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NK cells and chemokines

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CHAPTER CONTENTS

Introduction	203
Chemokine receptor expression by NK cells and chemokine-regulated NK cell functions in vitro	205
Signalling events controlling chemokine-regulated NK cell functions	207
In vivo regulation of NK cell functions by chemokines	208
Conclusions	210
Acknowledgements	210

Considerate la vostra semenza: fatti non foste a viver come bruti ma per seguir virtute e canoscenza.

Dante Alighieri

Consider ye your origin; ye were not made to live as brutes, but for pursuit of virtue and of knowledge.

Translation by Charles Eliot Norton

ABSTRACT

Natural killer (NK) cells represent a distinct population of circulating and tissue-resident lymphocytes that play an important role in the early phases of immune responses against microbial pathogens by exhibiting cytotoxic functions and secreting a number of cytokines and chemokines.

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Studies in man and mouse have shown that NK cells are distributed in several organs under normal conditions. Their frequency is comparatively high in nonlymphoid organs such as the lung, the liver and the mucosal tissue of maternal uterus, and rare in thymus and lymph nodes. Yet, these cells are rapidly recruited to the parenchyma of injured organs during inflammation, viral infections and tumour growth.

Chemotactic factors, including chemokines, play critical roles in the regulation of NK cell migration across endothelium and into the tissues. The differences in chemokine receptor expression together with distinct adhesive properties of different NK cell subsets as well as activated NK cells, imply that they have multiple routes of circulation and trafficking patterns.

Besides their role in the regulation of NK cell trafficking, chemotactic molecules can also affect NK cell effector functions by regulating their priming and their ability to kill and secrete cytokines.

KEY WORDS

Chemokine receptors, Chemokines, Chemotaxis, Lymphocyte homing, Signal transduction

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 (Trinchieri, 1989).

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 intimate contact between developing NK cells and stromal cells, which is required for their full maturation (Roth et al., 2007). In the mouse, a lymphoid precursor (NKP) committed to the NK cell lineage has been identified (Di Santo, 2006; Rosmaraki et al., 2001). NKP cells express the common IL-2R/IL-15R beta chain (CD122) and the activating receptor NKG2D, and lack lineage markers. At the next stage of maturation, immature NK cells (iNK) express NK1.1 and CD94, along with the alpha_v integrin chain (CD51) and low levels of the integrin chain CD11b, and have poor cytotoxic and cytokine secretion capacity (Kim et al., 2002; Vosshenrich et al., 2005). The subsequent acquisition of Ly49 receptors is followed by an expansion stage that is characterized by upregulation of the integrin CD49b (defined by the DX5 mAb clone) and by the loss of CD51 expression; among this population, cells with increased expression of CD11b and of the mucin-like molecule CD43 are considered fully functional/mature (mNK). CD11b^{low} NK cells predominate in BM and lymph nodes (LN), while CD11b^{high} NK cells prevail in the blood, liver, spleen and lungs. The observation that in addition to mNK cells, NKPs and iNK are found in the spleen, LN and liver, suggests that also the latter populations can exit the BM under normal conditions and that final maturation of BM-derived NK cell precursors can occur also in the periphery (Freud et al., 2005). In this regard, we recently demonstrated that iNK as well as mNK cells can exit the BM in response to mobilizing stimuli, such as the CXCR4 pharmacological antagonist AMD3100 (Bernardini et al., 2008).

Recent evidence indicates that a population of mouse NK cells that express the IL-7 receptor alpha chain (CD127) differentiates in the thymus and expresses low levels of CD11b and CD43 and a reduced repertoire of NK cell receptors than splenic NK cells. Differently from BM-derived NK cells, thymic-derived NK cells preferentially home to LN, suggesting that they selectively respond to LN homing molecules (Vosshenrich et al., 2006).

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 the mouse spleen, the majority of NK cells are found in the red pulp, while NK cells are preferentially present inside vessels or sinuses in the LN (Dokun et al., 2001; Walzer et al., 2007b). The same tissue distribution is also evident in the liver, where NK cells can be found in high frequency within sinusoid mononuclear cells (Bouwens et al., 1987).

