Am J Physiol Regul Integr Comp Physiol 307: R490–R492, 2014.
First published June 11, 2014; doi:10.1152/ajpregu.00176.2014.

EB 2014 | *Data Diuresis*

Role of decidual natural killer cells, interleukin-15, and interferon-γ in placental development and preeclampsia

Jenny L. Sones,[1] Heinrich E. Lob,[1] Catherine E. Isroff,[1] and Robin L. Davisson[1,2]

[1]*Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York; and* [2]*Cell and Developmental Biology, Weill Cornell Medical College, New York, New York*

Submitted 30 April 2014; accepted in final form 10 June 2014

Sones JL, Lob HE, Isroff CE, Davisson RL. Role of decidual natural killer cells, interleukin-15, and interferon-γ in placental development and preeclampsia. *Am J Physiol Regul Integr Comp Physiol* 307: R490–R492, 2014. First published June 11, 2014; doi:10.1152/ajpregu.00176.2014.—Preeclampsia is a hypertensive, proteinuric disease that affects 5–10% of all pregnancies and is a leading cause of maternal and perinatal morbidity/mortality (Soto et al., *J Matern Fetal Neonatal Med* 25: 498–507, 2011). The primary treatment for preeclampsia still is delivery of the fetus and placenta. The underlying mechanisms remain elusive. One possibility is inadequate uterine angiogenesis/vascularity (decidualization) at the time of implantation (Torry et al., *Am J Reprod Immunol* 51: 257–268, 2004). Here, we review evidence for dysregulation of decidual natural killer (dNK) cells, which secrete important angiogenic factors during decidualization, as a contributing factor in preeclampsia.

decidual natural killer cells; placenta; preeclampsia

IN RODENTS AND HUMANS, implantation is the first coordinated encounter between mother and baby (7). The process of decidualization is defined as the differentiation of uterine stromal cells into epithelial-like decidual tissue, beginning during the periimplantation period at *embryonic day* (*e*) *4.5* in mice (7). This transitory process is characterized by significant vascular remodeling of the uterus (*e5.5–e7.5*) to ensure proper placental blood flow (7). The decidua also modulates critical local immune responses (9).

Coincident with decidualization in humans and mice is the appearance of decidual natural killer (dNK) cells, the predominant immune cell at the maternal-fetal interface (4, 9, 10). Activation of dNK cells has been linked to uterine interleukin (IL)-15 (4). IL-15$^{-/-}$ mice show inappropriate decidualization, lack mature dNK cells, and, interestingly, produce low-birth-weight pups (3). Exogenous IL-15 administration in these mice restores dNK cell populations, thus confirming its importance in dNK cell maturation. In mice, mature dNK cells are recognized histologically by their lymphoid shape and cytoplasmic granules that react with *Dolichos biflorus* agglutinin (DBA) (5). DBA^{+} cells have been identified as early as *e5.5* in the decidua (12). They continue to proliferate and accumulate at the base of the placenta, obtaining peak numbers at midgestation (~*e12.5*) and declining thereafter (5). Mature dNK cells maintain decidual integrity and produce factors that directly modify decidual vessels (10). Upon stimulation by IL-15, dNK cells begin secreting key angiogenic factors, including interferon-γ (IFN-γ) (12) (Fig 1). In mice, IFN-γ provides the signals that transiently change spiral arteries from constricted to dilated high-capacitance vessels necessary for adequate placental perfusion (4). Endometrial IFN-γ expression mirrors dNK cell localization in the uterus (12). This expression profile was not observed in a mouse strain (tgε26; NK^{-}T^{-}B^{+}) having much reduced levels (only 1%) of normal dNK cell numbers (1). IFN-γ is also thought to promote senescent decline of dNK cells after midgestation (5). Implantation sites from *Ifng* null mice exhibit excessive numbers of incompletely differentiated dNK cells, widespread decidual necrosis, inappropriate spiral artery modifications (12), and significant fetal loss (1). Treatment of alymphoid mice with recombinant IFN-γ results in normal decidual and arterial morphology (12). Therefore, the production of IFN-γ by dNK cells is critical for gestational changes in the decidua and uterine vasculature.

Increased decidual IL-15 expression has been linked to recurrent miscarriage in women, suggesting impaired implantation and vascularization of the placenta (15). Other reports have demonstrated increased circulating IL-15 levels in the serum of preeclamptic mothers compared with healthy controls, where IL-15 levels were proportional to severity of disease presentation (11). These findings provide evidence that IL-15 may be involved in poor pregnancy outcomes. IFN-γ may also play a role in preeclampsia, since it is reported to be elevated in plasma, circulating leukocytes, and decidua from patients with preeclampsia (12). There is conflicting evidence regarding the significance of dNK cells in pregnancy outcomes. Some reports state that dNK cells are increased in

Address for reprint requests and other correspondence: R. L. Davisson, T9-014C Veterinary Research Tower, Cornell Univ., Ithaca, NY 14853-6401 (e-mail: robin.davisson@cornell.edu).

