*Commentary*

# Role of Uterine Natural Killer Cells and Interferon γ in Placental Development

By Raymond W. Redline

*From the Department of Pathology, University Hospitals of Cleveland and Case Western Reserve University, Cleveland, Ohio 44106*

Large granular lymphocytes migrate in large numbers to the pregnant uteri of a wide variety of mammalian species (1). These cells, also known as granulated metrial gland cells in rodents and endometrial granulocytes in primates, will for the purposes of this review all be referred to as uterine (u)NK cells. uNK cells are bone marrow–derived leukocytes, but their immediate precursors may migrate from the spleen. Cells expressing a similar panel of activation antigens are found in the spleens of pregnant but not nonpregnant mice, and only splenocytes derived from pregnant donors can populate the uteri of uNK-deficient recipients during pregnancy (1, 2). The signals that regulate migration of uNK to the uterus are not known. Homing precedes implantation in rodents and primates, so it is unlikely that the fetus plays a direct role. Circumstantial evidence implicates ovarian steroids and uterine decidualization, a metaplastic process that modifies the placental implantation site during pregnancy (3). Mice lacking genes for chemokines known to attract NK cells at other sites show no defects in uNK localization (1). After migration, uNK cells proliferate, differentiate, and accumulate in large numbers in specific areas of the uterus between days 2.5 and 12 of murine pregnancy (implantation occurs on day 4 and delivery on day 19). After day 12, uNK cells undergo extensive apoptosis (as defined by morphology and TdT tailing) and are dramatically decreased in number and activation through the remainder of pregnancy (4–6). uNK granules contain lytic molecules such as perforin and granzymes A and B, matrix components including osteopontin, and vasoactive factors such as inducible nitric oxide synthase (iNOS) and endothelial (e)NOS (6–8). Factors expressed in the uterus that are either bound by or otherwise implicated in the regulation of uNK cells include IL-15 (9), decidual prolactin-related peptide B (10), and IFN-γ. uNK cells can also be activated to secrete a variety of cytokines including GM-CSF, CSF-1, leukemia inhibitory factor, TGF-β1, TNF-α, and most importantly for the purposes of this discussion, IFN-γ (8).

Address correspondence to Raymond Redline, Institute of Pathology, Rm. 203, Case Western Reserve University, 2085 Adelbert Rd., Cleveland, OH 44106. Phone: 216-844-1813; Fax: 216-844-1810; E-mail: raymond.redline@uhhs.com

What is the justification for assigning uterine large granular lymphocytes to the NK lineage? Murine uNK cells express Thy 1.1, asialo-GM1, IL-15Rα, and at least two members of the CD94/NKG2 C-type lectin–like family of class I MHC receptors, NKR-P1 (NK1.1) and Ly49G2 (LGL-1), and can lyse YAC-1 target cells (5, 6). Human uNK cells express CD56 (polysialylated neural cell adhesion molecule), members of the CD94/NKG2 and killer inhibitory receptor (KIR)2D class I MHC receptor families, and can lyse K562 target cells (11, 12). Despite having these typical phenotypic and functional characteristics, some doubt persists as to whether uNK cells truly belong to the NK lineage. Part of the controversy is inherent to the definition of NK cells. In the absence of any one lineage specific marker, NK cells are essentially defined in negative terms, i.e., cells of appropriate phenotype and function that lack surface CD3/TCR molecules and show no molecular evidence of Ig/TCR rearrangement (9). Lineage diagrams depict an early separation of T lymphocytes and NK cells before expression of the recombinase activating genes RAG-1 and RAG-2. These definitions raise a more specific problem for uNK cells, the presence of an overlapping population of uterine large granular lymphocytes that express γ/δ TCRs. A significant fraction of $CD56^+$ human uNK cells either express γ/δ TCRs or can acquire γ/δ TCRs in culture (13). Based on these observations plus the unusually high expression of RAG-1 and RAG-2 in $CD56^+$ human uNK cells (14), it has been suggested that the human uterus could represent a site of extrathymic maturation for γ/δ T cells analogous to cryptopatches in the small intestine. A corresponding population of murine γ/δ T cells has also been isolated, but their exact phenotype and localization within the pregnant uterus has not been studied (15). Some evidence suggests that these cells may regulate pregnancy viability in early gestation (day 5.5–8.5) and produce cytokines in response to trophoblast antigens later in pregnancy (day 14–16) (16, 17). Whether these cells are distinct from uNK cells is unknown. However, as virtually all uterine $CD45^+$ leukocytes in the vicinity of the placenta are by morphologic and immunophenotypic criteria typical uNK cells, it is difficult to accommodate a substantial independent population of γ/δ T cells (6, 18). On balance, it seems reasonable at this point


J. Exp. Med. © The Rockefeller University Press • 0022-1007/2000/07/F1/04 $5.00
Volume 192, Number 2, July 17, 2000 F1–F4
http://www.jem.org/cgi/current/full/192/2/F1

to assign uterine large granular lymphocytes to the NK lineage, realizing that their taxonomy may change as further data becomes available.