During viral infections, inflammation, tumour growth and invasion, NK cells are rapidly recruited from the blood and accumulate in the parenchymas of injured organs (Biron, 1997; Fogler et al., 1996; Trinchieri, 1989); tissue-recruited 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. In addition, homing to LN of a particular subset of activated NK cells has been described both in human and mouse (Fehniger et al., 2003; Martin-Fontecha et al., 2004). NK cells enter into LN stimulated with LPS-matured DCs or with selected adjuvants, become activated and provide an early source of IFN- γ that is necessary for T helper 1 polarization (Lucas et al., 2007; Martin-Fontecha et al., 2004). This evidence suggests that NK cells enter into LN to acquire effector capabilities through interacting with other cells of the innate immune system, such as DCs, and to modulate adaptive T-cell responses.

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 (Lanier, 2005; Raulet et al., 2001). Therefore, activation of NK cell functions is the result of concomitant engagement of various activating and inhibitory receptors by the particular set of ligands on target cells (McQueen and Parham, 2002).

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. Their 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 of the Neural Cell Adhesion Molecule (NCAM), CD56.

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: About 90% are CD56^{low}CD16^{high}, whereas about 10% are CD56^{high}CD16^{low}. It has been proposed that CD56^{high} NK cells have a unique functional role in the innate immune response as a primary source of NK cell-derived immunoregulatory cytokines, whereas the CD56^{low}CD16^{high} subset represents the principal cytotoxic population (Cooper et al., 2001).

Populations of NK cells similar to the two main human NK cell subsets have been described in mice. Mouse CD11b^{high} NK cells can be dissected into CD27^{high} and CD27^{low} fractions that differ in terms of expression of NK cell inhibitory receptors and of chemokine receptors and are functionally different. CD27^{low} cells are mostly found in nonlymphoid organs (blood,

liver and lungs). As compared to CD27^{high} cells, they express a higher percentage of inhibitory receptors Ly49 I and C isoform and almost exclusively the inhibitory killer cell lectin-like receptor G1 (KLRG1), and their cytotoxic and cytokine production activities are more tightly regulated (Hayakawa and Smyth, 2006; Hayakawa et al., 2006). The CD27^{high} cells have many features similar to that of CD56^{high} cells. They predominate in the LN and are extremely responsive to IL-12 and IL-18; but unlike CD56^{high} cells, CD27^{high} cells are also highly cytotoxic. Considering that CD56^{high} and CD56^{low} NK cells were also recently shown to express high and low levels of CD27, respectively, it has been suggested that this marker can also be used to better identify the two major subsets of human NK cells (Silva et al., 2008).

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 immunoglobulin families, as well as chemokines (Kunkel and Butcher, 2002; Springer, 1994).

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 mediated 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. Their action is mediated by interaction with G-protein-coupled seven-transmembrane-domain receptors that initiate complex signalling 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 (Baggiolini et al., 1997; Clark and Brugge, 1995; Mantovani, 1999; Rossi and Zlotnik, 2000). In addition, by exerting a pro-adhesive function, a few chemokines can also modulate other steps of leukocyte migration into tissues, including firm adhesion to the endothelial layer (Fong et al., 1998).

Depending on the number and spacing of conserved cysteine residues in their amino acid sequence, chemokines have been classified into four major groups: the CXC (or alpha), CC (or beta), CX3C and C subfamilies (Rossi and Zlotnik, 2000).

The differential expression of chemokine receptors on distinct NK cell populations, together with their ability to regulate integrin expression and function, can be responsible for the recruitment of specialized NK cell subsets during inflammation.

Chemokine receptor expression by NK cells and chemokine-regulated NK cell functions in vitro

A large body of evidence indicates that NK cells can express several receptors for CXC, CC, C and CX3C chemokines, with great heterogeneity in the chemokine receptor repertoire among different NK cell populations and between resting versus activated NK cells (Gregoire et al., 2007; Hayakawa et al., 2006; Robertson, 2002).

