TRAFFICKING OF CIRCULATING PRO-NK CELLS TO THE DECIDUALIZING UTERUS; REGULATORY MECHANISMS IN THE MOUSE AND HUMAN

Marianne J. van den Heuvel[1], **Sirirak Chantakru**[2], **Xuemei Xie**[3], **Sharon S. Evans**[4], **Francis Tekpetey**[5], **Patricia A. Mote**[6], **Christine L. Clarke**[7], and **B. Anne Croy**

[1]Department of Pediatrics, University of Western Ontario, London, ON, Canada [2]Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand [3]Department of Biomedical Sciences, University of Guelph, ON, Canada [4]Department of Immunology, Roswell Park Cancer Institute, SUNY, Buffalo, New York, United States [5]Reproductive Endocrinology and Infertility Unit, London Health Sciences Centre, University of Western Ontario, London, ON, Canada [6]Westmead Institute for Cancer Research, University of Sydney, Westmead, New South Wales, Australia [7]Department of Anatomy and Cell Biology, Queen's University, Kingston, ON, Canada

Abstract

Natural Killer (NK) lymphocytes, strongly expressing CD56, become abundant in the human uterus three to five days after the mid-menstrual cycle surge in pituitary-derived luteinizing hormone (LH). The primary functions of LH are to initiate final oocyte maturation/ovulation and to contribute to decidualization of the uterine stroma. Decidualization is the transformation of estrogen-primed uterine stromal fibroblasts into large hormone producing cells under the influence of progesterone (P_4). Decidual CD56^{bright} (dNK) cells are a distinct, transient, tissue-specific NK cell subset that undergoes proliferation, terminal differentiation, and then death prior to menses. If pregnancy occurs, dNK cells increase during first trimester, then decline and are virtually absent in late pregnancy. In mouse models, pregnancy-associated uterine NK (uNK) cells appear coincident with onset of decidualization during embryonic implantation. Murine uNK cells traffic from the circulation to the anti-mesometrial side of the uterus and migrate to the mesometrial side of each implantation site. Here they proliferate and are implicated in regulation of mid-gestation structural changes to major arteries supplying the placenta, before dying in late gestation. Emerging data indicate that interactions between lymphocytes and endothelial cells within the uterine microenvironment are mediated by classical molecules associated with lymphocyte trafficking in immune surveillance and in response to inflammation. Here, we review factors influencing NK cell trafficking to decidualizing murine and human uteri and the differentiation and functions of these cells within the uterus.

Corresponding Author: Dr. Marianne van den Heuvel, Department of Pediatrics, University of Western Ontario, London, ON, Canada N6C 2V5Tel (519) 685 8500 Fax (519) 685 8186.

INTRODUCTION

The arrival of a population of NK cells in the mammalian uterus in association with decidualization and pregnancy represents an immunological enigma[75]. Despite great strides that have been made in defining interactions between maternal and fetal cells in primates and in rodents[19;40], the molecules regulating the trafficking of these cells to the uterus remain unclear. The original hypotheses postulated by Sir Peter Medawar to account for success of viviparity included anatomic separation of the mother and fetus, antigenic immaturity of the fetus and/or inertness of the maternal immune system[51]. It is well established that trophoblast, the cell lineage providing the fetal component of the placenta, separates the fetal and maternal circulations and permits only limited bi-directional cell traffic[48;49]. In humans, trophoblast expresses a unique and restricted pattern of HLA molecules[37;47] which is recognized by inhibitory and activating molecules of the NK receptor gene families known as killer cell immunoglobulin-like receptors (KIR for human)[33] or LY49 (mice)[60] expressed on CD56^{+} cells, that are transiently, the predominant uterine lymphocytes of early pregnancy[47]. Cells of the NK lineage constitute about 15% of peripheral blood lymphocytes but 70% of total lymphocytes in the decidualizing uterus. Although many of these decidual (d)NK cells proliferate in situ, there is mounting evidence that dNK precursors traffic from blood to the uterus in response to hormone-derived signals. In mice, adoptive cell transplantation has demonstrated that the uterine (u)NK cell lineage can be established from peripheral progenitor cells[11]. Here, we review the recent literature about the adhesion molecules, cytokines, chemokines and hormones thought to be involved in the trafficking of NK cells to the murine uterus.

