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COMMENT

A pregnancy to remember: trained immunity of the uterine mucosae

Dorien Feyaerts[1,2], Irma Joosten[1] and Renate G. van der Molen[1]

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Pregnancy is a unique situation where two histo-incompatible organisms peacefully co-exist, even though semi-allogeneic cells of fetal origin come into close contact with maternal immune cells in the uterine mucosa. The immune cells present in the non-pregnant uterine mucosa (i.e., endometrium) and in the uterine mucosa during pregnancy (i.e., decidua) have unique tissue specific phenotypes compared to their peripheral counterparts and exhibit dedicated functions. They play key roles in immune defense, tissue remodeling and repair, uterine receptivity, placentation, and maintaining tolerance toward fetal antigens[1]. A dysfunctional uterine immune system has been implicated in the pathophysiology of pregnancy complications, such as preeclampsia, recurrent miscarriages, recurrent implantation failure, preterm birth and uterine growth restriction[1]. It is well established that memory T cells will be formed during pregnancy, which are fetal-specific and are suggested to contribute to fetal tolerance[2]. Over the last decade, it has become apparent that immunological memory is not only a feature of the adaptive immune system, but also cells of the innate immune system can remember a previous immune insult[3]. This modulated immune response of innate immune cells is called "trained immunity" and manifests itself as an increased responsiveness and increased production of inflammatory mediators upon a second stimulus compared to untrained cells. This reprogramming of innate immune cells occurs through epigenetic modifications, and results in phenotypic and metabolic changes[3]. Trained immunity could provide an explanation as to why a more effective placentation can be observed in subsequent pregnancies[4–6] and why complications of placentation are less common in subsequent pregnancies compared to first pregnancies[7–11]. So far, there is little definitive evidence on this type of immune memory in secondary pregnancies, but it is tempting to speculate that indeed this may be the case. Recently, two studies indicated that at least in uterine Natural Killer (NK) cells such a feature may be found[12,13]. Trained immunity of NK cells in the context of pregnancy is especially interesting because of the specialized role of uterine NK cells in placentation[1]. In 2018, Gamliel et al. showed that an expanded population of decidual NK cells with a NKG2C$^+$LILRB1$^+$ memory phenotype, named pregnancy-trained decidual NK cells (PTdNK), can indeed be observed in the first trimester decidua of multigravida women[12]. These NKG2C$^+$LILRB1$^+$ PTdNK cells exhibited a distinct transcriptional and epigenetic profile. Upon interaction with HLA-E or HLA-G, these PTdNK cells showed enhanced IFN-γ and VEGFα secretion compared to decidual NK cells from primigravida women, which enhanced vascularization in in vitro and in vivo models. The expansion of NKG2C$^+$LILRB1$^+$ NK cells with an altered epigenetic signature persisted in the endometrium of multigravida women[12]. This suggests that pregnancy leads to the expansion of uterine NK cells bearing characteristics of trained immunity. How the induction of PTdNK cells is mediated is not yet clear. Possible mechanisms include the interaction of NKG2C and LILRB1 with their respective ligands expressed on trophoblast cells (i.e., HLA-E and HLA-G), high affinity binding of NKG2C to HLA-E loaded with HLA-G-derived peptide[14], or as yet undefined pathways. In this regard pregnancy hormones such as progesterone and hCG, known to influence immune responses[15,16], may be considered. Although trained immunity under the influence of pregnancy hormones has not been described so far, the ability of non-pregnancy related hormones such as aldosterone and insulin to induce trained immunity responses[17,18] suggests that pregnancy hormones might also have this potential. Another factor that should be considered is the influence of the maternal host-pathogen response. In the aforementioned study by Gamliel et al., all decidual and endometrial samples were derived from women who were cytomegalovirus seropositive (CMV^{pos})[12]. It is well known that CMV seropositive humans harbor an expanded population of long-lived peripheral blood NKG2C$^+$ NK cells[19,20]. This raises the question of whether CMV status may actually influence the induction of PTdNK cells. In a cohort of CMV^{pos} and CMV^{neg} nulli- and multigravida women[13], we confirmed the data of Gamliel et al. by showing an increased frequency of pregnancy-trained NKG2C$^+$LILRB1$^+$ NK cells in the endometrium of CMV^{pos} multigravida women. However, the frequency of these cells was not increased in the endometrium of CMV^{neg} multigravida women. These results imply that an underlying CMV infection might be a prerequisite for the induction of pregnancy-trained NK cells. In support of this notion, Pereira et al. showed that latent CMV infection of cells at the maternal-fetal interface is common[21]. Thirty-one out of 35 (89%) first trimester decidual samples, collected from healthy and uncomplicated pregnancies, showed detectable CMV DNA[21]. Co-culture of decidual NK cells with CMV-infected fibroblasts increased the percentage of NKG2C$^+$ expressing decidual NK cells, while LILRB1 expression decreased[22]. Upon exposure to CMV-infected cells, decidual NK cells become cytotoxic, which could clear intrauterine CMV infection during pregnancy[22–25]. Especially during the first trimester of pregnancy, when NK cells are abundantly present, the rate of congenital CMV infection is low[26]. In addition,

