The trafficking of natural killer cells

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Summary: Natural killer (NK) cells are large granular lymphocytes of the innate immune system that participate in the early control of microbial infections and cancer. NK cells can induce the death of autologous cells undergoing various forms of stress, recognizing and providing non-microbial 'danger' signals to the immune system. NK cells are widely distributed in lymphoid and non-lymphoid organs. NK cell precursors originate from the bone marrow and go through a complex maturation process that leads to the acquisition of their effector functions, to changes in their expression of integrins and chemotactic receptors, and to their redistribution from the bone marrow and lymph nodes to blood, spleen, liver, and lung. Here, we describe the tissue localization of NK cells, using NKp46 as an NK cell marker, and review the current knowledge on the mechanisms that govern their trafficking in humans and in mice.

Keywords: *innate immunity, anatomy of the immune system, natural killer cells*

Introduction

Natural killer (NK) cells are lymphocytes of the innate immune system that can induce the death of allogeneic cells and autologous cells undergoing various forms of stress, such as upon microbial infection and malignant transformation (1, 2). NK cells express an array of activating and inhibitory receptors, whose engagement allows them to discriminate between target and non-target cells (3–5). The repertoire of NK cell receptors complements that of other innate sensors, such as scavanger receptors, Toll-like receptors (TLRs) or nucleotide oligomerization domain (NOD) proteins. The strategies of NK cell recognition thus broaden the detection of pathogenic situations where microbial 'danger signals' are missing *in vivo*, such as in the case of poorly immunogenic tumors.

Consistent with their role in immune surveillance, NK cells are widely distributed in the body. It is unclear, however, whether this wide distribution is due to their recirculation, due to the existence of NK subsets with different homing capacities, or due to their development at multiples sites. NK cells can also be recruited in various tissues upon inflammation. However, in contrast to B and T cells, the mechanisms governing NK cell trafficking remain poorly dissected.

NK cellwide tissue distribution

It has been long appreciated that NK cells are widely distributed in mammals (6). However, few studies have precisely addressed this distribution. An early study in rat showed that the frequency of large granular lymphocytes (LGLs), including NK and T-cell subsets, was high in the lung and peripheral blood, superior to that in the spleen, peritoneal exudates, and lymph nodes. LGLs were also found to be absent in the thymus and bone marrow (7). Subsequent studies in the mouse have shown the presence of NK cells, defined as $NK1.1^+CD3^-$ or $DX5^+CD3^-$ by flow cytometry, in various organs but never focused on their distribution (8). We revisited this question and measured the percentage and number of mouse NK cells in various organs. Our results confirmed the wide distribution of NK cells in lymphoid and non-lymphoid organs (*Fig. 1*). The frequency of NK cells in lymphocytes was found to be the highest in non-lymphoid organs such as the lung and liver. A similar phenomenon has also been observed for effector memory $CD8^+$ T cells (9), suggesting similar mechanisms of trafficking for these two cell types and the existence of a niche for interleukin-15 (IL-15)-dependent lymphocytes in non-lymphoid organs. The order for NK cell frequency is lung > liver > peripheral blood mononuclear cells (PBMCs) > spleen > bone marrow (BM) > lymph node (LN) > thymus, where NK cells are almost undetectable. For comparison, $NK1.1^+$ T cells predominate in the liver, while T cells prevail in blood and LN (*Fig. 1*). Human NK cells also appear to be frequent in non-lymphoid organs (10, 11).

The largest number of mouse NK cells, 2–3 million, can be found in the spleen. Significant reservoirs of NK cells may also be found in all other organs tested excluding the thymus (*Fig. 1*). In humans, it was found that lymph node NK cells outnumber blood NK cells by 10:1 (12), whereas an estimated 1:1 ratio was observed in the mouse. The reason for this discrepancy could be linked to the expression of CCR7 on a subset of human but not on mouse NK cells, as discussed later.

The presence of NK cells in epithelial tissues has been poorly investigated. NK cell numbers are massively increased in the uterus during pregnancy both in humans and mice (13). In humans, $CD3^-CD56^+$ NK cells have been detected in the skin of healthy donors (14) and in the lymph draining this tissue (15). An infiltration of NK cells has been reported in lesional atopic dermatitis skin, in both epidermis and dermis, after *Malassezia* exposure (16), and in the inflammatory skin during