Lymphocyte Trafficking in Immune Surveillance and Inflammation

Mechanisms regulating lymphocyte homing, such as trafficking of naive T and B cells to secondary lymphoid tissues (lymph nodes (LN) and Peyer's Patches (PP)), are well described as a four step sequence of events, summarized in Figure 1[5;7;74;82]. First, a sequence of transient adhesive interactions occurs under shear force between adhesion molecules constitutively expressed on circulating lymphocytes (L-selectin) and their ligands [CD34, GlyCAM-1, podocalyxin, Sgp200, collectively known as peripheral node addressin (PNAd) and recognized by the mAb MECA-79] on specialized high endothelial venules (HEV)[67]. This causes lymphocytes to progressively slow, and roll, bringing the lymphocytes into close contact with endothelium. There, the second step, rapid activation of integrins [$\alpha_L\beta_2$ (LFA-1) or $\alpha_4\beta_7$ (L-PAM)] occurs in response to tissue-derived chemokines, enabling the third step, binding to endothelial ligands such as ICAM-1 or ICAM-2 in LN or mucosal addressin cell adhesion molecule (MAdCAM) in PP. Finally, cells undergo transendothelial migration along a chemokine gradient into tissue. Tightly regulated chemokines signals permit trafficking of specific subsets of lymphocytes into microdomains of lymphoid tissues, such that B cells migrate at follicular sites of LN and PP while T cells egress at interfollicular areas[6;14;30;59;83].

This classic homing pathway, while focusing on naive T and B cell recruitment, also applies to other leukocytes; specifically, migration of memory lymphocytes, dendritic cells, neutrophils and other leukocytes to tertiary sites in response to specific inflammation-

induced chemokine signals[63]. Inflammatory cytokines, secreted in response to injury or pathogens, simultaneously up-regulate expression of adhesion molecules such as E-selectin, VCAM-1 and ICAM-1 on vascular endothelium in the immediate area to facilitate egress of immune cells to sites of inflammation[6]. Studies on the expression, regulation and function of adhesion molecules and chemokines in homing of immune cells to the decidualizing uterus during normal pregnancy are limited.

Human Natural Killer Cells

The properties of NK cells found in human endometrium indicate that dNK cells are not simply in-transit, circulating cells. Circulating NK cells are subdivided by expression of CD3, CD16 and CD56. The majority of circulating NK cells express CD16 strongly and CD56 weakly (CD56^{dim}), but about 10% of blood NK cells have low expression of CD16 and strongly express CD56 (CD56^{bright})[66]. CD3 expressing CD56+ cells are designated NK-T cells as they also express the $\alpha\beta$ T cell receptor, thus considered T cells rather than NK cells. The CD56^{bright} subset is further differentiated from the dim subset by expression of high affinity IL-2R, higher expression of L-selectin and $\alpha_4\beta_1$ integrins, but lower expression of $\alpha_L\beta_2$ [27]. Table 1 summarizes expression of molecules on NK cell subsets.

CD56 bright and dim cells also differ in their biologic functions; CD56^{dim} cells have greater lytic ability, while CD56^{bright} cells have immunoregulatory roles mediated through secretion of cytokines[17]. NK cell function is regulated by receptors that recognize MHC class I molecules on target cells. Two structurally distinct families of NK receptors have been identified, KIRs, which are inhibitory receptors and the C-type lectin-like family (CD94/ NKG2A, NKG2D, CD69) which act as activating receptors. Human dNK cells are distinguished from peripheral blood NK cells by expression of CD56 at ten-fold higher levels, lack of expression of CD16 and reduced lytic ability[53], despite expression of a similar repertoire of activation markers[23;33]. In contrast to circulating CD56^{bright} cells that express high levels of the L-selectin homing receptor[26], dNK cells lack L-selectin[69;72;73], perhaps due to shedding during transit through endothelium[80].

Despite apparent hormonal regulation of dNK cell appearance in the uterus, hormone effects are thought to be indirect, since they do not express receptors to progesterone (PR)[39] or the predominant estrogen receptor (ERα), but do express ERβ[32]. Indeed, dNK cells do not respond to hormonal stimulation in culture by proliferation, cytokine secretion or gain in lytic ability[43]. Similarly, evidence of expression of ER or PR isoforms or luteinizing hormone receptor on blood NK cells is inconsistent.

In mammals with hemochorial placentation, which includes both primates and rodents, the maintenance of NK cells in decidualizing uterus is thought to require IL-15[41]. CD56+ cells have constitutive expression of the α β and γ chains of the IL15R[71] to drive further differentiation and proliferation[44;81]. IL-15 is synthesized by endometrial stromal cells in the decidua and its production appears to be regulated by P$_4$[58]. Transcripts of IL-15 in human perivascular endometrial stromal cells is detected primarily during the secretory phase of the menstrual cycle[44] and immediately prior to and after the onset of menses, and during the late proliferative phase[13]. In pregnancy, IL-15 mRNA is detected in endometrial stromal cells and in endometrial endothelium during the first trimester[44]. Interestingly, incubation of

CD16^{−} NK cells from peripheral blood with IL-15, results in increased expression of CD56[15] and development of a chemokine receptor repertoire similar to that found on dNK cells[31].

