Abstract - SRI

Title
Toll-like Receptor Expression, Function and Regulation In ILC3s During Murine and Human Pregnancies.

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Introduction
The recent description of an uterine microbiome drives our attention to the role of immune cell function in tolerance and pathogen defense during pregnancy. Innate lymphoid cells type 3 (ILC3s) are important for commensal bacteria tolerance in the gut. Their occurrence has been described in the uterus, although their local function remains unclear.
We aim to characterize the expression, function and regulation of Toll-like receptors (TLRs), relevant for microbe detection by ILCs at the fetomaternal interface (FMI) during pregnancy. We hypothesize that, in order to maintain immune homeostasis, TLR expression in ILC3s is locally regulated during pregnancy.

Methods
A mouse model was used to study the expression of TLRs and peripheral changes on uterine ILC3s ($CD45^+CD3e^-CD8a^-CD11b^-CD19^-Ter119^-CD127^+ROR\gamma t^+$) in non-pregnant mice and during the beginning and middle of pregnancy (N=7 per group). Subsequently, the impact of TLR-agonists on murine splenic and uterine ILCs (N≥5) was assessed *in vitro*. Further, the effects of local uterine factors (hCG, TGFβ, O_2 concentration) on TLR expression and function in human ILC3s ($CD45^+CD3^-CD14^-CD19^-CD20^-CD94^-CD127^+ROR\gamma t^+Nkp44^+$) obtained from umbilical cord stem cells (N=3) were evaluated *in vitro*. The expression of TLRs on ILC3s and their *ex vivo* activation upon treatment with TLR agonists was characterized in human *decidua basalis* and *parietalis* (N=10) from term births. TLR expression levels were determined by flow cytometry and qPCR. The data were analyzed by Student's *t*-test and a p-value <0.05 was considered as statistically significant.

Results
We could show that TLRs 2, 3, 4, 8 and 9 are expressed in uterine murine, human *in vitro* generated and human decidual ILC3s at RNA and protein level. Organ dependent differences occurred within TLR8 expression, which was significantly higher on murine uterine ILC3s compared to splenic ILC3s.
We observed that different local uterine factors (hCG, TGFβ, O_2) significantly modulated TLR expression on ILC3 *in vitro* (Table 1).

Table 1: significant changes of TLR expression in ILC3 upon treatment *in vitro*

TLR	O_2 concentration	hCG-stimulated	TGFβ-stimulated	TLR expression
2	21%	+	-	↑
	1%	+	-	↑
	1%	-	+	↑
	1%	-	-	↑
3	1%	-	+	↓
	1%	-	-	↑
4	21%	+	-	↓
9	21%	-	+	↑

Conclusion
Our data suggests that regulation of TLR expression on ILC3 by uterine factors may represent an important mechanism to maintain the immune homeostasis at the FMI. The high expression of TLR8 proposes that the distinction of dead versus live bacteria is especially important in the uterus. Breakdown of FMI's balance may jeopardize pregnancy outcome.

keywords: innate lymphoid cells, Toll-like receptors, fetomaternal tolerance