

# Migration of NK cells

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## Introduction

Natural killer (NK) cells belong to a distinct lineage of lymphocytes that play an important role in the early phase of immune responses against certain microbial pathogens by exhibiting cytotoxic functions and secreting a number of cytokines and chemokines.

NK cells develop from a common lymphoid precursor resident in the bone marrow (BM) that is considered the main site of their generation. The BM microenvironment provides a rich source of cytokines and growth factors and allows an intimate contact between developing NK cells and stromal cells, which is required for their full maturation [1]. However, final maturation of BM-derived NK cell precursors has been suggested to occur also at the periphery [2].

Mature NK cells mainly circulate in the peripheral blood, but are also resident in several lymphoid and non-lymphoid organs such as spleen, tonsils, liver, lungs, intestine and uterine decidua. In addition, homing to lymph nodes of a particular subset of activated NK cells has been described both in human and mouse [3, 4].

During viral infections, inflammation, tumor growth and invasion, NK cells are rapidly recruited from the blood and accumulate in the parenchyma of injured organs [1, 5, 6], where activated NK cells can kill target cells and release inflammatory cytokines and chemokines, thus participating in the recruitment and activation of other leukocytes and in the modulation of dendritic cell (DC) function.

Unlike B cells and T cells that express a single antigen-specific receptor, NK cells are endowed with a multiple cell surface receptor system encoded by genes that do not undergo recombination or sequence diversification. This complex receptor system is acquired during NK cell development, and consists of both activating and inhibitory receptors [7, 8].

The best studied among the activating receptors is the low-affinity Fc-receptor  $\gamma$  IIIA (CD16) that is responsible for antibody-dependent cellular cytotoxicity (ADCC) and allows NK cells to participate in the elimination of antibody-coated target cells [1]. Among the receptors capable of triggering natural killing, recent evi-

dence underscores the relevance of the C-type lectin family NKG2D receptor that recognizes the MHC class I-related A and B proteins (MICA and MICB) and the members of a family of proteins named UL16-binding proteins (ULBP) [9]. These ligands are mainly expressed on the surface of tumor cells of different histotypes, and infected or stressed cells, and are induced in response to DNA damage [10]. Other activating receptors, i.e., NKp46, NKp44, and NKp30, are Ig-like molecules and belong to the natural cytotoxicity receptor family, but their ligands are still unidentified [11].

In addition, NK cells express a number of receptors acting as activating or costimulatory molecules such as CD2, CD244 (2B4), NKp80,  $\beta_1$  and  $\beta_2$  integrins and DNAM-1 (CD226). Interestingly, DNAM-1 is associated with the  $\beta_2$  integrin LFA-1 and binds to the poliovirus receptor (PVR, CD155) and the nectin-2 (CD112), two members of the nectin family involved in the regulation of cell-cell interaction and leukocyte extravasation [12, 13].

NK cell functions are tightly regulated by inhibitory receptors that specifically interact with MHC class I antigens. In the human, they belong to two distinct groups: the killer cell Ig-like receptor (KIR) family that comprise molecules binding to groups of human leukocyte antigen (HLA)-A, -B, -C alleles, and the C-type lectin receptors (i.e., CD94/NKG2A) specific for the widely expressed non-classic HLA-class I molecule, HLA-E. Both receptor families include activating counterparts with similar specificity, but different ligand affinity. The functional role of these activating receptors as well as the identity of their ligands are at present, quite obscure.

Based on the receptor complexity, NK cell functions are thus the result of concomitant engagement of various activating and inhibitory receptors by the particular set of ligands on target cells. However, in most instances the inhibitory signals override the triggering ones [14].

All the receptors expressed by NK cells are not unique to this cell type, but are also present on cells of other lineages such as T cells or myeloid cells. The expression on NK cells is highly regulated, and some receptors are oligoclonally distributed or expressed on subsets of NK cells. Unlike peripheral blood human NK cells, some tissue-resident NK cells do not express CD16, but show high levels the NCAM adhesion molecule, CD56; in addition, CD16 and CD56 receptors can be expressed at different density on circulating blood NK cells.