Potential Mechanisms for Trafficking of NK Cells to the Decidualizing Uterus

Despite the reported paucity of NK cell migration to secondary lymphoid tissues[8], circulating CD56^{bright} cells express the necessary adhesion molecules and chemokine receptors to support homing to across HEV in secondary lymphoid organs (Figure 2, Table 1). CD56^{bright} cells have been found at low frequency in peripheral LN where they are speculated to provide a physiological link between adaptive and innate immune response s through secretion of IL-2[26]. Like other immune and inflammatory cells, NK cells leave the circulation to enter tissues through interactions with the vessel wall under flow conditions to arrest and transmigrate. NK cells respond to a variety of cytokines and chemokines, including IL-12, IFNα and β, CCL2, 3, 4, 5, 7, 8, CXCL8, and CX3CL1[9;42]. Thus, it is plausible that L-selectin, $\alpha_4\beta_1$, $\alpha_4\beta_7$, and $\alpha_L\beta_2$ integrins expressed by NK cells function during trafficking of NK cells or their precursors to the decidualizing uterus. We have taken advantage of the ability of adhesion molecules to recognize ligands across species to investigate trafficking potential of both human and murine NK cells using mouse tissue-based cell adhesion assays.

MURINE INVESTIGATIONS

Mice have a short 5 day estrous cycle and no post-ovulatory decidualization. Uterine decidualization is associated with blastocyst implantation at gestation day 4[54;68]. Implantation and primary uterine decidualization occur on the anti-mesometrial side of the uterus, the side opposite the major vascular connections to the uterine artery[54;68]. Decidualization rapidly moves towards the mesometrial side of the uterus and at gestation days 5–7 involves the entire implantation site. With subsequent growth of the fetus, most of the anti-mesometrial decidua is lost and the mesometrial decidua that remains associated with the developing placenta is given the term decidua basalis. An influx of NK cells, considered to be immature because they lack cytoplasmic granules, is observed within 24 hr of implantation[61]. These cells are first detected in the secondary, mesometrial decidua. Unlike their human counterparts, murine NK cell subsets have been discriminated by morphology and carbohydrate biochemistry rather than by immune surface phenotype[61]. By gestation day 6, large numbers of uNK cells are present, they are rapidly proliferating and acquiring their mature appearance of very large, heavily granulated lymphocytes. By gestation day 8, a few uNK cells are dying and by gestation day 12, cell division has ceased and most uNK cells are degenerating[20;61].

Functions of uNK Cells

Histological comparisons of implantation sites in alymphoid (NK[−], T[−], B[−]) mice to those in T and B cell deficient mice and to those in normal mice suggested that the NK cell influx contributed to maintenance of a fully differentiated decidua. Perhaps more importantly, uNK cells appeared to be essential for induction of the normal, pregnancy-associated structural changes in the convoluted arteries supplying placentae, that are called spiral arteries[29]. This

conclusion was confirmed by adoptive cell transfer from T and B cell deficient SCID mice to alymphoid mice which established the uNK cell lineage and function. Thus, in mice, NK cells are essential for normal vascular development of implantation sites[28]. Furthermore, the effects of uNK cells on the vasculature appeared to be mediated through uNK cell production of IFNγ since structural changes to vessels observed when pregnant alymphoid mice were reconstituted with NK cells could be fully replicated in alymphoid mouse pregnancies by cell free injections of mrIFN [2].

It is not clear whether uNK cells activate their terminal differentiation and cytokine production programs prior to, coincident with, or following their arrival in the decidualizing uterus. By gestation day 6 pregnancy-induced gains in expression of LY49 inhibitory receptors on uNK cells is complete [60]. However, transcription factors Eomes and t-bet which regulate expression of IFNγ, continue to rise as uNK cells differentiate from small, agranular cells (gd6) to mature, heavily granulated cells [78]. Uterine NK cell-derived IFNγ can be demonstrated by gestation day 6 and accounts for ~90% of the IFNγ produced in decidua basalis[1]. In addition to providing the IL-15 survival signal, decidualized mouse uterus also provides redundant pathways of regulatory signaling (ie, IL-12, 18, 23 and 27) for induction of IFNγ[85], suggesting that entry to the uterus triggers uNK cell function.