With respect to the CXCR and CX3CR families, it has been reported previously that human peripheral blood NK cells express both CXCR1 and CXCR2 as CXCL8 (IL-8) receptor (Casilli et al., 2005; Chuntharapai et al., 1994; Morohashi et al., 1995) and CX3CR1 as CX3CL1 (fractalkine) receptor (Imai et al., 1997; Yoneda et al., 2000). These observations have been further extended by Campbell and colleagues (2001) who provided the first evidence that distinct (CD56^{pos}CD16^{neg} and CD56^{pos}CD16^{pos}) peripheral blood NK cell subsets have a unique repertoire of chemokine receptors. CD16^{pos} NK cells uniformly express high levels of CXCR1 and CX3CR1, low levels of CXCR2 and CXCR3 and no detectable levels of CXCR5. In contrast, CD16^{neg} NK cells express high levels of CXCR3, express 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-1alpha/beta). With respect to the CC chemokine receptor family, the majority of NK cells lack expression of CCR1-7 and CCR9, and only the CD16^{neg} NK cell subset expresses high levels of CCR5 and CCR7, with the latter molecule mainly involved in the homing of lymphocytes to secondary lymphoid organs (Campbell et al., 2001).

Consistent with this expression profile, CXCL8 and soluble CX3CL1 preferentially attract the CD16^{pos} NK cell subset, which can also respond moderately to the CXCR3 ligands, CXCL11 (I-TAC) and CXCL10 (IP-10); by contrast, CD16^{neg} NK cells respond more dramatically to the CCR7 ligands, CCL19 (ELC/MIP-3beta) and CCL21 (SLC), as well as to the CXCR3 ligands, CXCL11 and CXCL10, and poorly to a CCR2 ligand, CCL2 (MCP-1), or CCR5 ligands, CCL4 (MIP-1beta) and CCL5 (RANTES). Both NK cell subsets strongly migrate in response to the ligand for CXCR4, CXCL12 (Campbell et al., 2001; Taub et al., 1995).

Moreover, Kim et al. (1999) have found that CD56^{high} CD16^{neg} cells respond more than CD56^{low} CD16^{pos} cells to CCL21 and CCL19 when used at high concentrations, although they observed that the two NK cell subsets express equal levels of CCR7 mRNA.

Besides chemokine receptors, human NK cells also express receptors for chemotactic molecules that do not belong to the chemokine superfamily and regulate NK cell trafficking under steady state and inflammatory conditions. In this regard, NK cells migrate in response to the proinflammatory plasma protein chemerin, and that CD56^{low}CD16^{pos}, but not CD56^{high}CD16^{neg}, expresses its receptor, ChemR23. Recently, a silent receptor for chemerin, CCRL2, has been also identified. CCRL2 is able to bind chemerin and to increase its local concentration and availability. Its expression and function on NK cells has not been clarified yet (Zabel et al., 2008). Sphingosine 1-phosphate (S1P) is a sphingophospholipid that influences lymphocyte trafficking as well as proliferation, adherence, and morphogenesis and is generated by the conversion of sphingomyelin into ceramide by sphingomyelinase (Matloubian et al., 2004). It was recently reported that human NK cells express the mRNA for S1P1, one of the five S1P G protein-coupled receptors, and that activation with IL-2 increases S1P1 and promotes S1P4 and S1P5 but not S1P2 expression; in addition, S1P but not sphingosine induces the chemotaxis of these cells (Kveberg et al., 2002; Maghazachi, 2003).

Interestingly, the selective expression of CXCR3 on CD56^{high} and of CX3CR1 on CD56^{low} NK cells is very similar to their expression pattern on the two main subsets of mouse mature NK cells defined as CD27^{high} and CD27^{low}. Indeed, as compared to CD27^{low} NK cells, CD27^{high} (both CD11b^{low} and CD11b^{high}) cells have higher expression of the chemokine receptor CXCR4, selectively express CXCR3 and has much lower expression of the chemoattractant receptor S1P5, a mouse NK cell-specific receptor for S1P (Hayakawa and Smyth, 2006; Walzer et al., 2007a). We recently extended this observation, showing that CXCR4 expression is very high on NKP cells and progressively decreases during NK cell differentiation, and that NKP cells functionally express also CXCR3 and CCR1 that may participate to their recruitment from BM to specific organs (Bernardini et al., 2008). In addition, using C57BL/6 mice in which a green fluorescent protein cDNA was knocked in to genes encoding CX3CR1, we and others observed selective expression of CX3CR1 on the KLRG1^{pos}/CD27^{low} NK cell subset (Gregoire et al., 2007; Bernardini, unpublished observations) (Table 15.1).