The recognition that uterine large granular lymphocytes are probably NK cells coincided with the finding that invasive trophoblasts in rodents and primates express class I MHC molecules (11, 18). These observations both occurred at a time of heightened interest in the mechanisms by which a semiallogeneic fetus might survive in the histoincompatible mother. It had previously been thought that trophoblasts lacked MHC expression, making them ineffective targets for a classical allogeneic response. The paired observations that invasive trophoblasts express class I MHC antigens and that uNK cells are prevalent in the pregnant uterus led to the formulation of a hypothesis that has remained pervasive in the field over the past decade: that trophoblasts resist NK lysis by expressing class I MHC molecules (later shown to the consequence of NK–class I MHC-specific KIR) and that recognition of trophoblasts by NK cells elicits the secretion of cytokines that both enhance placental growth and modulate local allogeneic responses (Th2 deviation). It is no exaggeration to state that the number of reviews, editorials, conference reports, and commentaries reiterating this hypothesis has far exceeded the number of studies that have directly tested it. Unfortunately, while heuristically satisfying, the uNK trophoblast class I MHC hypothesis has not been particularly useful thus far in explaining either normal placental development or reproductive disorders such as abortion, growth retardation, preeclampsia, and fetal loss.

Careful morphologic assessment of normal and abnormal pregnancies is a powerful tool to evaluate hypotheses regarding uNK function, and problems with the trophoblast class I MHC hypothesis are readily apparent. Most of the problems relate to the fact that for most of pregnancy, uNK cells and trophoblasts are not in close temporal or anatomic proximity to one another. Murine uNK cells accumulate before implantation away from the implanting conceptus and eventually concentrate in the metrial gland, a structure located deep within the uterine musculature (19). The metrial gland is never infiltrated by trophoblasts but rather surrounds large uterine arteries that supply the placenta (18). Only late in pregnancy after extensive apoptosis and downregulation of both activation antigens and lytic activity do uNK cells come in contact with trophoblasts in the decidua basalis. Even at this stage, the majority of uNK cells are in the metrial gland. Similarly, human uNK cells are primarily clustered around endometrial glandular epithelium and small arteries away from trophoblasts (20). The most intriguing observations relating to uNK function come from rats and hamsters, rodent species that, unlike the mouse, have deeply implanting placentas with uterine arteries that are remodelled by invading trophoblastic cells (discussed below). In these species, it has been shown that uNK cells infiltrate the walls of uterine arteries before their invasion by trophoblasts (21). Earlier in rat pregnancy, uNK cells appear to have second distinct role: infiltration of endometrial epithelium surrounding the implantation cylinder immediately before its incorporation into the endometrial vasculature and long before contact with placental trophoblasts (22). The weight of the anatomic evidence does not support a primary or direct role for uNK cells interacting with trophoblasts but rather suggests a role for uNK in the modification of uterine blood vessels and endometrial epithelium away from the zones of trophoblast invasion and placental morphogenesis.

Eschewing the trophoblast class I MHC hypothesis, Croy et al. (1) have over the past 10 years pursued an alternative genetic approach supplemented by careful morphologic analysis. This group has focused on two questions: What defects would be seen in pregnancies derived from mothers without uNK cells, and What manipulations would be required overcome uNK deficiency and restore normal morphology? Answering these questions was initially hampered by the lack of an appropriate model of uNK deficiency. Studies with SCID mice argued against a substantial role for adaptive immunity in placental morphology and pregnancy outcome. *beige/beige* mice with defective NK lytic activity also had essentially normal pregnancies, as did doubly mutant SCID/*beige* mice (8). Subsequent analysis of many immunodeficient mouse strains revealed two with absent uNK cells, tgε26 females having an insertional mutation involving multiple copies of the human CD3E gene and mice doubly mutant for p56 lck and IL-2Rβ. Six well defined pregnancy abnormalities were described in these animals: absence of uNK cells, no metrial gland, decreased placental size, increased fetal loss, decidual edema, and most importantly an arteriopathy involving the large maternal arteries supplying the placenta. This arteriopathy was characterized by hypertrophy of the muscular media and narrowing of the vascular lumen (defective vascular remodeling). All six defects were corrected by transplantation of normal bone marrow just before pregnancy. The results of the study by Ashkar et al. in this issue provide further insights into the etiology and mechanisms of uNK deficiency arteriopathy (23). For this study, a third uNK-deficient mouse, doubly mutant for RAG-2 and the common cytokine γ receptor subunit, was used. These mice received either bone marrow transplants from donors lacking key uNK-regulatory molecules or daily cytokine infusions to define the minimum requirements for restoration of normal morphology. Three conclusions emerged. First, low levels of endogenous (non–uNK-derived) IFN-γ are required to maintain decidual integrity at day 12–14 of pregnancy. Second, higher levels of IFN-γ, normally derived from uNK but substituted for by daily infusions of IFN-γ, are needed for vascular remodelling (day 10–12). Third, an intact IFN-γ signal transduction pathway is required in donor cells for normal uNK (and metrial gland) development. However, donors lacking IFN-γ signal transduction function were still able to appropriately remodel uterine arteries, suggesting that the uNK abnormalities observed in these mice did not interfere with the availability of IFN-γ.