Since the effects of uNK cells are mediated through IFNγ regulated genes in target cells, the midgestation distribution of murine uNK cells suggests that there are multiple target cell types with differing regulated genes. About 10% of midgestation uNK cells appear in the lumens of the spiral arteries, suggesting endothelium is a target. Another 25% are embedded within the vascular smooth muscle layers of the decidual arteries, suggesting smooth muscle cells are major targets. Most of the remaining cells are associated with decidual stroma and matrix, suggesting fibroblast cells are also targets. Many uNK cells in the decidua basalis are associated with larger, irregularly shaped cells that express PR (Figure 3)[55]. The identity of these PR+ cells is not yet known but the positional relationships are reminiscent of those with DC-SIGN+ dendritic cells in human endometrium and suggest regulatory interactions[35]. Murine uNK cells are also found at low frequency within the placenta, where fetally-derived trophoblast cells are potential targets for IFNγ regulated changes in gene transcription[62;76].

Sources of uNK Cell Progenitors

Murine uterine horn segment transplantation was used to determine that normal uterus (ie the graft) did not contain resident uNK precursor cells[11]. A uterine horn segment from a normal mouse was anastomosed into the horn of a mouse genetically deficient in NK and uNK cells, prior to breeding of the host mouse. During the resultant pregnancy, the graft decidualized but developed no uNK cells. Uterine NK containing grafts were found when the recipients were normal mice, indicating that precursors of uNK cells traffic from the circulation to the uterus at decidualization. Sources of uNK cell progenitors was further investigated *in vivo*, using adoptive transfer of cells from bone marrow, thymus, neonatal liver, spleen, and peripheral and mesenteric LN from virgin and pregnant wildtype (B6) mice into NK cell deficient mice[11]. All tissues, with the exception of LN that drain the pelvic organs (collected from pregnant donors), were able to re-constitute the uNK cell lineage.

Splenic cells derived from pregnant donors were the most competent source of uNK precursors. These results suggested a role for pregnancy or the hormones of pregnancy in regulating uNK cell precursor trafficking from reservoirs such as the spleen into the circulation and their retention in the uterus.

Adhesion Molecule Involvement in uNK Cell Recruitment to Decidua Basalis During Pregnancy

Since immune cells during pregnancy display many immunological markers associated with immune surveillance and inflammation (increased CD11b, CD14, and CD69 as well as increased intracellular reactive oxygen species), we postulated that molecules known to orchestrate leukocyte trafficking to inflamed sites also contribute to leukocyte homing to the uterus during early pregnancy[24;25;70]. The murine decidua basalis hosts an elegantly orchestrated succession of maternal leukocyte and fetal trophoblastic homing events that appear to be directed by vascular addressins on decidual endothelium. NK cells are found mainly in the central decidua basalis where the vascular endothelium expresses VCAM-1, a ligand for the $\alpha_4\beta_1$ integrins present on uNK cells. However, MAdCAM-1 (a ligand for $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins) PNAd, E-selectin and P-selectin were not found in the central decidua basalis using immunohistochemical techniques[45]. Endothelium at the leading edge of invading trophoblast expresses E-selectin, and attracts neutrophils from the circulation. The vascular zone, which separates the decidua basalis from the anti-mesometrial decidua expresses MAdCAM-1 and P-selectin and hosts monocytes. Thus, subcompartments within implantation sites in pregnant mice are differentially regulated to express highly specific patterns of adhesion molecules that would selectively recruit different leukocyte subsets from the circulation.

Hormone Effects on Endothelium

To investigate the relative roles of adhesion molecules in mediating trafficking to decidualizing uterus, we assessed lymphocyte adhesion to frozen uterine tissues collected from virgin, pregnant or ovariectomized, hormonally treated B6 mice, in *in vitro* assays[11;12]. Gestation or treatment of ovariectomized mice with estradiol (E_2), or P_4, or E_2+P_4 promoted gains in adhesive function by endothelial cells in an organ-restricted manner. These gains of function occurred in the central decidua basalis of the uterus and in specialized HEV of secondary lymphoid tissues (LN and PP) but not in squamous epithelium of extralymphoid tissues (pancreas)[11]. Interestingly, these gains were not seen if lymphocytes were pre-treated with function-blocking antibodies to L-selectin or $\alpha4$ integrin[12], the ligands of which (PNAd and MAdCAM, respectively) are reported to be absent from the decidua basalis[45]. We speculate either that vascular addressins are functionally displayed on decidual vessels at levels below detection by immunohistochemical techniques or that ligands activated or modified by pregnancy hormones or their products are not recognized by monoclonal antibodies.