Based on the receptor repertoire and surface receptor levels, phenotypically distinct NK cell populations have been identified, and suggested to represent specialized subsets capable of performing different functions and endowed with distinct migratory properties. Two major subsets of human peripheral blood NK cells have been described: the majority (about 90%) are CD56<sup>low</sup>CD16<sup>high</sup>, whereas about 10% of NK cells are CD56<sup>high</sup>CD16<sup>low</sup>. It has been proposed that CD56<sup>high</sup> NK cells have a unique functional role in the innate immune response as primary source of NK cell-derived immunoregulatory cytokines, whereas the CD56<sup>low</sup>CD16<sup>high</sup> subset represents the principal cytotoxic population [15].

It is still matter of debate as to whether these different NK cell populations represent functionally distinct subsets of mature NK cells, or whether CD56<sup>high</sup> NK cells are terminally differentiated cells indistinguishable from mature NK cells recently activated in response to cytokines such as IL-12 [16].

## NK cell adhesion molecules and chemokine receptors

The ability of leukocytes to traffic coordinately throughout the body is an essential requirement for the maintenance of immunosurveillance. NK cell migration across endothelium, as for other leukocytes, is a spatially and temporally integrated multi-step process regulated by a plethora of chemoattractants and adhesive molecules belonging to the selectin, integrin, and Ig families, as well as chemokines [17, 18].

Among adhesion molecules, both selectins and integrins contribute to the initial leukocyte tethering and rolling along vessel endothelium, while firm adhesion of the leukocyte to vascular endothelium and subsequent diapedesis into the underlying extravascular tissue is mainly mediate by integrins. The various steps of migration are tightly regulated; in fact, for migration to be effective, adhesion receptors must undergo cycles of attachment and detachment from their endothelial ligands.

Chemokines are a superfamily of inflammatory mediators that properly guide leukocyte recruitment and positioning into healthy or diseased tissues by interacting with seven-transmembrane-domain receptors and initiating complex signaling events that govern leukocyte migration, not only by eliciting a chemotactic response but also through a dynamic regulation of integrin adhesiveness for endothelial and extracellular matrix ligands [19–21]. Integrins can also regulate cell migration by initiating similar intracellular signal transduction pathways [22, 23].

## Adhesion molecules

In regard to selectin receptor family, human NK cells express L-selectin (CD62L), a molecule involved in the initial adhesion of leukocytes to peripheral lymph node high endothelial venules (HEV) [24, 25]. L-selectin has been found to be uniquely expressed on the CD56<sup>high</sup> subset of peripheral blood human NK cells at a density higher than that of all other peripheral blood leukocytes, including CD56<sup>low</sup> NK cells. NK cell activation results in modulation of L-selectin expression depending on the stimulus: phorbol esters, IL-2, IL-15, and TGF- $\beta$  down-regulate L-selectin on CD56<sup>high</sup> NK cell subset, whereas increased levels can be observed on both NK cell subsets in response to IL-12, IL-10, and IFN- $\alpha$ . In accordance with these observations, CD56<sup>high</sup> NK cells bind to the physiological L-selectin ligands on peripheral lymph node HEVs with higher efficiency as compared to the CD56<sup>low</sup> subpopulation, thus resulting in a selective advantage of this population in extravasation

across HEV [26]. There is also evidence from Uksila et al. [25] showing that a portion of CD16<sup>+</sup> NK cells express L-selectin, and that IL-2 treatment diminishes the expression of this molecule and concomitantly increases the levels of  $\alpha_4$  integrin and CD44, two major receptors involved in lymphocyte binding to mucosal HEV. Thus, IL-2 activation of NK cells decreases adherence to peripheral LN HEV, while increasing adherence to mucosal HEV.

NK cells can also express selectin ligands such as the sialyl stage-specific embryonic antigen 1, sialyl-Lewis<sup>x</sup> (sLe<sup>x</sup>) ligand and P-selectin glycoprotein ligand-1 (PSGL-1), which can bind to E- and P-selectin under static and flow conditions; this binding is up-regulated by IL-12 [27–30]. There is also evidence indicating that the sulfated lactosamine epitope expressed selectively on CD56<sup>low</sup>CD16<sup>+</sup> NK cells, PEN5, is a carbohydrate decoration of PSGL-1 that confers to PSGL-1 the ability of binding to L-selectin [31]. These results suggest that PEN5-L-selectin pair may promote cell-cell interactions and amplify the accumulation of NK cells at site of inflammation.