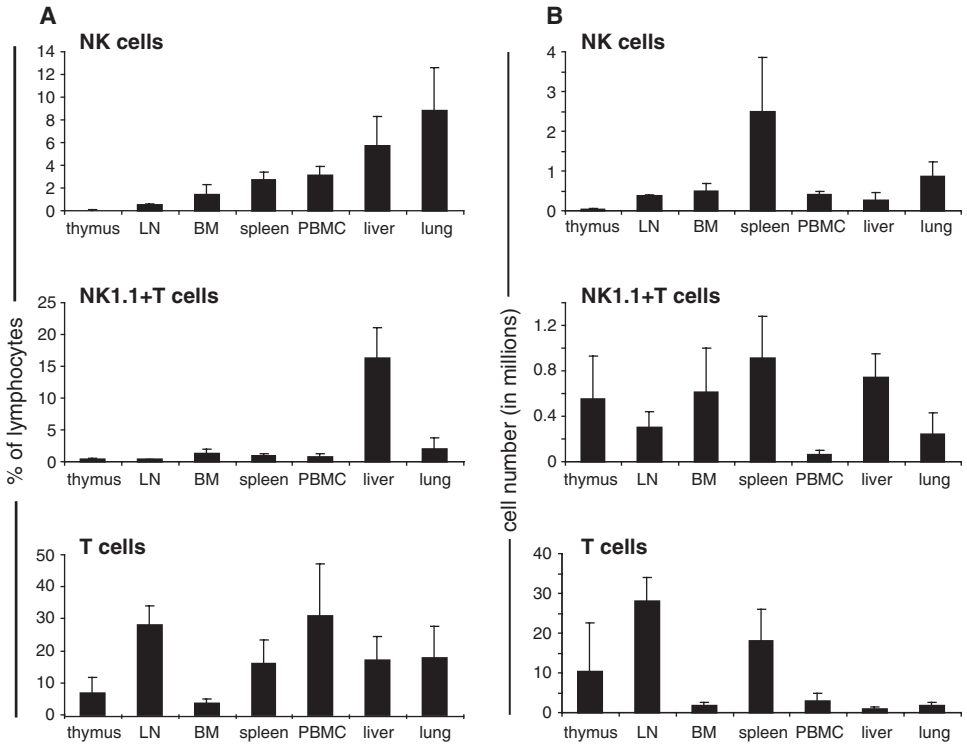

***Fig. 1.* Tissue distribution of natural killer (NK), $NK1.1^+$ T, and T cells.** Lymphocyte populations were isolated from the indicated organs of 6-week-old C57BL/6 female mice, as previously described (33). (A) The percentage of $NK1.1^+CD3^-$ (NK cells), $NK1.1^+CD3^+$ ($NK1.1^+$ T cells), and $NK1.1^-CD3^+$ (mature T cells) cells was measured by flow cytometry. (B) Cell numbers of the indicated subsets were obtained by multiplying the respective frequency of each subset by the total number of lymphocytes in the organ. For blood and lymph nodes, we used an estimate number of 10 million cells in peripheral blood mononuclear cells and 100 million cells in LNs. Results show the mean ± standard deviation (SD) of six mice for each organ.

the elicitation phase of contact hypersensitivity in a mouse model of allergic contact dermatitis (17). Furthermore, several groups have reported an accumulation of cells expressing an NK cell phenotype in skin psoriatic lesions (18–20). However, further investigations are required to clearly determine whether infiltrating cells are 'true' NK cells or rather NK-like T cells. In a normal human intestine, $CD3^-$ intraepithelial leukocytes expressing NK cell markers such as CD122, CD161, CD2, CD94, CD56, or CD16 have been described (21–24). Finally, $CD3^-NK1.1^+$ cells have been recently identified in cell suspensions prepared from mouse vaginal tissue (25). Moreover, IL-$15^{-/-}$ and/or RAG$2^{-/-}\gamma_c^{-/-}$ mice are more sensitive to genital herpes simplex virus 2 (HSV-2) infection, suggesting a potential implication of NK cells in the control of infection spreading in mucosal tissues (26, 27).

NK cells in sinuses

In situ visualization of NK cells has been hampered by the lack of specific reagents. Previous attempts to identify NK cells *in situ* were based on adoptive transfers of fluorescently labeled cells or staining with anti-NK1.1, anti-Ly49G2, or anti-CD49b (28–32). However, none of the aforementioned antibodies are NK cell specific (NK1.1, CD49b) or expressed on all NK cells (Ly49G2, CD49b). We recently described that the cell surface expression of the activating NK cell receptor NKp46 (CD336) defines at best NK cells across mammalian species, providing the opportunity to look at NK cells *in situ* on tissue sections using anti-NKp46 antibodies (33).

In the spleen, NK cells are mostly found in the red pulp at a steady state, thus excluded from the T and B lymphocyte-rich area (defined by CD3 and CD19 staining) (*Fig. 2A*).

Staining for metallophilic macrophages [sialoadhesin (Sn)] (*Fig. 2B*) and marginal zone macrophages (SignR1) (*Fig. 2C*) reveals that a few NK cells are also present in the marginal zone but do not go beyond this zone toward the white pulp. Antibodies to NKp46 and CD31 [platelet-endothelial cell adhesion molecule-1 (PECAM-1), an endothelial marker] stain the same regions, suggesting that most splenic NK cells are in fact located inside blood sinuses (*Fig. 2D*). Occasional $NKp46^+$ cells are found in the white pulp, but staining for CD31 suggests that many of them are in fact located within sinuses or vessels (*Fig. 2D*). Many other cell types can be found in the vicinity of the 'NK cell zone'. In particular, most $CD11b^{high}$ cells (including macrophages) (*Fig. 2E*) are found in the red pulp and the marginal zone, whereas many $CD11c^{high}$ cells (including dendritic cells) (*Fig. 2F*) are found not only in the white pulp but also in the marginal zone and the red pulp. Cell–cell contacts between NK cells and macrophages (either of the red pulp or of the marginal zone) can be seen on these static images. These observations support the existence of interactions between NK cells and macrophages, as well as NK cells and dendritic cells. Such interactions have been extensively documented *in vitro* (34–37) and lead to the mutual regulation of these different cell types in the orchestration of immune responses.

At a steady state, NK cells are also found in the peripheral LN, mostly excluded from T- and B-cell zones (33, authors' unpublished data), consistent with previous studies (29). NK cells are found in perifollicular regions, in the paracortex, and especially in the medulla zone within lymphatic sinuses (29). In the perifollicular region, NK cells are found again in sinuses surrounding the follicles and especially between these follicles, at the site where presumably dendritic cells migrating from tissues arrive to the draining LN through an afferent lymph (29).

Within secondary lymphoid organs, the three types of lymphocytes (T, B, and NK cells) thus localize in distinct compartments. NK cells are preferentially found inside vessels or sinuses, either blood or lymphatics. The same conclusion could also be reached for the liver, as it is known that most hepatic lymphocytes are present in the sinusoids (38) and are absent from the parenchymal space.

NK cell development and maturation

Compelling evidence points to the BM as the primary site of NK cell development in adults at a steady state (6). Moreover, recent articles suggested that LN and thymus could be alternative sources of NK cells (39, 40). The relative contribution of these organs to the pool of NK cells is not known but is expected to be low at a steady state. Indeed, this extramedullary 'NK-poiesis' appears to produce phenotypically distinct NK cells, expressing the α chain of the IL-7 receptor (40). Such NK cells make up $< 5\%$ of the total NK cells. Moreover, a careful examination of thymic NK cells reveals that many of them express CD3 intracellularly and share phenotypic and functional features with $NK1.1^+$ T cells, suggesting that they are in fact NK-like T cells (41). However, in mice where T-cell development is blocked by genetic means, a very high number of NK cells develop in the thymus, showing the ability of T-cell precursors to develop into NK cells under particular conditions (40). Furthermore, the normal liver contains a substantial number of immature NK cells, suggesting that NK cell precursors originating in the BM could seed the periphery and develop *in situ* (42). Thus, it is possible that extramedullary NK cell development may take place under lymphopenic or other conditions that remain to be dissected.