[1]Department of Laboratory Medicine, Laboratory for Medical Immunology, Radboud University Medical Center, 6500 HB Nijmegen, The Netherlands
Correspondence: Renate G. van der Molen (Renate.vandermolen@radboudumc.nl)
[2]Present address: Department of Anesthesiology, Perioperative and Pain Medicine, Stanford University School of Medicine, Stanford, CA 94121, USA

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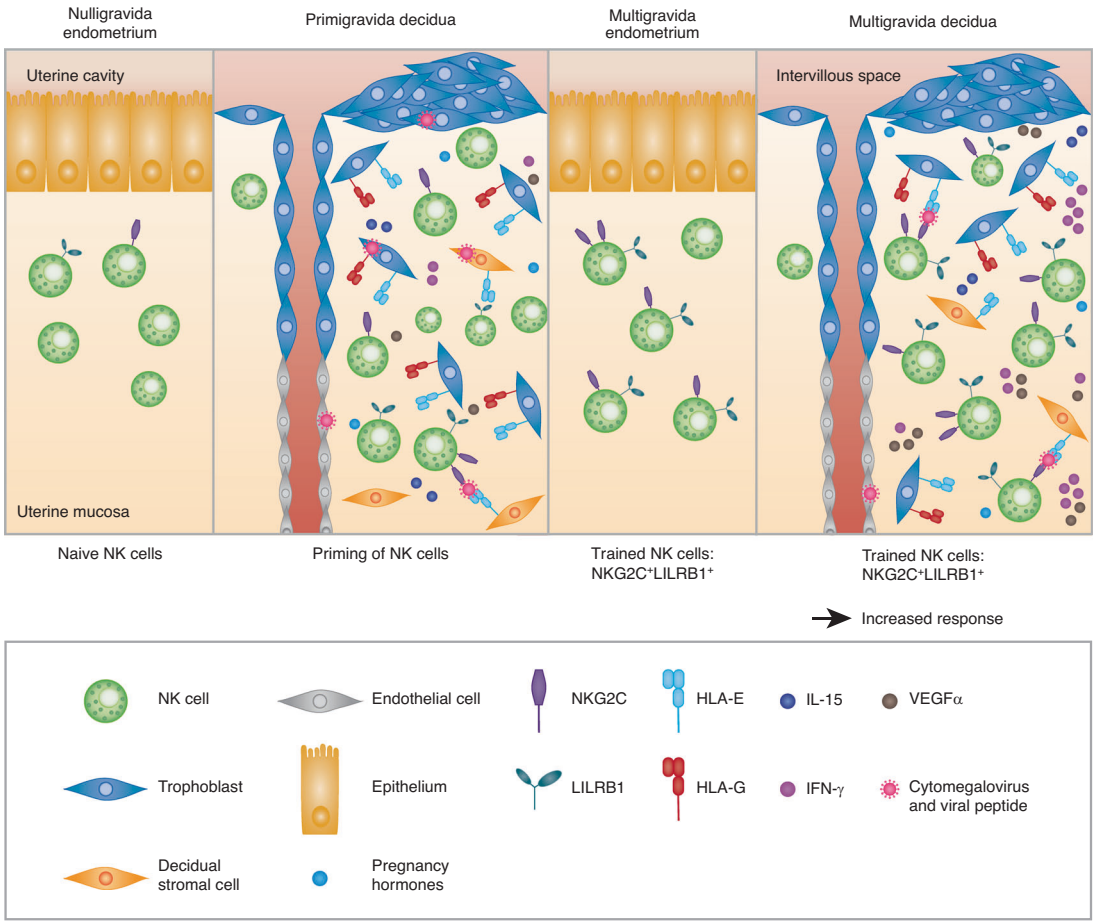