On the NK cell surface, another carbohydrate modification of PSGL-1, CLA has been also found, which is a marker for tissue infiltrating leukocytes. Notably, expression of PEN5 and CLA on NK cells is mutually exclusive, suggesting that distinct NK cell subsets exhibit different trafficking properties [31].

In regard to integrins, human NK cells express various members of the  $\beta_1$ ,  $\beta_2$  and  $\beta_7$  families. Among the  $\beta_1$  integrins, freshly isolated peripheral blood NK cells express  $\alpha_5\beta_1$  and  $\alpha_4\beta_1$  as fibronectin and VCAM-1 receptors, and  $\alpha_6\beta_1$  as laminin receptor [32]. The pattern of  $\beta_1$  integrin expression changes upon NK cell activation, in that activated NK cells acquire  $\alpha_1\beta_1$  and  $\alpha_2\beta_1$  integrins, and down-regulate the expression of  $\alpha_6\beta_1$  [24, 33–35].  $\alpha_4\beta_1$ -VCAM-1 adhesive pathway is involved in the adhesion and migration of resting or IL-2-activated NK cells across IL-1 $\beta$ -, IFN- $\gamma$ -, TNF- $\alpha$ -activated, but not resting, endothelial cells. Integrin-mediated NK cell interaction with endothelial cells is characterized by a peculiar structural feature: the formation of podosomes that represent dot-shaped protrusions of the cellular ventral membrane provided with adhesive properties and formed by particular cytoskeletal architecture [36]. Recent evidence indicates that the interaction of  $\alpha_4\beta_1$  integrin with VCAM-1 on porcine endothelial cells is also required for both rolling and firm adhesion of human NK cells to porcine endothelial cells [37].

$\alpha_4$  integrin subunit can also associate with another  $\beta$  chain, the  $\beta_7$ , to give a functionally distinct integrin receptor capable of binding the mucosal vascular addressin MAdCAM-1.  $\alpha_4\beta_7$  is expressed on NK cells and mediates NK cell binding to mucosal HEV [25, 38, 39]. Functional evidences indicate, however, that NK cells expressing both  $\alpha_4\beta_7$  and  $\alpha_4\beta_1$  bind well to VCAM-1 but poorly to MAdCAM-1, suggesting that regulation of MAdCAM-1 *versus* VCAM-1 expression might critically control the recruitment of NK cell subsets to distinct tissues [40].

NK cells express all members of the  $\beta_2$  integrin family (CD11a–d/CD18), which are leukocyte-associated adhesion molecules mainly involved in the regulation of

cell-cell interactions [41]. The leukocyte function-associated antigen 1 (CD11a/CD18 also known as LFA-1) is the receptor for the intercellular adhesion molecules (ICAM-1, 2, 3), and plays a crucial role in mediating NK cell adhesion to target cells as well as NK cell binding and transmigration across endothelial cells [36]. The expression and function of  $\beta_2$  integrins on NK cells is highly regulated. In this regard, it has been shown that the levels of LFA-1 are higher on the CD56<sup>low</sup> subset compared with the CD56<sup>high</sup>, while  $\alpha_M\beta_2$  (CD11b/CD18 also known as Mac-1) and  $\alpha_X\beta_2$  (CD11c/CD18) integrins are expressed on all and one-half of NK cell population, respectively [25, 26]. NK cell activation by cytokines, such as IL-2 or IL-12, results in up-regulation of LFA-1 expression and function, while CD11b and CD11c are down-regulated [24, 42] (Tab. 1).

The differential expression of adhesion molecules on NK cells together with quantitative and qualitative regulation of integrin expression and function occurring following NK cell activation, can be responsible for the recruitment of specialized NK cell subsets during inflammation.

### Chemokine receptors and chemokine-induced *in vitro* NK cell migration

A large body of evidence indicates that NK cells can express several receptors for CXC, CC, C and CX3C chemokines, with a great heterogeneity in the chemokine receptor repertoire among different NK cell populations and between resting *versus* activated NK cells.