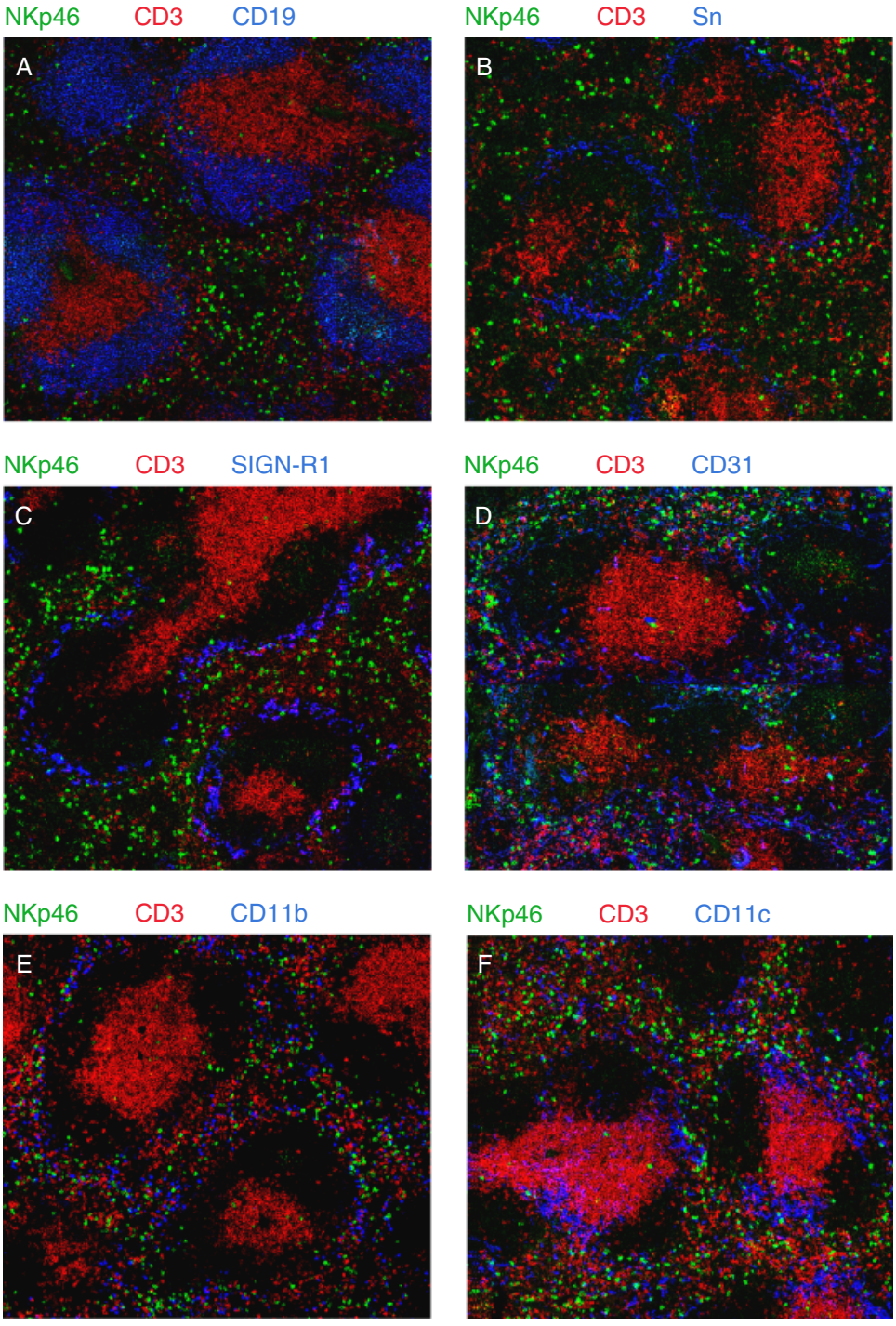

***Fig.* 2. Localization of natural killer (NK) cells in the spleen.** Frozen sections of spleen were fixed with acetone and stained with fluorescently coupled antibodies or biotinylated antibodies revealed with fluorochrome-coupled streptavidin. Anti-CD3 (145-2C11), anti-CD19 (1D3), anti-CD31 (MEC13-3), anti-CD11b (M1/70), and anti-CD11c (HL3) monoclonal antibodies were from BD Pharmingen (San Diego, CA, USA). Anti-sialoadhesin (MOMA-1 for metallophilic macrophages) and anti-Sign-R1 (ER-TR9 for marginal zone macrophages) monoclonal antibodies were obtained from AbD Serotec (Raleigh, NC, USA) and BMA Biomedicals (Augst, Switzerland), respectively. Polyclonal goat anti-NKp46 (R&D Systems, Minneapolis, MN, USA) was revealed with donkey-anti-goat antibody (Invitrogen, Carlsbad, CA, USA), Sections were visualized by confocal microscopy (Zeiss LSM 510 META, Iena, Germany). Panels A–F show representative images for the indicated staining.

Upon commitment to the NK cell lineage, NK cells go through a complex maturation process that leads to the gradual acquisition of effector functions. Three stages of NK cell maturation can be defined in the mouse based on the expression of CD11b and CD27: $CD27^{high}CD11b^{dull}$ (abbreviated as $CD11b^{dull}$, the most immature), double-positive

$CD11b^{high}CD27^{high}$ (abbreviated as DP), and $CD11b^{high}$ $CD27^{dull}$ (abbreviated as $CD27^{dull}$, the most mature) (43–45). DP and $CD27^{dull}$ NK cells display stronger effector functions than $CD11b^{dull}$ NK cells (113). The repartition of the three NK cell subsets varies with the tissue distribution. Whereas $CD11b^{dull}$ NK cells predominate in BM and LN, DP and especially $CD27^{dull}$ NK cells prevail in the blood, liver, spleen, and lung (44). As discussed later, the expression of different chemotactic receptors participates in the distinct homing capabilities of the NK cell subsets. In human, two subsets of NK cells have been described, $CD56^{dim}$ and $CD56^{bright}$ NK cells, that share many characteristics with $CD11b^{dull}$ and DP/$CD27^{dull}$ NK cells, respectively (45). This resemblance and other observations (12, 46) suggest that $CD56^{bright}$ could be precursors of $CD56^{dim}$ NK cells. Like their mouse counterparts, human $CD56^{bright}$ NK cells are preferentially found in the LN, whereas $CD56^{dim}$ cells are more abundant in the blood and spleen (12).