The expression of chemokine receptors on NK cells can be modulated upon cytokine stimulation. A significant decrease of CXCR3 expression on human NK cells treated for 6h or 24h with IL-2 and IL-12 alone or in combination has been reported, and the decreased

expression was associated with reduced chemotaxis to CXCL10. Similarly, CCR7 receptor is down-regulated on NK cells after IL-2 activation, whereas the CCR4 and CX3CR1 molecule is induced on IL-2 activated NK cells. The same treatment did not affect the expression of other chemokine receptors such as CCR1, CCR2 or CXCR4 (Hodge et al., 2002). However, previous reports have shown that short-term exposure of freshly isolated NK cells to IL-2 can positively modulate CCR2 mRNA expression (Polentarutti et al., 1997), and long-term (8–10 days) IL-2 stimulation results in increased expression of CCR1, CCR2, CCR4, CCR5 and CCR8 (Inngjerdingen et al., 2001). CXCR4 and CX3CR1 have been also shown to be down-regulated through stimulation with different cytokines, including IL-15, whereas TGF-beta has been proven to be a potent inducer of CXCR4 on NK cell subsets (Barlic et al., 2003; Inngjerdingen et al., 2001).

In agreement with these observations, IL-2 activated NK cells can migrate in response to many CC chemokines, such as CCL2, CCL8 (MCP-2), CCL7 (MCP-3), CCL3 (MIP-1 alpha), CCL4, CCL5 and CCL22 (MDC) (Allavena et al., 1994; Godiska et al., 1997; Inngjerdingen et al., 2001; Loetscher et al., 1996).

NK cell treatment with IL-18, differently from IL-2, results in selective induction of CCR7 expression on the CD56^{low} NK cell subset but does not affect CCR7 expression on the CD56^{high} subset; increased expression of CCR7 on CD56^{low} NK cells is associated with reduced levels of CD16 and enhanced capability to migrate in response to the LN associated chemokine CCL21 (Mailliard et al., 2005).

Besides migration, other human and mouse NK cell functions can be affected by chemokines. Indeed, chemokines can activate an NK cell defence machinery that may directly counteract an infectious agent by performing cytotoxicity or by secreting pro-inflammatory cytokines that recruit and activate other effector cells (Taub et al., 1995). Those effects are well exemplified by the multifunctional role of the chemokine CX3CL1 in NK cell activation. Soluble and membrane-bound fractalkine induce IFN- γ production by NK cells and affect NK cell ability to kill tumour cells both in vitro and in vivo (Guo et al., 2003; Yoneda et al., 2003; Zeng et al., 2007). The CX3CL1-mediated pro-adhesive function is relevant in CX3CL1-promoted granule exocytosis and IFN- γ production by NK cells during endothelial cell (EC) contact (Yoneda et al., 2000). These findings suggest that the expression of fractalkine at the site of inflammation can attract and activate NK cells through CX3CR1 and that NK cells, once activated, can lyse neighbouring ECs, promoting vascular injury (Umehara et al., 2004). This effect may have important implications in several pathological conditions such as graft rejection and tissue damage promoted by chronic infections

Table 15.1 Chemoattractant receptor expression on the main subsets of human and mouse NK cells subsets

	Human		Mouse	
	CD56 ^{low} CD16 ^{high}	CD56 ^{high} CD16 ^{low}	CD27 ^{high}	CD27 ^{low}
CXCR1	++ ¹	- ³	ND ⁴	ND
CXCR2	+ ²	-	ND	ND
CXCR3	+	++	++	+/- ⁵
CXCR4	++	++	++	+
CCR1	-	-	ND	++
CCR2	-	-	++	+
CCR3	-	-	ND	ND
CCR4	-	-	ND	ND
CCR5	-	++	+	++
CCR6	-	-	ND	ND
CCR7	-	++	ND	ND
CCR9	-	-	ND	ND
CX3CR1	++	+	-	++
ChemR23	++	+/-	?	?
S1P5	?	?	+/-	++

¹Indicates high levels of expression.