With respect to the CXCR and CX3CR families, it has been previously reported that human peripheral blood NK cells express both CXCR1 and CXCR2 as CXCL8 (IL-8) receptor [43–45] and CX3CR1 as CX3CL1 (fractalkine) receptor [46, 47]. These observations have been further extended by Campbell and colleagues [48] who provided the first evidence that distinct (CD56<sup>+</sup>CD16<sup>+</sup> and CD56<sup>+</sup>CD16<sup>-</sup>) peripheral blood NK cell subsets have a unique repertoire of chemokine receptors. CD16<sup>+</sup> NK cells uniformly express high levels of CXCR1 and CX3CR1, low levels of CXCR2 and CXCR3 and no detectable levels of CXCR5. By contrast, CD16<sup>-</sup> NK cells express high levels of CXCR3, low levels of CX3CR1, and are negative for CXCR1, CXCR2 and CXCR5; moreover, both NK cell subsets express high levels of CXCR4, the receptor for CXCL12 (SDF-1 $\alpha/\beta$ ). With respect to the CC chemokine receptor family, these authors found that the majority of NK cells lack the expression of CCR1-7 and CCR9, and only the CD16<sup>-</sup> NK cell subset expresses high levels of CCR5 and CCR7, the latter molecule mainly involved in the homing of lymphocytes to secondary lymphoid organs [48].

Consistent with this expression profile, CXCL8 (IL-8) and soluble CX3CL1 (fractalkine) preferentially attract the CD16<sup>+</sup> NK cell subset which can also respond moderately to the CXCR3 ligands, CXCL11 (I-TAC) and CXCL10 (IP-10); by contrast, CD16<sup>-</sup> NK cells respond more dramatically to the CCR7 ligands, CCL19

Table 1 - Adhesion molecules and chemokine receptor expression on peripheral blood NK cell subsets

	CD56 <sup>low</sup> CD16 <sup>high</sup>	CD56 <sup>high</sup> CD16 <sup>low</sup>
CD62L (L-selectin)	+ <sup>a</sup>	++ <sup>b</sup>
PSGL-1/PEN5	+	- <sup>c</sup>
PSGL-1/CLA	-	+
CD49dCD29 ( $\alpha$ 4 $\beta$ 1)	+	+
CD49eCD29 ( $\alpha$ 5 $\beta$ 1)	+	+
CD49fCD29 ( $\alpha$ 6 $\beta$ 1)	+	+
CD49d $\beta$ 7 ( $\alpha$ 4 $\beta$ 7)	+/- <sup>d</sup>	+
CD11aCD18 ( $\alpha$ L $\beta$ 2)	++	+
CD11bCD18 ( $\alpha$ M $\beta$ 2)	+	+
CD11cCD18 ( $\alpha$ X $\beta$ 2)	+/-	+/-
CXCR1	++	-
CXCR2	+	-
CXCR3	+	++
CXCR4	++	++
CCR1	-	-
CCR2	-	-
CCR3	-	-
CCR4	-	-
CCR5	-	++
CCR6	-	-
CCR7	-	++
CCR9	-	-
CX3CR1	++	+

<sup>a</sup>indicates intermediate levels of expression

<sup>b</sup>indicates high levels of expression

<sup>c</sup>indicates undetectable levels of expression

<sup>d</sup>indicates low levels of expression

(ELC/MIP-3 $\beta$ ) and CCL21 (SLC), as well as to the CXCR3 ligands, CXCL11 (I-TAC) and CXCL10 (IP-10), and poorly to a CCR2 ligand, CCL2 (MCP-1), or CCR5 ligands, CCL4 (MIP-1 $\beta$ ) and CCL5 (RANTES). Both NK cell subsets strongly migrate in response to the ligand for CXCR4, CXCL12 (SDF-1 $\alpha$ / $\beta$ ) [48, 49].

Moreover, Kim et al. [50] have found that CD56<sup>high</sup> CD16<sup>-</sup> cells respond more than CD56<sup>low</sup> CD16<sup>+</sup> cells to CCL21 (SLC) and CCL19 (ELC) when used at high

concentrations, although they observed that the two NK cell subsets express equal levels of CCR7mRNA.

