BIOLOGY OF UTERINE NATURAL KILLER CELLS AT THE FETO-MATERNAL INTERFACE

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Granular lymphocytes (GLs) are common inhabitants of mammalian uteri including not only the deciduata but the nondeciduata. In women and mice, these cells are transient, appear to terminally differentiate, and are hormonally dependent lymphocytes of a natural killer (NK) cell lineage. NK cells in the peripheral blood and spleen contribute to both innate and adaptive immunity by their prompt secretion of cytokines, including IFN-γ, and lysis of virally-infected and tumor cells without previous sensitization. The differentiation and function of tissue-based NK cells, such as uterine NK (uNK) cells, remain to be fully understood. Recent studies using NK$^-$T$^-$ mice have established that uNK cells have critical functions in pregnancy success. We will address here some aspects of uNK cells regarding their differentiation and function based on our studies.

1) When do uNK cell precursors first appear in the uterus?

Ly49G2, the earliest marker of uNK cells, was used to enumerate putative precursors of uNK cells in the maturing, nonpregnant uterus of mice. Ly49G2$^+$ cells were not seen until the mice were 2 wks of age and 10 fold increase in these cells occurred between wks 2-3 of age. A second significant increase of Ly49G2$^+$ cells occurred at wk 5 which achieved a frequency of Ly49G2$^+$ cells equal to that in the virgin, adult uterus. These data suggest that the appearance of Ly49G2$^+$ cells in the uterus is a postnatal event, independent of either puberty or pregnancy.

2) Are uNK cell precursors resident in the uterus?

The previous experiment suggests that uNK cell precursors reside in the uterus. To confirm this, a transplantation technique was developed to assess uNK cell differentiation in the uterine tissue. The uterus is cut into small cubes, enclosed within a diffusion chamber and transplanted to the abdomen of an adult mated female on the day of the copulation plug. This study shows that uNK cell precursors reside in 7 wk old virgin uteri and that their lifespan is as long as 12 days. However, when uterine tissues from normal mice was transplanted by end to end anastomosis into the uterus of uNK cell-deficient mice, on 18 days after transplantation deciduomata formed but they had no uNK cells. Thus,

the uterus does not have a long-lived, self-renewing pool of uNK cell precursors, and the major population of uNK cells is replenished by precursors from other tissues.

3) Which factors influence the differentiation of uNK cells?

To determine whether B or T cells influence uNK cell differentiation, Ly49G2$^+$ cells were counted in the uteri of SCID mice between birth and 10 wks of age. Ly49G2$^+$ cells were absent until wk 5 of age and did not reach adult levels until 10 wks of age. Comparing to immunocompetent barrier-reared, flora-defined mice, these times are each delayed by 3 wks. Thus, uNK cells differentiate in the absence of B and T cells, but the appearance of their putative precursors is delayed. To evaluate the influence of macrophages and CSF-1 on uNK cell differentiation, the metrial gland was evaluated from pregnant mice of genotype *op/op*, which produce no CSF-1 and have a deficit in macrophages. Metrial glands and uNK cells of this mice were normal, suggesting that uNK cells do not require CSF-1 for their differentiation or to maintain cell viability of precursors.

4) What are the interactions between uNK cells and extracellular matrix (ECM)?

Several studies have been undertaken to define interactions between uNK cells and the ECM. In vivo, anomalies in uNK cell distribution occurred in the mutant *Tsk/+*, which produces excess collagen. Also, delay of uNK cell differentiation occurred in the mutant *aly/aly*, which is genetically deficient in lymph nodes and Peyer's patches due to a lymphoid-associated mesenchymal disorder. These indicate that ECMs can affect both distribution and differentiation of uNK cells. In culture, viability of uNK cells was only sustained in the presence of ECM substrates or metrial gland explant tissue. Viability was best on laminin, while on fibronectin uNK cells underwent dramatic changes in shape and elongate. Besides, cultured uNK cells migrated in response to the ECM and the ectoplacental cone that is known to produce FN, while no response to IL-2, LIF nor B cell conditioned. Vitronectin and laminin intensively induced the chemotaxis of uNK cells. These suggest that viability and chemotaxis of uNK cells are closely related with ECMs. Immunohistochemical analyses revealed that uNK cells express VLA-β1 at all stages of pregnancy. VLA-α1 and α3 chains (collagen/laminin receptors) are expressed by Ly49G2+ cells until day6 of pregnancy, but not by day 8-15 uNK cells which express the fibronectin receptor a4. Thus, ECMs regulate cell mobility, cell viability and cytokine gene expression, and other cell surface and cytoplasmic proteins may be altered in their expression by uNK cells during implantation/placentation period.