²Indicates intermediate levels of expression.

³Indicates undetectable levels of expression.

⁴Not done.

⁵Indicates low levels of expression.

(Bolovan-Fritts and Spector, 2008; Robinson et al., 2000). Enhancement of NK cell degranulation has also been demonstrated in response to other chemokines belonging to the CC family and to CXCL10 (Maghazachi et al., 1996; Taub et al., 1996). In addition, CC chemokines-mediated redistribution of adhesion molecules on NK cell surfaces may also increase NK cell cytotoxicity by promoting effector-target cell interaction (Nieto et al., 1998).

CX3CL1 has been found to be a pivotal molecule driving immune synapse formation during DC/NK cell interaction leading to efficient NK cell activation (Pallandre et al., 2008). This is similar to what is described for other chemokines that can act as T cell costimulators by prolonging the duration of T cell-antigen presenting cell interaction and by avoiding premature cell splitting. Importantly, the absence of CX3CR1, or CX3CL1-blockade, abrogated the ability of NK cells to produce IFN- γ when exposed to DC, underlying a key role of this chemokine during DC-mediated NK cell priming.

Thus, the CX3CR1/CX3CL1 axis regulates NK cell functions at different levels promoting their migration through endothelial vessels, their activation and their ability to kill or secrete cytokines.

Signalling events controlling chemokine-regulated NK cell functions

Despite increasing evidence of a prominent role of chemokines and integrins in the dynamic regulation of leukocyte adhesion and migration, the signalling 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 the migrating cell and retraction at the cell rear (Ridley et al., 2003; Vicente-Manzanares and Sanchez-Madrid, 2004).

Thus, NK cell migration, as for all leukocytes, depends on a highly integrated signalling network culminating in coordinate activation and functional cooperation between different pathways triggered by integrin and chemokine receptors. Chemokine receptors are mainly coupled to G α_{i} -dependent heterotrimeric G proteins, as in most cases Pertussis Toxin X (PTX) inhibits the biological activities induced by chemokines, including cell migration. A PTX-sensitive signalling pathway has been shown to be involved in CX3CL1-activated NK cell polarization leading to NK cell priming by DC- or NK cell-mediated target cell lysis (Pallandre et al., 2008). IFN- γ production by NK cells exposed to mature DCs was shown to be dependant on the CX3CL1 ability to promote redistribution of lipid rafts on NK cell membrane that excluded KIR2DL1 inhibitory receptor from the immune synapse leading to inhibition of ligand-induced KIR phosphorylation and recruitment of SHP1.

However, chemokine-induced NK cell chemotaxis is coupled also to PTX-insensitive G-protein such as G α_{q} (Maghazachi, 1997; Soede et al., 2001).

PI3K and its products are signalling intermediates that play a crucial role in cell migration. In this regard, evidence is available on the involvement of PI3K in chemokine-mediated NK cell chemotaxis. In particular, it has been reported that wortmannin, as well as Ab directed against PI3K- γ but not PI3K- α 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- γ into NK cell membranes

in response to CCL5 stimulation has been reported (al-Aoukaty et al., 1999).

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

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-induced NK cell chemotaxis has been described. In accordance with these results, NK cell stimulation with CXCL12 leads to tyrosine phosphorylation and activation of Lck (Inngjerdingen et al., 2002). Activation of Lck can be PI3K γ -dependent or -independent, as Galph α_s can directly couple to Src family PTKs, and lead to Vav phosphorylation, leading to activation of Cdc42 and Rac small GTPases and lamellipodia formation.

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- β (CAK- β), or related adhesion focal tyrosine kinase (RAFTK). They are non-receptor PTKs 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, MAP kinases, PKC and inositol phosphate metabolism (Lev et al., 1995).