As above mentioned, the expression of chemokine receptors on NK cells may be modulated upon cytokine stimulation. A significant decrease of CXCR3 expression on NK cells treated for 6 or 24 h with IL-2 and IL-12 alone or in combination has been reported, and the decreased expression was associated with reduced chemotaxis to CXCL10 (IP-10). The same treatment did not affect the expression of other chemokine receptors such as CCR1, CCR2 or CXCR4 [51]. However, previous reports have shown that short-term exposure of freshly isolated NK cells to IL-2 can positively modulate CCR2 mRNA expression [52], and long-term (8–10 days) stimulation of NK cells with IL-2 results in increased expression of CCR1, CCR2, CCR4, CCR5 and CCR8 [53]. In agreement with these observations, IL-2-activated NK cells can migrate in response to many CC chemokines, such as CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), CCL5 (RANTES), and CCL22 (MDC) [53–56].

A recent report indicates that NK cell treatment with IL-18, differently from IL-2, results in selective induction of CCR7 expression on the CD56<sup>low</sup> NK cell subset but not affects CCR7 expression on the CD56<sup>high</sup> subset; increased expression of CCR7 on CD56<sup>low</sup> NK cell subset is associated with reduced levels of CD16 and enhanced capability to migrate in response to the lymph node-associated chemokine CCL21 (SLC) [57] (Tab. 1).

## Signaling events controlling chemokine-induced integrin-supported NK cell migration

Despite the increasing evidence on the prominent role of chemokines and integrins in the dynamic regulation of leukocyte adhesion and migration, the signaling pathways responsible for the integrin-supported leukocyte migration elicited by chemokines are not yet completely defined. The propagation of the migratory signals depends on a complex interplay among molecules that regulate actin, myosin and other cytoskeleton components, and results in the formation of protrusive structures at the front of migrating cell and retraction at cell rear [58, 59].

Thus, NK cell migration, as for all leukocytes, depends on a highly integrated signaling network culminating in coordinate activation and functional cooperation between different pathways triggered by integrin and chemokine receptors.

Activation of protein tyrosine kinases (PTK) is a prerequisite event for leukocyte migration, controlling both integrin adhesiveness and chemotactic response. The involvement of tyrosine kinases belonging to the Src and Syk/Zap families in cell migration have been largely documented for T lymphocytes and cells of myeloid lineage.

In regard to NK cells, it has been reported that LFA-1 engagement results in both Src and Syk kinase activation but these events have been associated with the cyto-

toxic function rather than with the migratory ability of NK cells [60, 61]. Using PTK inhibitors such as the general tyrosine kinase inhibitor herbimycin A, the specific Lck inhibitor damnacanthal, and the Syk inhibitor piceatannol, a role for the Src kinase Lck but not for Syk in CXCL12 (SDF-1 $\alpha/\beta$ )-induced NK cell chemotaxis has been described. In accordance with these results, NK cell stimulation with CXCL12 (SDF-1 $\alpha$ ) leads to tyrosine phosphorylation and activation of Lck [62].

More recently, a role for the focal adhesion kinases as cytoplasmic mediators of motility events in multiple cell types has been reported. The focal adhesion kinase family comprises two members that share an amino acid identity of almost 50%, the p125 focal adhesion kinase (p125Fak) and the proline-rich tyrosine kinase 2 (Pyk-2) also known as cell adhesion kinase- $\beta$  (CAK- $\beta$ ), or related adhesion focal tyrosine kinase (RAFTK). They are non-receptor PTK capable of coupling several receptors including integrins and chemokine receptors, with a variety of downstream effectors, such as small GTP-binding proteins belonging to the Ras and Rho families, MAPK, PKC and inositol phosphate metabolism [63, 64].