Fig. 1 Proposed model for pregnancy-trained NK cells in the uterine mucosae. During first pregnancy, decidual NK cells get primed. Which signals contribute to priming remains unclear, but we hypothesize that next to HLA-E and HLA-G expressed on trophoblast cells, HLA-E on decidual stromal cells, priming by CMV-infected decidual cells, and/or pregnancy hormones might be important to induce pregnancy-trained NK cells. Trained NK cells with enhanced NKG2C and LILRB1 expression reside in the endometrium between pregnancies. During subsequent pregnancies, trained decidual NK cells (i.e., NKG2C^{+}LILRB1^{+} NK cells) expand rapidly. These trained decidual NK cells secrete greater amounts of IFN-γ and VEFGα, thereby facilitating more effective placenta development. In addition, we hypothesize that these trained decidual NK cells could provide improved protection against intrauterine CMV infection.

epidemiological studies showed that a positive CMV status during previous pregnancies reduced the risk of congenital CMV infections in future pregnancies[27,28]. The risk of vertical transmission is 30–40% in case of primary infection during pregnancy, while transmission risk is only 2–4% in case of a non-primary maternal infection[28,29]. This may suggest that the observed pregnancy-trained decidual NK cells could be induced by decidual CMV exposure, which might aid in the protection against intrauterine CMV disease in subsequent pregnancies.

Apart from NK cells, the evidence for pregnancy-related trained immunity is scant. In mice, uterine innate lymphoid cells (ILC) with memory-marker CXCR6 expanded in second pregnancies compared to first pregnancies[30]. Whether these memory ILC1s show enhanced functionality or whether pregnancy-trained uterine ILC1s are present in humans remains to be investigated. For other innate cell types no evidence is available so far.

In conclusion, trained immunity of innate immune cells in the uterine mucosae may provide another piece in the complex immunological puzzle of successful pregnancy, and could offer a compelling explanation as to why placentation is more effective, and complications of placentation are less frequent in subsequent pregnancies. So far, two studies support the presence of pregnancy-trained decidual and endometrial NK cells with enhanced functional capacity and signs of epigenetic reprogramming. It is unclear which signals are involved in the training of these cells. We propose that next to pregnancy-specific factors, priming of decidual NK cells by decidual CMV exposure during pregnancy is one of the key factors in this process (Fig. 1). To support this notion, it will be essential to show that trained NK cells are absent in the first trimester decidua of multigravida women who are CMV^{neg} compared to CMV^{pos} multigravida women, which has currently only been shown for endometrial NK cells. In addition, the use of advanced tissue imaging techniques on the decidual tissue of CMV^{neg} and CMV^{pos} multigravida women might pinpoint the exact location of these NKG2C^{+}LILRB1^{+}-trained decidual NK cells, i.e., in close proximity to invading trophoblast cells and/or CMV-infected decidual cells. This will aid in understanding their functional role in the uterine mucosae. The question is whether they are involved in supporting placentation, or whether they protect against intrauterine CMV disease, or possibly both? In addition, it will be intriguing to investigate whether other innate immune cells in the decidua and endometrium of multigravida women, such as monocytes/macrophages and ILCs, show trained characteristics compared to nulligravida women.

Unraveling the properties of trained immunity at the maternal-fetal interface may open up new possibilities for therapeutic interventions to treat complications of inadequate placentation by manipulating and expanding trained immune cells in the uterine mucosae, or on the other hand, might contribute to the

generation of a maternal vaccine that could control congenital CMV infection.

AUTHOR CONTRIBUTIONS

D.F. wrote the article. R.G.M. and I.J. reviewed and edited the article. All authors approved the final version of the article.

ADDITIONAL INFORMATION

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