The expression of Fak family members on NK is controversial. p125Fak is expressed on NK cells with $\beta 1$ integrin engagement results in activation of this kinase and its association with Fyn and Zap-70 PTKs (Rabinowich et al., 1996). In 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. 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. By 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 that 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 signalling, and Pyk-2/Rac pathway plays a pivotal role in the control of NK cell transendothelial migration (Gismondi et al., 2003).

These results are consistent with findings indicating that Pyk-2 can colocalize with the microtubule-organizing centre at the trailing edge of migrating NK cells and in the area of the NK cell membrane that faces target cells (Sancho et al., 2000).

In vivo regulation of NK cell functions by chemokines

NK cells express several chemotactic receptors that are involved in the control of NK cell migration across endothelium and in the correct tissue positioning of lymphocytes, but only recently, we are beginning to appreciate the contribution of chemotactic factors on NK cell trafficking and tissue distribution under steady-state and pathological conditions (Figure 15.1).

Although the differential expression of chemokine receptors and chemotactic responsiveness of the CD27^{low} and CD27^{high} subsets strongly suggests a correlation with their tissue distribution under normal conditions, few chemoattractant receptors have been demonstrated to play a role in NK cell subset distribution in vivo at present. A drastic decrease in NK cell numbers has been observed in the blood, spleen, and lungs of SIP5-deficient mice, associated with an increased number of NK cells in the BM and LNs. This altered distribution has suggested that SIP5 provides an egress signal to NK cells, promoting their exit from BM and LN (Walzer et al., 2007a). In addition, the observation that CX3CR1-deficient mice display a selective reduction of NK cell number in the lung strongly suggests that this receptor is important for the accumulation of at least one subset of NK cells in this organ under steady-state conditions (Yu et al., 2007). Also the expression of CXCR4 profoundly affects NK cell subset distribution likely by contributing to the maintenance of an NK cell pool within BM, as shown by the recruitment of BM iNK and mNK cells into circulation and spleen following in vivo delivery of the CXCR4 pharmacological antagonist AMD-3100 (Bernardini et al., 2008).

Interestingly, altered distribution of NK cell subsets has been revealed also in mice defective for transcription factors (Boos et al., 2007). Indeed, although normal number and function of BM NK cells were found in Id2^{-/-}E2a^{-/-} mice, very few mature NK cells were found in the spleen, whereas liver-specific homing of BM NK cells was reduced in the absence of Gata-3 (Samson et al., 2003). Overall, these findings suggest