5) Does apoptosis account for the loss of uNK cells in late pregnancy?

uNK cells increase in number until mid gestation, but disappear from the uterus in late gestation. To determine whether such a decrease of uNK cells is caused from apoptosis, morphology, in situ

detection and electrophoresis were conducted. These data indicate that the loss of uNK cells after 15 days of pregnancy is due to apoptosis. Indeed, in pregnant *lpr/lpr* mice which lack genetically Fas gene, uNK cells were seen in significant number also in late pregnancy. The Fas antigen on uNK cells was expressed on day 15 of gestation, while not on day 12. Besides, the Fas ligand could cause apoptosis in day 16 uNK cells, while not on day 12. Thus, it is most likely that the Fas/Fas ligand system is the common apoptosis mediator also in uNK cells.

In an infertile strain (*IF*) among various strains of Japanese wild mice, *Mus musculus molossinus,* some uNK cells still persisted in the postpartum uterus. Japanese wild mice mate on the day of litter birth. Infertility in *IF molossinus* mice occurred in second pregnancy as a failure of embryo implantation. Thus, persistence of mature uNK cells into the second pregnancy may have detrimental effects on pregnancy outcome, suggesting that uNK cell apoptosis in late pregnancy is an important event.

6) Do uNK cells have detrimental effects on pregnancy outcome?

To assess whether uNK cells are associated with abortion, due to perforin within their cytoplasmic granules, uteri were collected from abortion models, i.e., spontaneous abortion of DBA/2-mated CBA/J female mice and artificial abortion of IL-2-administrated B6 female mice. In both abortion models, morphology, localization and influence of perforin$^+$ uNK cells were similar to those of normal mice, suggesting that uNK cells have no functions in abortion. On the other hand, in Tg2Rβ, IL-2 receptor β chain overexpressed transgenic mice, which are completely deficient in NK1.1$^+$NK cells and Thy-1$^+$dendritic epidermal cells, on day 12 of pregnancy all of fetuses were resorbing comparing to normal littermates. Surprisingly, many uNK cells infiltrated to the labyrinthine zone, and day 8 uNK cells in Tg2Rβ were similar to day 12 uNK cells of normal littermates, suggesting that Tg2Rβ uNK cells differentiated at earlier stage of pregnancy. Thus, a lytic activity of uNK cells, that was usually latent, may be enhanced by overexpression of IL-2Rβ.

7) How do uNK cells play crucial roles in pregnancy success?

In mice deficient in uNK cells (TgE26), the placental size was significant smaller than normal and the metrial gland was poorly developed. In TgE26, a sudden onset of fetal loss began at day 10 of gestation. In TgE26 reconstituted with SCID bone marrow, uNK cells were recovered at the normal level and the fetus was alive, suggesting that uNK cells have crucial roles in pregnancy success. The structure of decidua, architecture of spiral arterioles and IFN-γ levels were more pathological or lower in TgE26 than normal and other immunodeficient mice. Since a major product of NK cells, including uNK cells, is IFN-γ, and since IFN-γ plays an important role in decidua formation, the mechanisms of action of uNK cells may be IFN-γ-mediated and the decidua ant its

vasculature may be the targets of the physiological actions of uNK cells.

Further studies are required to establish both differentiation and function of uNK cells at the feto-maternal interface during successful pregnancy.

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