It is still unknown how and where NK cells complete their maturation. As the three NK cell subsets are found in all organs, it is likely that NK cell precursors or immature NK cells ($CD11b^{dull}$ stage) are seeded from the BM to the periphery, where they further develop *in situ* under the influence of micro-environmental factors.

NK cell recirculation

Few data are available on the recirculation of NK cells between organs. Do NK cells traffic through peripheral organs? Do they exit lymphoid and non-lymphoid organs, or do they reside in tissues? An early study (47) showed that upon intravenous transfer into recipient rats, radiolabeled LGLs preferentially homed into the alveolar walls of the lung and the red pulp of the spleen. LGLs were found, however, to be absent from the thoracic duct and LN (47). Later, adoptive transfer experiments in mouse showed that splenic NK cells can home to the spleen, liver, and BM of recipient mice (48–50). To gain an insight into NK cell trafficking, we performed an intravenous adoptive transfer of carboxyfluorescein succinimidyl ester (CFSE)-labeled splenocytes into syngeneic, non-irradiated recipient mice. Twenty-four hours post-transfer, the percentage of NK cells within lymphocytes was measured in the blood, spleen, LN, BM, lung, and liver. The results showed that the distribution of transferred NK cells paralleled that of recipient NK cells (*Fig.* 3), as did the distribution of $NK1.1^+$ T cells. Moreover, the relative proportion of NK cell subsets ($CD11b^{dull}$ to $CD27^{dull}$) was similar between recipient and donor NK cells in every organ (data not shown). This simple experiment shows that (i) splenic NK cells are not programmed to home to the spleen but instead recirculate through all NK cell-containing organs and (ii) NK cell recirculation appears to be subset-specific: LN and BM are preferentially repopulated by $CD11b^{dull}$ NK cells and the blood, spleen and lung by $CD27^{dull}$ NK cells.

The relative contribution of the factors that regulate tissue homing and egress, such as chemotactic receptors or homeostatic mechanisms, in the distribution of NK cells, remains to be addressed. Nevertheless, multiple approaches

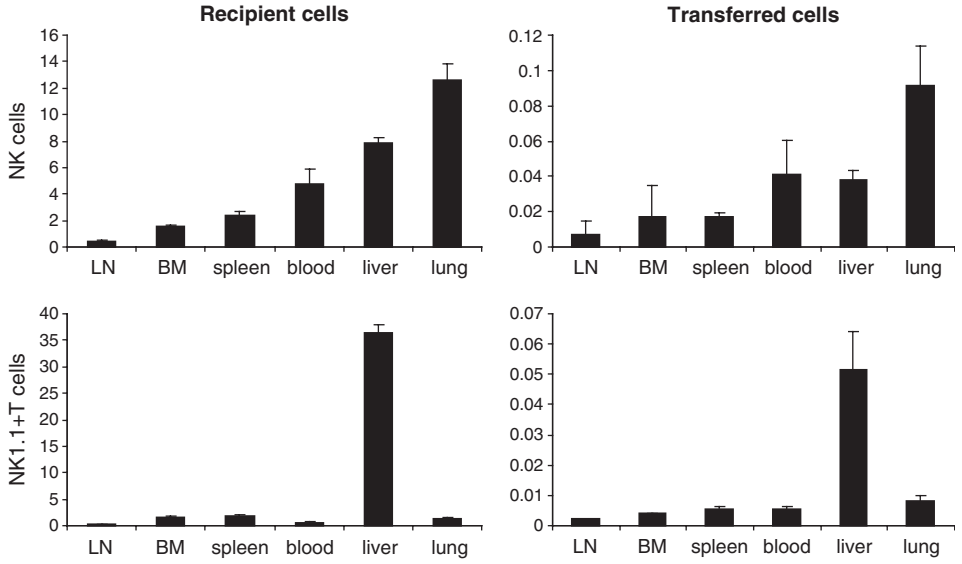

***Fig.* 3. Recirculation of natural killer (NK) cells.** C57BL/6 spleen cells were labeled with 3 μM 5-(and 6-)-carboxyfluorescein succinimidyl ester (CFSE) and injected retro-orbitally to C57BL/6 recipient mice. One day after transfer, lymphocyte populations were isolated from the indicated organs. The percentage of $NK1.1^+CD3^-$ (NK cells) and $NK1.1^+CD3^+$ ($NK1.1^+$ T cells) in gated $CFSE^+$ (transferred) and $CFSE^-$ (recipient) cells was measured by flow cytometry. Results are the mean of six transferred mice in two independent experiments.