Human lymphocytes or murine cell lines with known adhesion molecules were used as indicator cells for these assays. When human NK cells were pre-labelled with a fluorescent antibody to CD56, we found CD56^{bright} cells adhered only to the decidua basalis and were preferentially enriched amongst adherent cells (70% of adherent cells were CD56^{bright}

although the starting suspensions contained less than 3% CD56^{bright} lymphocytes). This NK subset is characterized by extraordinarily high levels of L-selectin, as compared to naïve T and B lymphocytes, neutrophils, monocytes and CD56^{dim} lymphocytes[26]. Both pregnancy and the pregnancy–related hormones enhanced lymphocyte-endothelial interactions. Function-blocking antibodies to PNAd/L-selectin or MAdCAM-1/VCAM/α4 established that observed pregnancy-related enhancement in NK cell adhesion were mediated through these molecules, but the extracellular matrix protein, fibronectin, (which contains integrin binding sites) did not contribute to CD56+ cell adhesion[11;12]. Although pregnancy increased the total number of CD56^{bright} cells binding to HEV of lymphoid organs, no enrichment of this subset was observed relative to the total leukocyte population. These findings, together with evidence that identical molecular interactions between lymphocytes and endothelium are regulated by both pregnancy or pregnancy-associated hormones strongly suggests that decidual endothelium is a unique, extralymphoid site specialized for recruitment of NK cells. Our histological observations that uNK cells appear to be in transit across endothelium in arteries and are localized in large numbers around arteries, but not veins in rodent implantation sites raises the possibility that unique sites of extravasation are involved in uterine trafficking (Croy, unpublished). Intravital microscopy is required to establish the direction of transit through these vessels.

Hormone Effects on Lymphocytes

In concert with endothelial changes in response to pregnancy and pregnancy hormones, a coordinated change in adhesive capability of lymphocytes from treated mice was observed. Adhesion by splenocytes from pregnant and hormone-treated ovariectomized mice was significantly higher than by splenocytes from virgin or ovariectomized and-placebo treated mice on a common LN substrate. The enhanced adhesion was blocked by pre-treatment of the splenocytes with function blocking antibodies to L-selectin[12]. A role for L-selectin is further supported by recent studies showing that lymphocytes from L-selectin-deficient mice exhibit impaired short-term trafficking (0.5–1 h) to lymphoid and uterine tissues in pregnant animals[84]. It is tempting to speculate that pregnancy or their associated hormones activate receptors on lymphocytes such that they increase in trafficking potential to uterus and secondary lymphoid organs.

Role of Chemokines and their Receptors in NK Cell Homing to the Uterus

Individualized patterns of chemokine receptor expression have been described on NK cell subsets from blood[9;34;46] as well as from decidua [31;46;64] and these are summarized in Figure 2. Histological and morphometric analyses of implantation sites in mice genetically deleted for the chemokine receptors CCR2 and CCR5 (ligands MCP-2, MIP-1β and RANTES) showed no alteration in uNK cell numbers, positioning or function (assessed as spiral artery modification) when these chemokine mediated pathways were eliminated[10]. The chemokine receptor CXCR3 contributes to leukocyte chemotaxis and is expressed on a wide variety of lymphocytes including human CD16^{−}CD56^{bright} NK cells and dNK cells[31] and mouse uNK cells[84]. Compared with CD16^{+}CD56^{dim} NK cells, CD16^{−}CD56^{bright} NK cells express higher levels of CXCR3[8] and respond more vigorously to its ligands, Mig (CXCL9) and IP-10 (CXCL10) which are present in decidua[31]. Since these chemokines are IFNγ induced, it seems probable that uNK cells promote their own enrichment in the

decidua, through secretion of IFNγ. In mice, genetic loss of CXCR3 function resulted in fewer NK cells only in the mural aspect of the decidua basalis, a microdomain normally enriched in more immature, proliferating uNK cells[84]. This was associated with some but not total impairment of spiral artery modification. These studies, summarized in Table 2, suggest that L-selectin-based interactions may initiate homing of leukocytes while CXCR3-based interactions participate in positioning of uNK cells within the uterine microdomains during early pregnancy.

PREGNANCY HORMONES, DECIDUALIZATION AND CHEMOTAXIS

Effects of Progesterone On Stromal Cells, Endothelial Cells and Lymphocytes

Uterine decidualization is dependent upon P_4 activation of E_2 primed endometrial stroma[50]. Progesterone also regulates decidual production of cytokines such as IL-15[58;81], leukemia inhibitory factor (LIF), required for implantation and IL-11 which contributes to further decidualization in an autocrine manner[21;65]. Progesterone induces stromal cell production of CXCR3 ligands, CXCL10 and CXCL11 at the time NK cells are known to accumulate at implantation sites (Figure 3)[42]. Chemokine receptors which are greatly up-regulated in decidua during the luteal (P_4 dominant) phase include CXCR1, CCR2b and CCR5[22]. Protein products such as renin, prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) and extra-cellular matrix proteins, laminin and fibronectin, established as a peri-cellular rim, are also produced by decidua under the influence of P_4 to facilitate implantation and invasion of trophoblast[36]. Thus major roles for P_4 include induction of decidualization, induction of chemokines capable of recruiting NK cells and induction of IL-15 to drive and maintain terminal uNK cell differentiation.