The expression of Fak family members on NK is controversial. Rabinowich and colleagues [65] have reported that p125Fak is expressed on NK cells and that  $\beta_1$  integrin engagement results in activation of this kinase and its association with Fyn and Zap-70 PTK. By contrast, we demonstrated that human peripheral blood NK cells express Pyk-2 that is constitutively associated with the cytoskeletal protein paxillin, but not p125 FAK. Engagement of  $\beta_1$  or  $\beta_2$  integrins on human NK cells results in rapid tyrosine phosphorylation of both Pyk-2 and paxillin. Moreover, we demonstrated that Pyk-2 acts as an upstream mediator of  $\beta_1$  and  $\beta_2$  integrin-triggered MAPK cascades, and controls the development of NK cell-mediated natural cytotoxicity [66–68]. More recently, we have reported that NK cell binding to endothelium activates Pyk-2 and the small GTP-binding protein Rac, a key regulator of actin cytoskeleton dynamics. Both Pyk-2 and Rac activation are coupled to integrins and chemokine receptors. Using recombinant vaccinia viruses encoding dominant negative mutants of Pyk-2 and Rac, we demonstrated that both Pyk-2 and Rac are functionally involved in chemokine-induced NK cell migration through endothelium or ICAM-1 or VCAM-1 adhesive proteins. We also found that Pyk-2 is associated with the Rac guanine nucleotide exchange factor Vav, which undergoes tyrosine phosphorylation upon integrin triggering, but not with PIX, another exchange factor for Rac that is associated with paxillin through p95 PKL. Collectively, these results indicate that Pyk-2 acts as a receptor-proximal link between integrin and chemokine receptor signaling, and the Pyk-2/Rac pathway plays a pivotal role in the control of NK cell transendothelial migration [68]. These results are consistent with a report by Sancho et al. [69] indicating that Pyk-2 can colocalize with the microtubule-organizing center at the trailing edge of migrating NK cells and in the area of the NK cell membrane that faces target cells.

PI3K and its products are other signaling intermediates that play a crucial role in cell migration. In this regard, evidence is available on the involvement of PI3K on



chemokine-mediated NK cell chemotaxis. In particular, it has been reported that wortmannin as well as antibody to PI3K- $\gamma$ , but not PI3K- $\alpha$ , can inhibit C, CC, and CXC chemokine-induced NK cell chemotaxis, suggesting that PI3K IB plays a crucial role in chemokine-induced activation of NK cells. In agreement with these results, recruitment of PI3K- $\gamma$  into NK cell membranes in response to RANTES stimulation has been reported [70].

### ***In vivo* NK cell migration**

Although NK cells express several adhesion molecules and chemotactic receptors that are involved in the control of NK cell migration across endothelium, and in their correct positioning into different lymphoid and non lymphoid organs, very little is known about the molecular events that govern NK cell trafficking *in vivo* under physiological or pathological conditions.

It has been reported that during murine cytomegalovirus infection, NK cells migrate through a CCL3 (MIP1- $\alpha$ )-dependent mechanism to the site of liver infection, where they contribute to antiviral defense [71]. The involvement of CCL3 (MIP1- $\alpha$ ) in the recruitment of NK cells in the liver is further supported by the demonstration that CCL3 (MIP1- $\alpha$ )-deficient mice show decreased resistance to cytomegalovirus infection that is associated with a dramatic reduction of NK cell accumulation and IFN- $\gamma$  production in the liver [72].

A role for CCL3 (MIP1- $\alpha$ ) in recruitment of NK cells has been also demonstrated by intrapulmonary transient transgenic expression of this chemokine that resulted in increased *Klebsiella pneumoniae* lung clearance associated with NK cell activation and accumulation in this organ [73]. Accumulation of NK cells in the lungs has also been observed in mice with invasive aspergillosis. In this model, however, NK cell recruitment was mediated by CCL2 (MCP-1), as neutralization of this chemokine resulted in reduced NK cell numbers in the lungs and impaired clearance of the pathogen from this organ [74]. Among CXC chemokines, CXCL10 (IP-10) has been reported to promote innate defense mechanisms following coronavirus infection in the central nervous system by recruiting and activating NK cells [75]. The involvement of CX3CL1 (fractalkine) in supporting NK cell migration *in vivo* has been provided by the evidence that CX3CL1-transfected tumor cells exhibit a reduced growth capability that is mediated by an increased recruitment of activated NK cells [76, 77]. In addition, using an *in vivo* model of NK cell-mediated lung tumor cell clearance and blocking antibodies against CX3CL1 or CX3CR1, it has been demonstrated that decreased clearance of tumor cells following perturbation of CX3CL1/CX3CR1 interaction is attributable to defective NK cell recruitment to the lung [78].

Defective recruitment of NK cells has been described in mice deficient for chemokine receptors. Using a model of *in vivo* pulmonary injury in CXCR3-defi-

cient mice, Jiang et al. [79] have reported that NK cells fail to migrate to the lungs. Using CXCR3-knockout mice, recruitment of NK cells in the lungs has also been shown to participate to the pulmonary host defense against *Bordetella bronchiseptica* [80]. In addition, a specific defect of NK cell recruitment to pulmonary granulomas has observed in CCR1-deficient mice [81]. Recently, using selective depletion and adoptive transfer experiments, Martin-Fontecha et al. [4] have reported that DC-induced recruitment of NK cells into lymph nodes occurs in a CXCR3-, but not CCR7-dependent manner.