Vascular remodelling is a relatively recent concept now believed to occur in a variety of situations characterized by

inadequate perfusion or increased vascular demand (24). Mediated by changes in local cytokines, growth factors, vasoregulatory substances, matrix components, and matrix proteases, this process culminates in permanent structural alterations, including thinning of the vascular muscle wall and with luminal dilatation, which increase local perfusion. Although unfamiliar to many immunologists, vascular remodelling has been of considerable recent interest in reproductive biology. It has long been known that maternal arteries supplying the human placenta are invaded by fetal trophoblasts, which replace the muscular media with a fibrinoid matrix, leading to increased blood flow (21). Recent data have shown that this trophoblast-dependent process is preceded by a trophoblast-independent phase of vascular remodelling (25). As shown most convincingly by Ashkar et al. (23), trophoblast-independent remodelling of uterine arteries in rodents appears to be uNK dependent. Although human uNK cells have not yet been implicated in vascular remodelling, it is notable that ~10% of human uNK cells cluster around uterine arteries undergoing vascular remodelling (20). Recent data has shown that decreased oxygen tension leads to impaired trophoblast differentiation and trophoblast-dependent arterial remodelling, both of which are characteristic of the human pregnancy disorder preeclampsia (26). In light of these recent data, the following hypothesis is suggested. Defects in early vascular remodelling related to uNK dysfunction lead to local hypoxia, which retards trophoblast differentiation and inhibits trophoblast-dependent vascular remodelling. The resulting reduction in uteroplacental perfusion would then increase the risk of preeclampsia and related pregnancy disorders such as growth retardation and unexplained fetal loss. The uNK-deficient murine model, although lacking the trophoblast-dependent vascular remodelling component, supports such a model and implicates uNK-derived IFN-γ as a possible mediator.

With regard to future investigation, three obvious questions stand out: the relationship of murine uNK to γ/δ T cells throughout gestation and their relative functionality, the downstream pathways mediating murine IFN-γ–dependent vascular remodelling, and the relationship of human uNK cells to abnormal vascular remodelling in preeclampsia and related disorders.