Effects of Estradiol on Endothelial Cells and Lymphocytes

The ovarian hormone estrogen exerts effects through interactions with ER found in many cell types, including endothelial cells throughout the cardiovascular system. Estradiol induces vasodilation or relaxation in vessels[52;77]. These effects are particularly profound in the pregnant uterus, where uterine arteries elongate and become tortuous while undergoing major dilation with the result of having increased flow of maternal nutrients to the feto-placental unit. A direct role of E_2 in NK cell activation and/or differentiation is controversial. Bone marrow transplant experiments in which alymphoid mice received grafts from mice deficient in ERα or ERβ showed apparently normal differentiation of the uNK cell lineage and its full function as assessed by spiral artery modification[3], indicating that E_2 was not essential to recruitment or differentiation of uNK cells.

Model of dNK Precursor Cell Recruitment to the Human Uterus

From information provided by murine models, human uterine immunohistology and in situ hybridizations and assays of human blood and uterine NK cell functions, a model of redundancy in cell origin and in recruitment pathways has emerged for uNK cells. To date, the only non redundant processes defined are the absolute requirement for IL-15 in early uNK cell differentiation, the absolute requirement for co-differentiation of decidua and the need for uNK cell derived IFNγ to effect spiral artery modification. Figure 4 demonstrates our proposed model of uNK cell trafficking; under the influence of hormones or hormone-

derived factors uNK cell precursors enter the circulation from various lymphoid reservoirs, particularly spleen during pregnancy. Once circulating, CD56^{bright} cells enter LN where they interact with other immune cells, priming them to respond to chemotactic signals from the decidualizing uterus/conceptus. Egress from blood vessels is accomplished using classic adhesion molecules, which includes but is not restricted to L-selectin and α_4 integrin, to adhere to endothelium in the decidua. It appears probable that at implantation, this initially occurs anti-mesometrially at the site of implantation and first decidualization, but that as decidualization rapidly progresses, entry is restricted to the decidua basalis by gestation day 6. Final uNK cell positioning in the decidua and mural regions is effected by CXCR4 and CXCR3 molecules respectively on NK cells.

We conclude that trafficking of pro-uNK cells to the uterus utilizes well-established trafficking molecules in response to a unique set of stimulating molecules consisting of hormones (or their induced factors) and specific combinations of chemokines to correctly attract and position uNK cells within the uterus. Aberrant regulation of these pathways may contribute to implantation failure or arrest and subsequent failure of developing conceptuses.

Acknowledgments

Abbreviations

B6	Wildtype C57BL6 mice
dNK	decidual Natural Killer cells of human pregnancy
E$_2$	estradiol
ER	estrogen receptor
HEV	high endothelial venule
ICAM-1	intercellular adhesion molecule-1
LFA-1	leukocyte function associated molecule -1
LIF	leukemia inhibitory factor
LH	luteinizing hormone
LN	Lymph Node
mAb	monoclonal antibody
MAdCAM	mucosal addressin cell adhesion molecule
NK	Natural Killer cell
PNAd	peripheral lymph node addressin

| PP | Peyers Patches |
P_4	progesterone
PR	progesterone receptor
uNK	uterine NK cell of mouse pregnancy
VCAM-1	vascular cell adhesion molecule-1