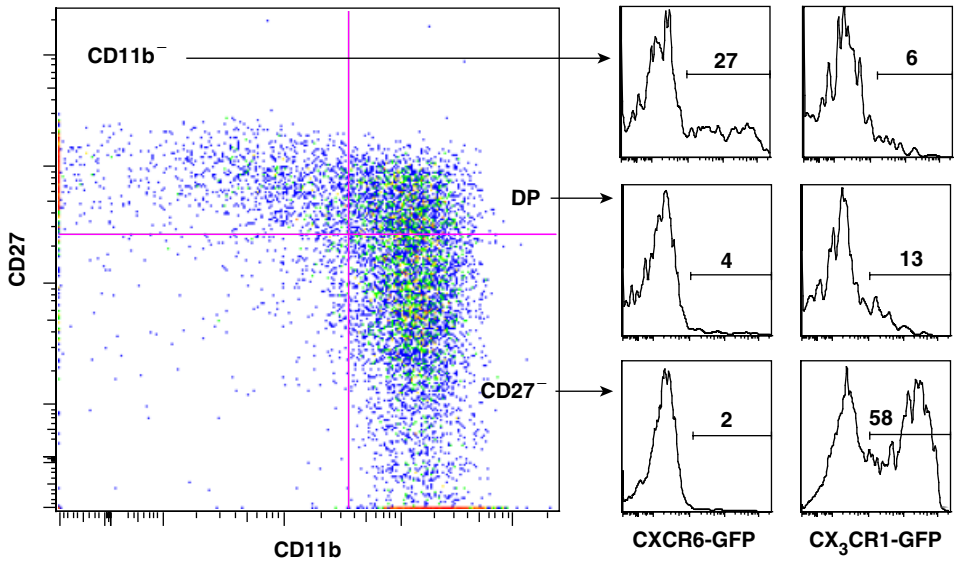

***Fig. 4.* Expression of CXCR6 and CX_3CR1 by mouse natural killer (NK) cell subsets.** Spleen cells from $CXCR6^{gfp/gfp}$ and $CX_3CR1^{gfp/gfp}$ knockin mice were stained for CD3, NK1.1, CD11b, and CD27 and analyzed by flow cytometry. Left panel shows the representative expression of CD11b and CD27 in gated $NK1.1^{+}CD3^{-}$ NK cells. NK cell subsets were gated as indicated and CXCR6-GFP or CX_3CR1-GFP was measured. Results shown are representative of three mice in each group.

have been used to address the expression of chemotactic receptors in NK cells. First, several studies have measured the expression of chemotactic receptors on NK cells by flow cytometry, when antibodies were available (51–57). Second, several strains of mice were created in which a green fluorescent protein cDNA was knocked in to genes encoding chemokine receptors (58, 59). Using such mice, we found that CXCR6 is only expressed by a fraction of $CD11b^{dull}$ NK cells, whereas CX_3CR1 is acquired with maturation, almost selectively expressed by $CD27^{dull}$ NK cells (*Fig.* 4).

Third, microarray experiments have been performed to measure gene expression at the pan-genomic level in human (60, 61) and mouse NK cell subsets (*Table* 1). The findings obtained with these different approaches reveal several trends (*Fig.* 5).

First, a strong similarity in the expression pattern of chemokine receptors on human and mouse NK cell subsets emphasizes the homology between mouse $CD11b^{dull}$ NK cells and $CD56^{bright}$ NK cells, on the one hand, and mouse DP/$CD27^{dull}$ and human $CD56^{dim}$ NK cells on the other. There are, however, important differences. In particular, CCR7 is expressed by human $CD56^{bright}$ NK cells, but no *Ccr7* transcripts are detected in mouse $CD11b^{dull}$ NK cells. This difference might account for the distinct representation of NK cells in LN in humans (5% of lymphocytes) and in mice (0.5% of lymphocytes). Second, NK cells are poised to be rapidly recruited on sites of inflammation, as they express receptors for a broad range of inflammatory chemokines (CCR2, CCR5,

***Table* 1. Relative levels of selectin and integrin transcripts in mouse natural killer (NK) cell subsets**

	other name	$CD11b^{-}$	DP	$CD27^{-}$
Integrin subunits				
Itga1	CD49a	+++	+++	+++
Itga2	CD49b (DX5)	++	+++	+++
Itga3	CD49c	+++	+++	+++
Itga4	CD49d	++	+++	+++
Itga5	CD49e	+++	+++	+++
Itga6	CD49f	++	++	+++
Itga7		+++	+++	+++
Itgae	CD103	++	++	+++
Itgal	CD11a	+++	+++	+++
Itgam	CD11b	**+**	**++**	**+++**
Itgav	CD51	+++	++	++
Itgax	CD11c	+++	+++	++
Itgb1	VLA-4 b	++	+++	+++
Itgb2	CD18	++	++	+++
Itgb3	CD61	**+++**	**++**	**+**
Itgb5		+++	+++	++
Itgb7		++	++	+++
Selectins				
L-selectin	CD62L	+++	+++	+++
P-selectin ligand	PSGL1	+++	+++	+++

Spleen cells from C57BL/6 mice were stained for NK1.1, CD3, CD27 and CD11b expression. NK cell subsets were sorted by flow cytometry, and total RNA was extracted using Qiagen RNAmicro kit (Valencia, CA, USA). The quality of total RNA was assessed using an Agilent Bioanalyzer (Santa Clara, CA, USA). Biotinylated antisense cRNA was prepared by using two cycles of *in vitro* amplification according to the Affymetrix Small Sample Labeling Protocol II (Affymetrix, Santa Clara, CA, USA). Biotinylated cRNA (15 μg) was fragmented and hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 arrays. All data analyses were performed by using Bioconductor version 1.5 for the statistical software R. Expression values were background corrected, normalized, and summarized by using the default settings of the *gcrma* package. Accession numbers: The complete microarray data set is available on the CIML website (Vivier lab: http://www.ciml.univ-mrs.fr/Lab/Vivier/Resource.htm). Changes in the level of expression between subsets are highlighted in bold.