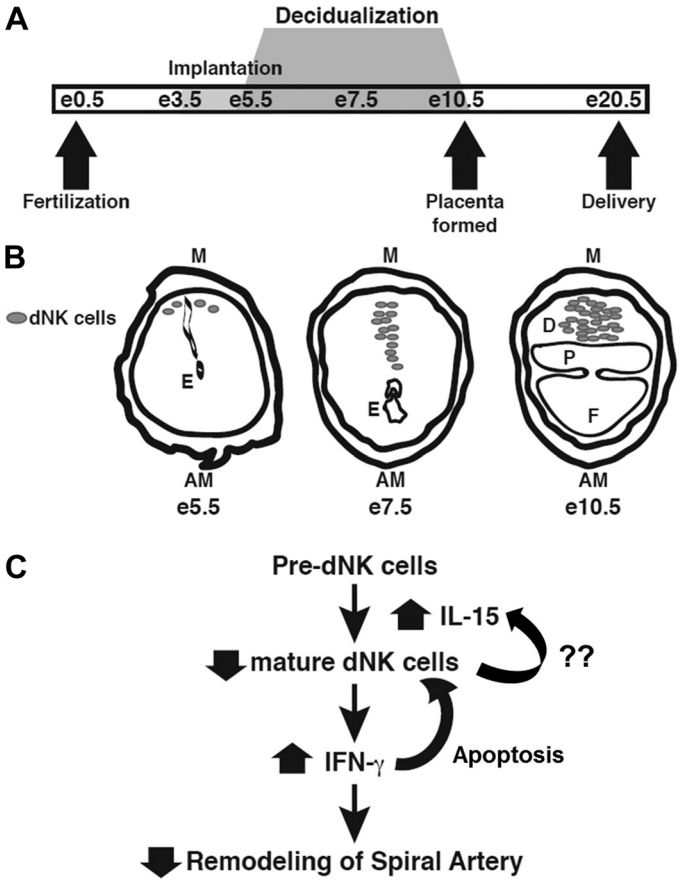

Fig. 1. Decidual natural killer (dNK) cell recruitment during decidualization is necessary for establishing blood flow to the placenta. *A*: window of implantation in mice is between *embryonic days* (*e*) *e3.5* and *e5.5* (rectangle). Decidualization begins at *e4.5*, peaks at *e7.5*, and declines thereafter as the placental unit is formed by *e10.5* (trapezoid). *B*: coincident with decidualization is the appearance of dNK cells into the uterus at *e5.5* with cell numbers increasing to reach maximum between *e10.5* and *e12.5*. *C*: pre-dNK cells are activated by uterine IL-15 to mature dNK cells that produce angiogenic factors, including IFN-γ, which is important in spiral artery remodeling for adequate placental blood flow. Evidence in human preeclamptic pregnancies and our BPH/5 mouse model of preeclampsia indicates dysregulation in mature dNKs, IL-15, and IFN-γ (bold arrows). M, mesometrial pole; AM, anti-mesometrial pole; E, embryo; D, decidua; P, placenta; F, fetus.

preeclampsia, albeit with an altered phenotype (2), whereas others show dNK cells are decreased in preeclamptic placental samples (17). Interestingly, others propose that dNK cell function, rather than number, may be altered in preeclampsia (16).

Because dNK cells are critical for uterine angiogenesis and spiral artery remodeling, gestational processes that are often pathological in preeclamptic pregnancies, we asked whether dNK cell activation and function were dysregulated before placenta formation in our model of preeclampsia, BPH/5. These mice spontaneously develop the maternal signs of preeclampsia, including hypertension and proteinuria, as well as similar placental defects observed in human preeclamptic placentae such as inadequate remodeling of spiral arteries (6, 8). We found dNK cell mRNA expression in BPH/5 implantation sites was similar to C57Bl/6 controls at *e4.5* but dramatically reduced at *e5.5*. This finding was confirmed by a marked decrease in mature dNK cell numbers at *e5.5* in BPH/5 implantation sites as measured by flow cytometry and immunohistochemistry for DBA^+ cells. Furthermore, IL-15 mRNA was significantly increased in BPH/5 implantation sites compared with controls at *e4.5* and *e5.5*, when decidualization first begins, while other pro-inflammatory cytokines were unchanged. One hypothesis is that high levels of IL-15 may be induced to help promote more dNK cell activation in BPH/5; however, this is currently under investigation. We speculate that an early increase in IFN-γ mRNA in *e4.5* BPH/5 implantation sites contributes to dNK cell loss. These findings support the hypothesis that periimplantation events may lead to early impaired uterine angiogenesis and placental development long before the development of preeclampsia in this model.

Perspectives and Significance

Both dNK cell activation by IL-15 and dNK secretion of IFN-γ are crucial for placental development. Interestingly, BPH/5 mice show elevations in IL-15 and IFN-γ mRNA as well as a dramatic reduction in mature dNK cells. Other mouse models lacking dNK cells, IL-15, or IFN-γ have significant placental and/or fetal abnormalities. Furthermore, women with pregnancy-related disorders also show dysregulation in IL-15, IFN-γ, and dNK cells. Therefore, understanding the significance of dNK cells and related cytokines in preeclampsia and other pathological pregnancies with abnormal placenta formation is of utmost importance. Mouse models such as the BPH/5 offer critical tools for investigating this at the earliest stages of pregnancy.