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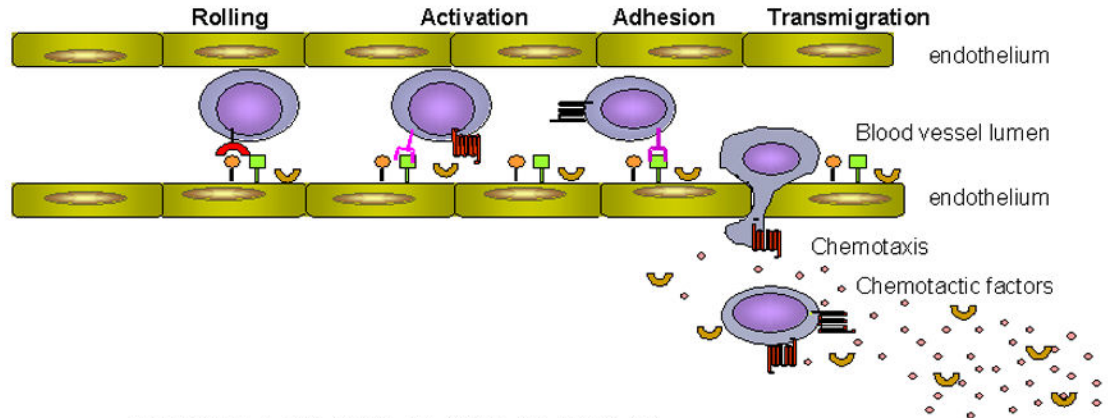
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Figure 1.
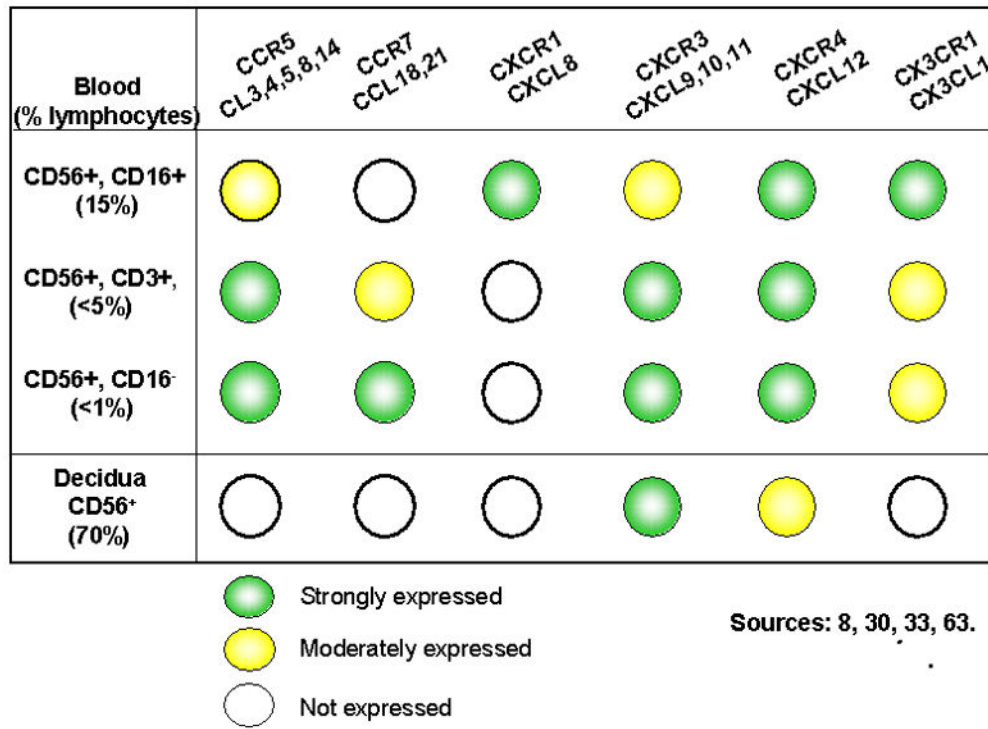Lymphocyte Extravasation into Tissues

