s, $CD34^+$ stem cells were isolated from umbilical cord blood and differentiated *in vitro* into ILC3. Sorted NCR^+ ILC3 were stimulated with IL-1β, IL-23, PMA and Ionomycin. CM of ILC3 with and without blocking of IL-22 was used to perform HUtMEC tube formation and scratch assay. Additionally, the CM was used to stimulate DSC to produce VEGF. Experiments were performed at least in duplicates and repeated six times. Microscopic images were analysed with Image J. Data was analyzed by repeated measures ANOVA and Dunnett's post test or Student's *t*-test. A p-value <0.05 was considered statistically significant.

Results

The presence of IL-22RA1 could be detected in HUtMECs. After stimulation with IL-22 there was a significant concentration dependent increase in tube formation. Measured with PCR, increasing concentrations of IL-22 induced the expression of VEGF in HUtMECs. VEGF could not be detected in supernatants. MMP-2 was significantly induced by IL-22 in a dose-dependent manner. There was no influence of IL-22 on TIMP-1 expression. The expression of IL-22RA1 was significantly induced by 100 ng/mL IL-22 under 1% oxygen. IL-22 showed no effect to endothelial cell migration. Although the addition of 25 % supernatant of ILC3 significantly increased tube formation compared to the addition of 25 % ILC3 control medium, the effect was still lower than with HUtMEC 100 % basal medium.

Conclusion

Our data show that IL-22 supports an angiogenic phenotype on uterine microvascular endothelial cells. More experiments are needed to clarify the role of ILC3–derived IL-22 on uterine vascular remodeling.