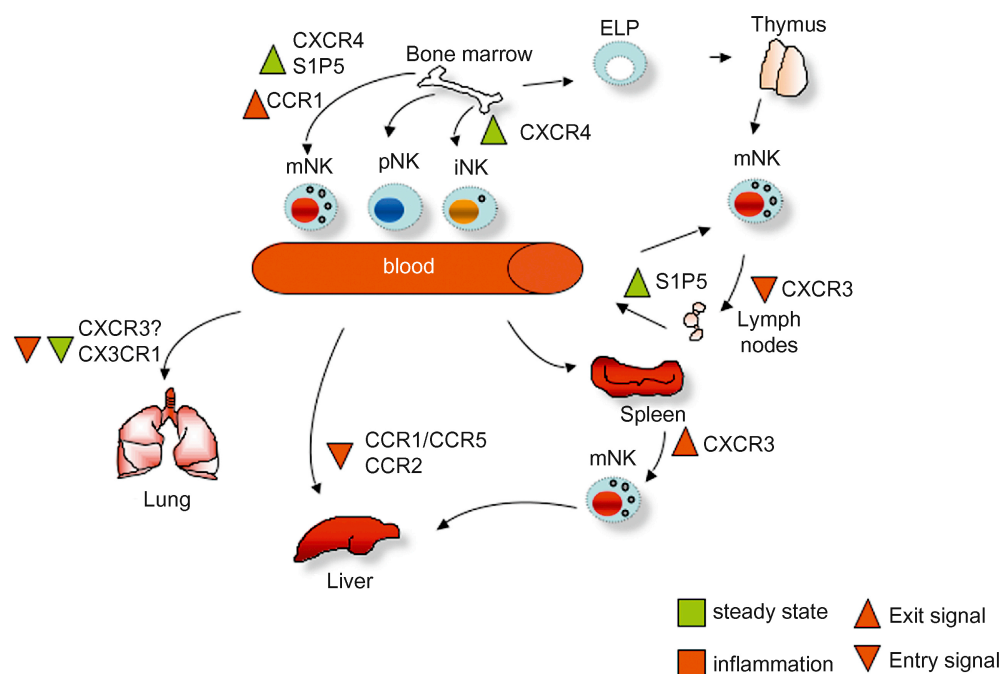


Figure 15.1 • Chemoattractant receptor involvement in the trafficking of mouse NK cells. The majority of NK cells develop in the BM, and a subset that is characterized by CD127 expression develops into the thymus. Recruitment into circulation from different locations of NK cells at different stages of maturation is directed by chemoattractants both under normal (light grey triangles) or inflammatory (dark grey triangles) conditions. Once into circulation, the differential expression of chemoattractant receptors on different NK cell subsets or differently activated NK cells regulate their homing into normal or inflamed tissues.

that these transcription factors are strongly involved in the expression of tissue homing receptors on NK cells either by directly regulating their transcription or by affecting the differentiation of selected NK cell subsets expressing such receptors.

While the contribution of chemokines in the regulation of NK cell tissue distribution during homeostasis has been poorly addressed so far, a number of studies indicate that selected chemokines play a critical role in the orchestration of NK cell trafficking during inflammation. During murine cytomegalovirus infection, NK cells migrate through a CCL3-dependent mechanism to sites of liver infection where they contribute to antiviral defence (Salazar-Mather et al., 1998). This evidence was further supported by the demonstration that CCL3-deficient mice show decreased resistance to cytomegalovirus infection that is associated with a dramatic reduction of NK cell accumulation and IFN- γ production in the liver. The recruitment of NK cells to the liver during infection required the receptor for CCL3, CCR5, and the receptor for CCL2, CCR2 (Hokeness et al., 2005; Salazar-Mather et al., 1998). IFN- γ -dependent recruitment of NK cells to liver has also been described in a model of Concanavalin A-induced hepatitis. In this case, NK cell entry in the liver was reduced in the absence of CCR1 and involved NK cells that used CXCR3 to exit the spleen (Wald et al., 2006).

A role for CCL3 in recruitment of NK cells has also been 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 (Zeng et al., 2003). Accumulation of NK cells in the lungs has been also observed in mice with invasive aspergillosis. In this model, however, NK cell recruitment was mediated by CCL2, as neutralization of this chemokine resulted in a reduced NK cell number in the lungs and impaired clearance of the pathogen from this organ (Morrison et al., 2003). In addition, using an in vivo model of NK cell-mediated lung tumour cell clearance in CX3CR1-deficient mice or after treatment with blocking antibodies against CX3CL1 or CX3CR1, it has been demonstrated that decreased clearance of tumour cells following perturbation of CX3CL1/CX3CR1 interaction is attributable to defective NK cell recruitment to the lung (Robinson et al., 2003; Yu et al., 2007). Using CXCR3-knockout mice, recruitment of NK cells in the lungs also participates in the pulmonary host defence against *Bordetella bronchiseptica* (Widney et al., 2005). In addition, a specific defect of NK cell recruitment to pulmonary granulomas was observed in CCR1-deficient mice (Shang et al., 2000).

In the central nervous system (CNS), CXCL10 promotes innate defence mechanisms following coronavirus infection by recruiting and activating NK cells, while

CX3CR1GFP/GFP mice showed a selective deficiency of NK cell recruitment in the CNS during experimental autoimmune encephalomyelitis that was accompanied by very severe disease (Trifilo et al., 2004). The results of this study suggested that loss of the regulatory influence of NK cells accounts for the severe EAE phenotype in CX3CR1GFP/GFP mice. Coincidentally, alterations of CX3CR1^{pos} NK cell numbers are observed in patients with multiple sclerosis. Whether or not CX3CR1 can be used in multiple sclerosis therapy remains to be seen, but the beneficial effects observed during anti-CD25-based therapy of multiple sclerosis have been attributed to activated CD56^{high} cells (Infante-Duarte et al., 2005).