*Submitted: 26 May 2000*
*Accepted: 8 June 2000*

## References

1. Croy, B.A., J.P. DiSanto, J.D. Greenwood, S. Chantakru, and A.A. Ashkar. 2000. Transplantation into genetically alymphoid mice as an approach to dissect the roles of uterine natural killer cells during pregnancy--a review. *Placenta*. 21: Suppl A:S77–S80.
2. Linnemeyer, P.A., and S.B. Pollack. 1991. Mab4H12 recognizes subsets of adherent-lymphokine activated killer cells and splenic NK cells from pregnant and neonatal mice. *J. Immunol*. 146:3729–3734.
3. Sharma, R., D. Bulmer, and S. Peel. 1986. Effects of exogenous progesterone following ovariectomy on the metrial glands of pregnant mice. *J. Anat*. 144:189–199.
4. Croy, B.A., L.A. Villeneuve, K. Kusakabe, Y. Kiso, and M. Vandenheuvel. 1997. Characterization of the cells that migrate from metrial glands of the pregnant mouse uterus during explant culture. *J. Reprod. Immunol*. 32:241–263.
5. Linnemeyer, P.A., and S.B. Pollack. 1994. Stage-specific expression of activation antigens on NK cells at uterine implantation sites in mice. *J. Immunol*. 153:1478–1485.
6. Parr, E.L., L.H.Y. Young, M.B. Parr, and J.D.-E. Young. 1990. Granulated metrial gland cells of pregnant mouse uterus are natural killer cells that contain perforin and serine esterases. *J. Immunol*. 145:2365–2372.
7. Nomura, S., A.J. Wills, D.R. Edwards, J.K. Heath, and B.L. Hogan. 1988. Developmental expression of 2ar (osteopontin) and SPARC (osteonectin) RNA as revealed by in situ hybridization. *J. Cell Biol*. 106:441–450.
8. Guimond, M.J., B. Wang, and B.A. Croy. 1999. Immune competence involving the natural killer cell lineage promotes placental growth. *Placenta*. 20:441–450.
9. Liu, C.-C., B. Perussia, and J.D.-E. Young. 2000. The emerging role of IL-15 in NK-cell development. *Immunol. Today*. 21:113–116.
10. Muller, H., B. Liu, B.A. Croy, J.R. Head, J.S. Hunt, G.L. Dai, and M.J. Soares. 1999. Uterine natural killer cells are targets for a trophoblast cell-specific cytokine, prolactin-like protein A. *Endocrinology*. 140:2711–2720.
11. King, A., S.E. Hilby, L. Gardner, S. Joseph, J.M. Bowen, S. Verma, T.D. Burrows, and Y.W. Loke. 2000. Recognition of trophoblast HLA class I molecules by decidual NK cell receptors-a review. *Placenta*. 21:Suppl A:S81–S85.
12. Ferry, B.L., P.M. Starkey, I.L. Sargent, G.M.O. Watt, M. Jackson, and C.W.G. Redman. 1990. Cell populations in the human early pregnancy decidua:natural killer activity and response to interleukin-2 of CD56-positive large granular lymphocytes. *Immunology*. 70:446–452.
13. Mincheva-Nilsson, L., M. Kling, S. Hammarstrom, O. Nagaeva, K.-G. Sundqvist, M.-L. Hammarstrom, and V. Baranov. 1997. γδ T cells of human early pregnancy decidua. *J. Immunol*. 159:3266–3277.
14. Hayakawa, S., S. Saito, N. Nemoto, F. Chishima, K. Akiyama, H. Shiraishi, J. Hayakawa, M. Karasaki-Suzuki, K.T. Fujii, M. Ichijo, et al. 1994. Expression of recombinase-activating genes (RAG-1 and 2) in human decidual mononuclear cells. *J. Immunol*. 153:4934–4939.
15. Heyborne, K.D., R.L. Cranfill, S.R. Carding, W.K. Born, and R.L. O'Brien. 1992. Characterization of γδ T lymphocytes at the maternal-fetal interface. *J. Immunol*. 149:2872–2878.
16. Arck, P.C., D.A. Ferrick, D. Steele-Norwood, P.J. Egan, K. Croitoru, S.R. Carding, J. Dietl, and D.A. Clark. 1999. Murine T cell determination of pregnancy outcome II. Distinct Th1 and Th2/3 populations of V gamma 1(+)delta 6(+) T cells influence success and failure of pregnancy in CBA/J x DBA/2J matings. *Cell. Immunol*. 196:71–79.
17. Heyborne, K., Y.-X. Fu, A. Nelson, A. Farr, R. O'Brien, and W. Born. 1994. Recognition of trophoblasts by γδ T cells. *J. Immunol*. 153:2918–2926.
18. Redline, R.W., and C.Y. Lu. 1989. Localization of fetal major histocompatibility complex antigens and maternal leukocytes in murine placenta. *Lab. Invest*. 61:27–36.
19. Stewart, I., and S. Peel. 1978. The differentiation of the decidua and the distribution of metrial gland cells in the preg-

nant mouse uterus. *Cell Tissue Res*. 187:167–179.
20. Mincheva-Nilsson, L., V. Baranov, M.M.-W. Yeung, S. Hammarstrom, and M.-L. Hammarstrom. 1994. Immunomorphologic studies of human decidua-associated lymphoid cells in normal early pregnancy. *J. Immunol*. 152:2020–2032.
21. Pijnenborg, R., W.B. Robertson, I. Brosens, and G. Dixon. 1981. Review article:trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. *Placenta*. 2:71–92.
22. Welsh, A.O., and A.C. Enders. 1993. Chorioallantonic placenta formation in the Rat. III. Granulated cells invade the uterine luminal epithelium at the time of epithelial cell death. *Biol. Reprod*. 49:38–57.
23. Ashkar, A.A., J.P. DiSanto, and B.A. Croy. 2000. Interferon $\gamma$ contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J. Exp. Med*. 192:259–269.
24. Gibbons, G.H., and V.J. Dzau. 1994. The emerging concept of vascular remodeling. *N. Engl. J. Med*. 330:1431–1438.
25. Craven, C.M., T. Morgan, and K. Ward. 1998. Decidual spiral artery remodelling begins before cellular interaction with cytotrophoblasts. *Placenta*. 19:241–252.
26. Caniggia, I., H. Mostachfi, J. Winter, M. Gassmann, S.J. Lye, M. Kuliszewski, and M. Post. 2000. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGF$\beta_3$. *J. Clin. Invest*. 105:577–587.