These data strongly support the *in vivo* relevance of number of chemokine receptor-ligand interaction, including CX3CR1-CX3CL1, CXCR3-CXCL10/CXCL11 (IP-10/I-TAC), CCR5-CCL3/CCL4 (MIP-1 $\alpha$ / $\beta$ ), CCR5-CCL5 (RANTES), shown to mediate human NK cell chemotactic response.

## Uterine NK cells

NK cells are the predominant lymphocyte population present in the uterus. Their number increases drastically in the late secretory phase during the menstrual cycle and early pregnancy of humans, and at the implantation site in rodents [82, 83]. They accumulate as single cells or aggregates around endometrial glands and vessels playing a crucial role for the normal development of placenta and/or its vasculature [84].

Uterine NK cells exhibit a particular transcriptional profile [85], but their origin is still unknown. It is debated whether they are recruited from blood and/or arise from an NK cell progenitors found in the uterus or recruited from other tissues.

The analysis of the molecules potentially involved in the control of NK cell accumulation in the uterus has shown that first trimester human decidual NK cells, which are characterized by high levels of CD56 but fail to express CD16 (CD56<sup>high</sup>CD16<sup>-</sup>), express a distinct repertoire of adhesion molecules and chemokine receptors as compared to their peripheral blood counterpart [82, 85, 86]. In particular, they exhibit high levels of  $\alpha_E\beta_7$ ,  $\alpha_1\beta_1$ ,  $\alpha_X\beta_2$ ,  $\alpha_D\beta_2$ , whereas they do not express  $\alpha_6\beta_1$  laminin receptor. In addition, uterine NK cells also display the  $\beta_5$  integrin subunit and selectively express high levels of tetraspan 5, CD151, and CD9 tetraspanins that are constitutively associated with integrins and modulate integrin function [87].

In regard to chemokine receptors, first trimester human decidual NK cells express higher levels of CCR1, CCR3, CXCR3, CXCR2, and lower levels of CCR7, CXCR4, CX3CR1 as compared with CD56<sup>high</sup>CD16<sup>low</sup> peripheral blood NK cells [88]. This receptor profile is consistent with evidence showing the ability of uterine NK cells to migrate in response to CXCL9, CXCL10 and CXCL12 that have shown to be produced by the trophoblast or by the endometrial cells [88–90].

Evidence so far available in the mouse models has not allowed the identification of a particular chemokine receptor-ligand system involved in the control of uterine

NK cell accumulation. Indeed, no changes in NK cell localization and activation have been observed in mice genetically ablated for CCR2, CCR5, and MIP-1 $\alpha$  or mice doubly deleted for CCL3 (MIP-1 $\alpha$ ) and CCR5 [91]. This may be attributable to the known redundancy of the chemokine system as well to differences in the human *versus* mouse pregnancy.

By contrast, at day 11 of gestation at implantation sites, altered size and frequency of uterine NK cells have been observed in mice either lacking adhesion molecules such as P-selectin or  $\beta_7$ -integrin or treated with blocking monoclonal antibodies against MAdCAM-1 or  $\alpha_4\beta_7$  integrin [92, 93]. Whether this finding is related to the absence  $\alpha_4\beta_7^+$  leukocytes other than NK cells involved in uterine NK cell differentiation/accumulation is presently unknown.

## Conclusions

NK cells were initially thought to be endowed with a particular migratory pattern and to mainly circulate in the blood. The recent findings reviewed here highlight that NK cells can exhibit different predilections for tissue compartments, i.e., lymph nodes, inflamed tissues, etc., where they can play an important role as active participants in directing DC maturation and T cell response polarization and/or as cytotoxic effector cells.

The mechanisms regulating tissue-selective NK cell homing and functional specialization are just starting to be unraveled, but the importance of tissue microenvironment is becoming increasingly clear. An example is given by decidual NK cells whose differentiation, accumulation and functional program are under hormonal influence.

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