GRANTS

This study was supported by National Institutes of Health Grants HL-63887 and HL-84207 (to R. L. Davisson); and by American Heart Association Grants 12POST11250010 (to J. L. Sones) and 12SDG9160010 (to H. E. Lob).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.L.S., H.E.L., and R.L.D. conception and design of research; J.L.S., H.E.L., and C.E.I. performed experiments; J.L.S., H.E.L., and C.E.I. analyzed data; J.L.S., H.E.L., and R.L.D. interpreted results of experiments; J.L.S. and H.E.L. prepared figures; J.L.S. drafted manuscript; J.L.S., H.E.L., and R.L.D. edited and revised manuscript; J.L.S., H.E.L., and R.L.D. approved final version of manuscript.

REFERENCES

1. **Ashkar AA, Croy BA.** Interferon-gamma contributes to the normalcy of murine pregnancy. *Biol Reprod* 61: 493–502, 1999.
2. **Bachmayer N, Rafik Hamad R, Liszka L, Bremme K, Sverremark-Ekstrom E.** Aberrant uterine natural killer (NK)-cell expression and altered placental and serum levels of the NK-cell promoting cytokine interleukin-12 in pre-eclampsia. *Am J Reprod Immunol* 56: 292–301, 2006.
3. **Barber EM, Pollard JW.** The uterine NK cell population requires IL-15 but these cells are not required for pregnancy nor the resolution of a Listeria monocytogenes infection. *J Immunol* 171: 37–46, 2003.
4. **Burke SD, Barrette VF, Gravel J, Carter AL, Hatta K, Zhang J, Chen Z, Leno-Duran E, Bianco J, Leonard S, Murrant C, Adams MA, Croy BA.** Uterine NK cells, spiral artery modification and the regulation of blood pressure during mouse pregnancy. *Am J Reprod Immunol* 63: 472–481, 2010.
5. **Croy BA, Chantakru S, Esadeg S, Ashkar AA, Wei Q.** Decidual natural killer cells: key regulators of placental development (Review). *J Reprod Immunol* 57: 151–168, 2002.

6. **Davisson RL, Hoffmann DS, Butz GM, Aldape G, Schlager G, Merrill DC, Sethi S, Weiss RM, Bates JN.** Discovery of a spontaneous genetic mouse model of preeclampsia. *Hypertension* 39: 337–342, 2002.
7. **Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H.** Molecular cues to implantation. *Endocr Rev* 25: 341–373, 2004.
8. **Dokras A, Hoffmann DS, Eastvold JS, Kienzle MF, Gruman LM, Kirby PA, Weiss RM, Davisson RL.** Severe feto-placental abnormalities precede the onset of hypertension and proteinuria in a mouse model of preeclampsia. *Biol Reprod* 75: 899–907, 2006.
9. **Erlebacher A.** Immunology of the maternal-fetal interface. *Annu Rev Immunol* 31: 387–411, 2013.
10. **Herington JL, Bany BM.** Effect of the conceptus on uterine natural killer cell numbers and function in the mouse uterus during decidualization. *Biol Reprod* 76: 579–588, 2007.
11. **Hu W, Wang H, Wang Z, Huang H, Dong M.** Elevated serum levels of interleukin-15 and interleukin-16 in preeclampsia. *J Reprod Immunol* 73: 166–171, 2007.
12. **Murphy SP, Tayade C, Ashkar AA, Hatta K, Zhang J, Croy BA.** Interferon gamma in successful pregnancies. *Biol Reprod* 80: 848–859, 2009.
13. **Soto E, Romero R, Kusanovic JP, Ogge G, Hussein Y, Yeo L, Hassan SS, Kim CJ, Chaiworapongsa T.** Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal underperfusion. *J Matern Fetal Neonatal Med* 25: 498–507, 2011.
14. **Torry DS, Hinrichs M, Torry RJ.** Determinants of placental vascularity. *Am J Reprod Immunol* 51: 257–268, 2004.
15. **Toth B, Haufe T, Scholz C, Kuhn C, Friese K, Karamouti M, Makrigiannakis A, Jeschke U.** Placental interleukin-15 expression in recurrent miscarriage. *Am J Reprod Immunol* 64: 402–410, 2010.
16. **Wallace AE, Host AJ, Whitley GS, Cartwright JE.** Decidual natural killer cell interactions with trophoblasts are impaired in pregnancies at increased risk of preeclampsia. *Am J Pathol* 183: 1853–1861, 2013.
17. **Williams PJ, Bulmer JN, Searle RF, Innes BA, Robson SC.** Altered decidual leucocyte populations in the placental bed in pre-eclampsia and foetal growth restriction: a comparison with late normal pregnancy. *Reproduction* 138: 177–184, 2009.