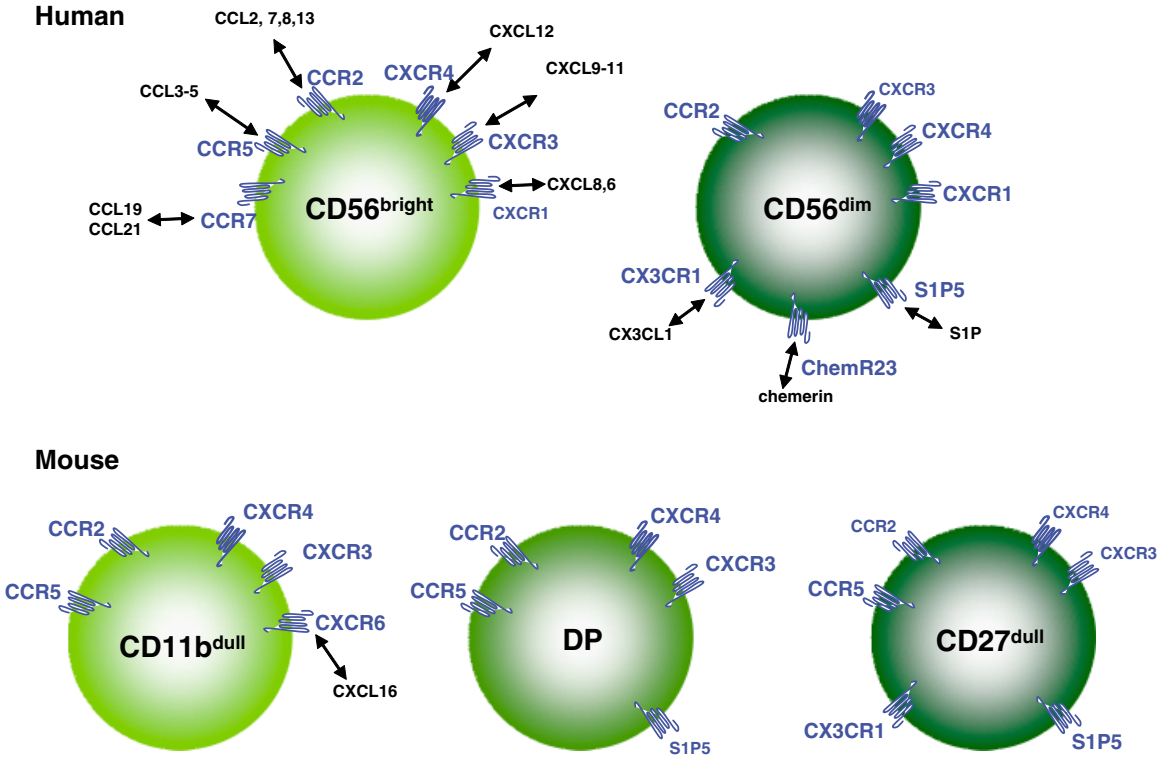

***Fig. 5.* Expression of chemotactic receptors by natural killer (NK) cells.** The pattern of expression of chemotactic receptors displayed by human (top) and mouse (bottom) NK cell subsets is based on the reported expression at the protein level by flow cytometry or at the mRNA level using microarray experiments (see references in text). Chemokine aliases: CCL19: ELC, CCL21: SLC, CCL3: MIP1-α, CCL4: MIP1-β, CCL5: RANTES, CCL2: MCP1, CCL8: MCP2, CCL7: MCP3, CCL13: MCP4, CXCL12: SDF1, CXCL9: MIG, CXCL10: IP10, CXCL11: I-TAC, CXCL8: IL-8, CXCL6: GCP-2, CX3CL1: fractalkine.

CXCR3, CX_3CR1). Third, the expression of chemotactic receptors switches over maturation in NK cells. In particular, $CD27^{dull}/CD56^{dim}$ NK cells lose CXCR3 (and also CXCR4 in mouse) but acquire CX_3CR1 (and also ChemR23 in human) expression, an array of receptors that are anticipated to influence their recirculation and their recruitment upon inflammation.

NK cell trafficking at a steady state

How do NK cells leave the bone marrow?

A role for CXCR4 in human NK cell homing to the BM of reconstituted NOD (nonobese diabetic)/SCID (severe combined immunodeficient) mice was reported (62), suggesting that loss of CXCR4 could contribute to the export of newly produced NK cells. However, CXCR4 seems to be uniformly expressed by NK cells from all organs. Moreover, a recent study showed that mouse treatment with a selective inhibitor of CXCR4 did not induce NK cell mobilization from the BM (63). Another study showed that NK cells were virtually absent from the periphery of CXCR3 knockout mice (64). However, three other studies did not report any defect in the distribution of NK cells in these mice (65–67), questioning the role of CXCR3 in the emigration of NK cells from BM. More recently, we found that NK cell maturation correlated with the acquisition of $S1P_5$, one of the five sphingosine-phosphate (S1P) G-protein-coupled receptors (113). S1P is a secreted lysophospholipid bound extensively to albumin and other plasma proteins. Coordinated activities of biosynthetic (sphingosine kinases) and biodegradative (sphingosine lyase and phosphatases) enzymes maintain S1P gradients *in vivo*, with high S1P concentrations in extracellular fluids and low S1P concentrations in tissues (68, 69). In $S1p_5$-deficient mice, a drastic decrease in peripheral NK cell counts is observed in the blood, spleen, and lung. This defective homing is NK cell-intrinsic and correlates with an increased number of NK cells in the BM and LN. $S1P_5$ operates in NK cells as a chemotactic receptor for S1P *in vitro*, promoting NK cell homing in the blood, spleen, and lung *in vivo*. The extent of NK cell accumulation in BM and LN correlates with the level of $S1P_5$ expression (113). Altogether, these observations suggest that $S1P_5$ provides an egress signal to NK cells, allowing both their export from the BM and their exit from LN (*Fig.* 6). Interestingly, $Id2^{-/-}E2a^{-/-}$ mice have normal BM NK cells but very few mature NK cells in the spleen (70), prompting an investigation

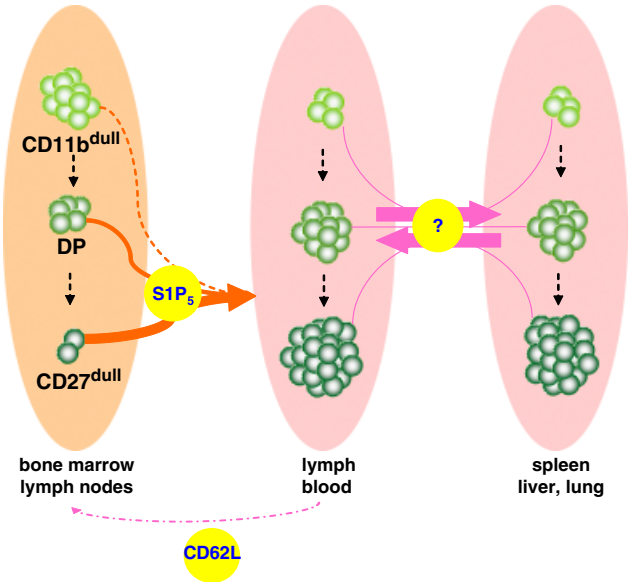

***Fig. 6.* Model of mouse natural killer (NK) cell circulation at steady state.** NK cells develop mostly in the bone marrow (BM) and, for the CD127^{+} fraction, also in lymph node (LN) and thymus. NK cells mature from the CD11b^{dull} stage to the double positive CD11b^{high}CD27^{high} (DP) and further to the CD27^{dull} stage in all organs, starting in the BM (vertical dotted arrows). Upon maturation, they acquire $S1P_5$ expression and exit the BM in a $S1P_5$-dependent manner. In this model, the more NK cells express $S1P_5$, the more they exit the BM. Once in the periphery, they may return to BM and LN, through a CD62L-dependent mechanism for the LN.

of whether these transcription factors control $S1P_5$ expression and/or function.