Figure 2.
Chemokine Receptors on CD56+ cell subsets
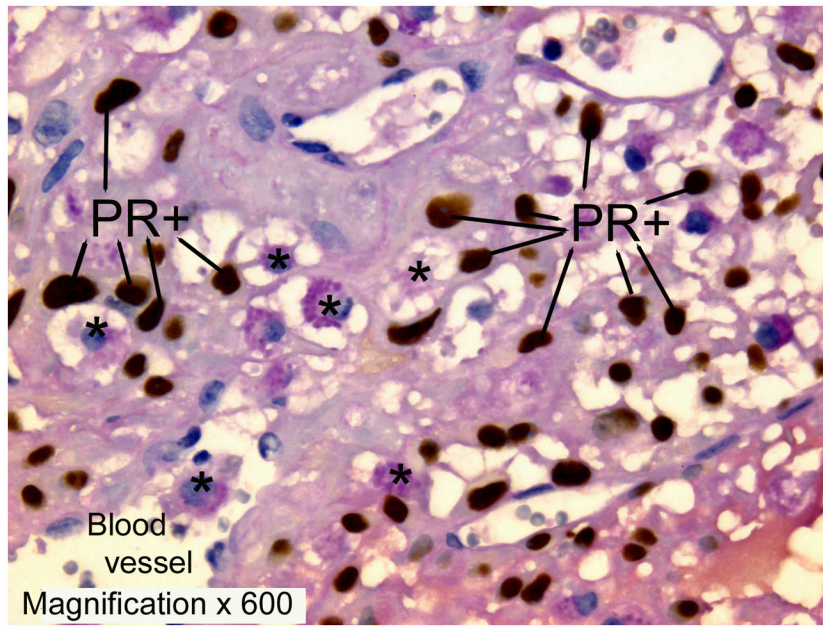
Figure 3.
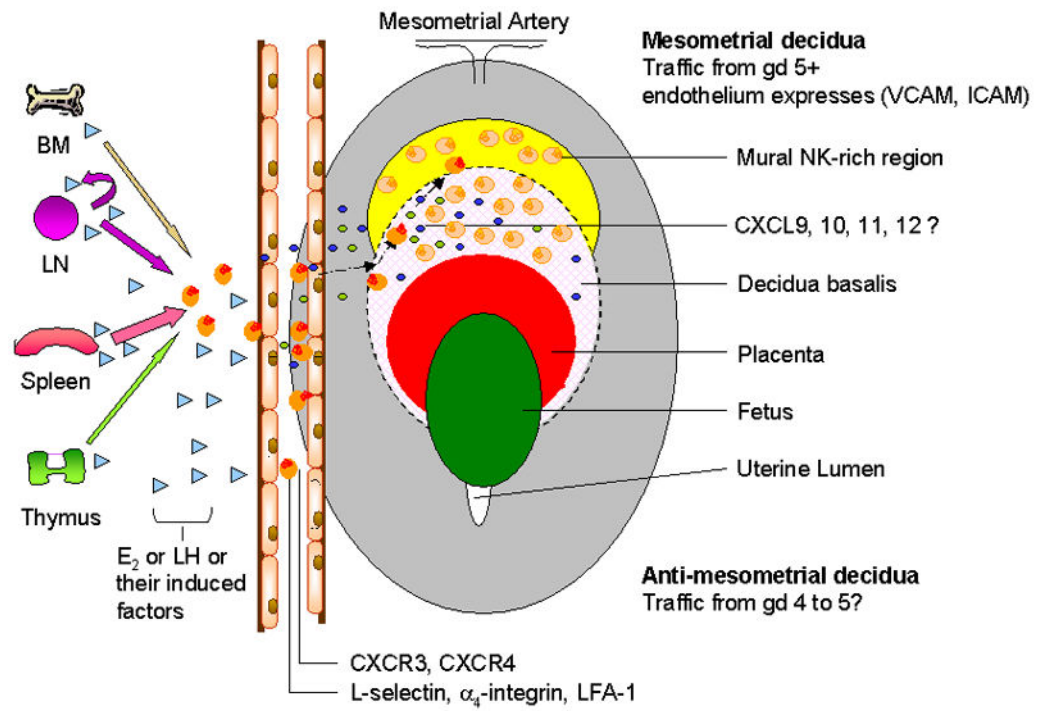
Figure 4.

Table 1

Expression of surface molecules on NK cell subsets

Molecule	Blood		UNK
	CD56^{Dim}	CD56^{Bright}	CD56^{Bright}
CD2	✓	✓	✓
CD11a (LFA-1)	✓ ✓	✓	✓
CD16	✓	×	×
CD18 (β2)	✓	✓	✓
CD29 (β1)	✓	✓	✓ ✓
CD49a (α1)	✓	✓	✓ ✓
CD49d (α4)	✓	✓	✓ ✓
CD57	✓	✓ ✓	×
CD62L	✓	✓ ✓ ✓	×
CD69	×	×	✓
CD94	✓	✓ ✓	✓ ✓
KIR	✓	×	✓ ✓
IL 2Rβ	✓	✓ ✓	✓ ✓
c-kit	×	✓	✓

References:[4;16;38;56;57;79]

Table 2

Effect of gene deletion on murine uNK cell homing, distribution and function at gestation day 10.

Deleted Gene Products	uNK Cell Density	uNK Cell Positioning	Spiral Artery Modification	Reference
Trafficking Molecules				
L-selectin	Reduced <6h	Normal	Normal	[84]
CCR2	Normal	Normal	Normal	[10]
CCR5	Normal	Normal	Normal	[10]
CXCR3	Reduced	Deficit in MLAp	Slightly impaired	[84]
Activation Molecules				
IL-12 p40 (B6)	Normal	Normal	Impaired	[18]
IL18 (B6)	Normal	Normal	Impaired	[85]
IL-12–/–/18–/– (B6)	Normal	Normal	Impaired	
IL-15 (B6)	No uNK cells	No uNK cells	None	[1]