The involvement of CXCL10 and CX3CL1 in supporting NK cell function in vivo has also been provided in antitumour immunity. CXCL10 promotes a strong antileukemic NK cell-mediated response via enhanced induction of cytotoxicity and expression of the T cell costimulatory molecule B7-H1 (Saudemont et al., 2005). In addition, a link between IFN- γ induction of CXCR3 ligand expression during the antitumour immune response and CD27^{high} NK cell recruitment within the tumour mass was found and shown to be critical for host survival (Wendel et al., 2008). CX3CL1-transfected tumour cells exhibit a reduced growth capability that is mediated by an increased recruitment and activation of NK cells (Robinson et al., 2003).

The selective role of chemokine/chemokine receptor interaction in NK cell migration in vivo suggests that different NK cell subsets may be independently recruited in distinct inflammatory settings, as the two major subsets of mature NK cells, CD27^{high} and CD27^{low}, can be characterized by the mutually exclusive expression of CXCR3 and CX3CR1.

Recently, by using selective depletion and adoptive transfer experiments, Martin-Fontecha et al. have reported that DC-induced recruitment of NK cells into LN occurs in a CXCR3- but not CCR7-dependent manner (2004). The role of CXCR3 has also been emphasized within the spleen in the recruitment of red pulp-localized NK cells to the white pulp during TLR ligand stimulation in vivo (Gregoire et al., 2008). Interestingly, the entry of NK cells into the splenic red pulp is PTX-insensitive, thus suggesting that NK cell localization into this organ is regulated by a chemokine-independent or a PTX-insensitive chemokine receptor. The entry of NK cells within the T cell area of secondary lymphoid organs has several implications allowing their correct priming by DCs as well as an efficient T-cell polarization. Using a genetic approach to in vivo deplete CD11c^{high} DC, Lucas and coworkers showed that NK cell responses to viral and bacterial pathogens in vivo depend on their interaction with CD11c^{high} DC activated by IFN type I signals within the secondary lymphoid organs (2007).

These data collected in mice strongly support the in vivo relevance of a number of chemokine receptor-ligand interactions, including CX3CR1-CX3CL1, CXCR3-CXCL10/CXCL11, CCR5-CCL3/CCL4 and CCR5-CCL5, which have been shown to mediate human NK cell chemotactic response in vitro.

Inflammatory conditions can induce drastic changes in the pattern of chemokine receptor expression on human NK cells and the regulation of the expression of their ligands in inflamed or injured tissues, thus altering NK cell subset recruitment and redistribution. Indeed, granulomatous lesions in the skin and the respiratory tract of patients with transporter associated with antigen processing 2 (TAP-2) (transporter of activated peptide 2) deficiency display an accumulation of activated NK cells hyper responsive to CCR2 ligands (Hanna et al., 2005). In addition, a strong correlation between ChemR23 expression and colocalization of NK cells with DCs was observed in human biopsies of lichen planus in areas where chemerin is present (Parolini et al., 2007).

Conclusions

Individual NK cell subsets displaying unique functional features, and tissue locations have been identified both in mouse and humans. Although this suggests that the expression of specific homing receptors is involved, the role of chemokines in the trafficking of NK cells in normal and disease conditions is only now starting to be elucidated.

In the steady state, NK cells are present at a high frequency in the circulation, ready to extravasate to tissues under inflammatory conditions. The recent findings reviewed herein highlight that NK cells respond to several chemoattractants regulating the maintenance of resting NK cells in the circulation (i.e. S1P) or the recruitment of activated NK cells into the sites of diseases and inflammation. In these locations, NK cells can play an important role as active participants in directing DC maturation and T-cell response polarization and/or as cytotoxic effector cells.

Involvement of chemokines in the regulation of DC-mediated NK cell priming and effector functions has also been documented and should be taken into account when analyzing the role of chemokines in NK cell-dependent immune responses.

Acknowledgements

This work was supported by grants from Istituto Pasteur Fondazione Cenci Bolognetti, MIUR-PRIN, Centro di Eccellenza BEMM, EC NoE EMBIC contract # 512040.

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