How do NK cells reach lymphoid organs?

Like other lymphocytes, NK cells enter LNs through high endothelial venules (HEVs) and the spleen through the marginal sinus. CD56^{bright} human NK cells express L-selectin (CD62L) that allows interaction with glycosylated L-selectin ligands on HEVs (71). CD62L is expressed at a similar level on mouse NK cell subsets isolated from the LN (44) and is required for mouse NK cell entry in LNs (72) (*Fig.* 6). Whether CD62L is sufficient for the entry is not known. As discussed previously, most mouse NK cells localize in the sinusoids around T and B-cell areas, a location reminiscent of that in spleen where NK cells are found in the red pulp surrounding the white pulp. One possibility is that this area is reached by default, in the absence of CCR7 expression. Consistent with this model, human LN CD56 bright NK cells that do express CCR7 are localized, at least in part, in the T-cell cortex (73). Recently, it was shown that CCR7 is induced on CD56^{dim} cells by IL-18 *in vitro* (74), suggesting that under certain conditions, these cells could also traffic to LN.

Transfer experiments have shown that peripheral NK cells may also return to the BM. Central memory T cells preferentially home in this tissue by a mechanism that depends both on CXCL12 (CXCR4 ligand) and on E/P/L selectins (75). Whether this is also the case for NK cells requires further investigation.

How do NK cells reach non-lymphoid organs?

The distribution of NK cell subsets in the blood, spleen, liver, and lung is very similar, with a majority of CD27^{dull} NK cells. $S1P_5$ deficiency affects NK cells from these compartments in the same way (113). This finding suggests, after their $S1P_5$-dependent export to the periphery, that NK cells are carried by the blood flow in a $S1P_5$-independent manner (*Fig.* 6). A fraction of CD11b^{dull} NK cells expressing CXCR6 is enriched in the liver. Similar to what was shown for NKT cells, these CXCR6 NK cells could patrol hepatic sinusoids through the interaction of CXCR6 with endothelium-bound CXCL16 (58).

NK cell trafficking under inflammatory conditions

Upon inflammation, NK mouse cells may be recruited in various organs such as the LN, lung, liver, or central nervous system (CNS), and can then extravasate in the parenchyma or cavities (6). The migration of leukocytes from the vascular lumen to tissues depends on a series of sequential molecular interactions between leukocytes and endothelial cells, involving selectins, integrins, and chemokine receptors.

Selectins

In the mouse, L-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) are expressed at similar levels on NK cell subsets (*Table* 1). L-selectin is required for mouse NK cell homing and recruitment to the LN (72). In human, CD56^{bright} but not mature CD56^{dim}CD16^{+} NK cells also express L-selectin (71, 76). Sialyl stage-specific embryonic antigen 1 and an uncharacterized sLe^{x}-bearing receptor may serve as E-selectin ligands (77, 78). Although PSGL-1 is expressed on freshly isolated human NK cells, only a minor population of NK cells binds P-selectin-immunoglobulin (Ig) (78, 79). Human NK cell differentiation is accompanied by the cell surface expression of a mucin-like glycoprotein bearing an NK cell-restricted keratan sulfate-related lactosamine, the PEN5 epitope (80). The PEN5 carbohydrate decorates PSGL-1, creating a unique binding site for L-selectin, which is independent of PSGL-1 tyrosine sulfation (76). By analogy to the ability of rolling neutrophils to capture free-flowing neutrophils in a PSGL-1:L-selectin-dependent manner (81), the PEN5 epitope

Table 2. **Experimental models of natural killer (NK) cell trafficking**

Stimulus	Organ where NK cells are recruited	Mechanisms	Reference
Human			
Granulomas in TAP2-deficient patients	Skin lung	**CCR2?**	Hanna *et al.* (93)
Oral lichen planus	Oral mucosa	**ChemR23**	Parolini *et al.* (57)
Hemolytic uremic syndrome	Kidney	**CX3CR1**	Ramos *et al.* (95)
Psoriasis	Skin	**CXCR3/CCR5**	Ottaviani *et al.* (20)
Menstruation	Uterus	**CXCR3?**	Sentman *et al.* (96)
Invasive trophoblasts	Decidua	**CXCL12/CXCR4**	Hanna *et al.* (94)
IL-18	LN?	**CCR7**	Mailliard *et al.* (74)
Mouse			
TLR7/8 ligands, injection sc	Draining LN	**CXCR3**	Martin-Fontecha *et al.* (87)
ConA-induced hepatitis	Blood, mobilization from spleen	**CXCR3**	Wald *et al.* (65)
ConA-induced hepatitis	Liver	**CCR1**	Wald *et al.* (91)
MCMV, i.p.	Liver	MCP1/**CCR2**	Hokeness *et al.* (83)
MCMV or poly(I:C), i.p.	Liver	MIP1-a/**CCR5?**	Salazar Mather *et al.* (89)
Toxoplasma gondii	Liver, spleen	**CCR5**	Khan *et al.* (85)
Dengue virus	Liver	CXCL10/**CXCR3?**	Chen *et al.* (82)
Bordetella bronchiseptica	Lung	**CXCR3**	Widney *et al.* (66)
Bleomycin-induced lung fibrosis	Lung	**CXCR3**	Jiang *et al.* (64)
Intracerebral coronavirus infection	CNS	CXCL10/**CXCR3?**	Stiles *et al.* (90)
MOG (s.c.)-induced EAE	CNS	**CX3CR1 not CXCR3**	Huang *et al.*, Liu *et al.* (84, 67)
None	Lung	**CX3CR1**	Yu *et al.* (92)
B16-F10	Lung	**CX3CR1**	Yu *et al.* (92)
Invasive aspergillosis	Lung	MCP1/**CCR2**	Morrison *et al.* (88)
EL4 tumor cells, s.c.	Tumor site	**CX3CR1**	Lavergne *et al.* (86)
HSV2 genital infection	CNS	**CCR5**	Thapa *et al.* (25)

Compilation of data from the literature describing the recruitment of NK cells in various organs in response to the indicated stimuli. In each case, the receptor involved is indicated in bold.
HSV, herpes simplex virus; CNS, central nervous system; MCMV, mouse cytomegalovirus; LN, lymph node.

might allow NK cells to interact with other L-selectin$^+$ leukocytes (e.g. neutrophils, monocytes, or T/B lymphocytes) attached to the inflammatory endothelium to amplify the immune response.

Chemotaxis

The role of chemokine receptors in mouse NK cell recruitment to inflammatory sites has been studied using knockout strains and blocking antibodies (25, 64–67, 82–92). In humans, the expression of chemokines and their receptors at inflammatory sites has also provided some information (20, 57, 74, 93–96) (*Table* 2).

Four receptors appear to play a key role in mouse NK cell recruitment following an inflammatory stimulus: CCR2, CCR5, CXCR3, and CX_3CR1. These receptors allow NK cells to respond to a large array of inflammatory chemokines such as CCL2, CCL3, CCL5, CCL7, CCL8, CCL9, CCL11, CCL13, CXCL9-11 and CX3CL1. This broad responsiveness could warrant NK cell recruitment in situations where limited sets of chemokines are expressed. Thus, NK cell recruitment in the same tissue can be mediated by different chemokine receptors. For example, human NK cell recruitment to the epithelia appears to be dependent on CCR2, CCR5, CXCR3, or ChemR23, depending on the inflammatory conditions (20, 57, 93). Not only may NK cells respond to different chemokines, but it also seems that chemokines may act in concert to recruit them. CCR2 and CCR5 are both required for NK cell recruitment to the liver of mouse cytomegalovirus (MCMV)-infected mice (83, 89). A similar phenomenon had been observed previously for monocytes (97). Importantly, NK cell subsets could also be differentially recruited, depending on the stimulus. Indeed, CX_3CR1 but not CXCR3 is required for NK cell recruitment in the CNS in a model of experimental autoimmune encephalomyelitis (67, 84). Conversely, CXCR3 has a central role in NK cell recruitment to the inflammatory LN (87). As CXCR3 and CX_3CR1 are expressed in a quasi-mutually exclusive fashion on NK cell subsets (*Fig.* 5), this finding suggests that different NK cell subsets may be independently recruited in distinct inflammatory settings. Such a division of labor has already been shown for other types of leukocytes, including monocytes (98) and memory CD8$^+$ T cells (99). Besides chemokines, NK cells have been shown to respond to various other chemotactic compounds that are either induced or augmented upon inflammation. These include lysophosphatidic acid (100), *N*-formyl-methionyl-leucyl-phenylalanine (f-MLP) (101), leukotrienes (102), and

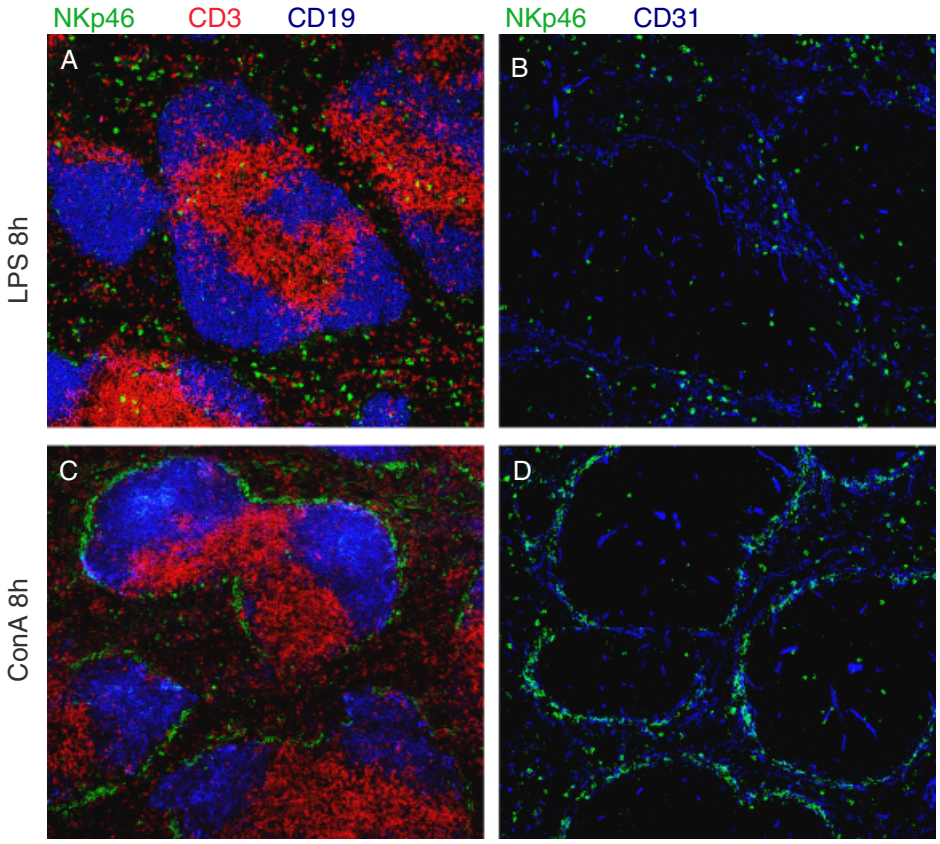

***Fig. 7.* Relocalization of natural killer (NK) cells in the spleen in response to Con-A injection.** C57BL/6 mice were treated with indicated stimuli [Con-A, Sigma (St. Louis, MO, USA): 300 μg i.v., lipopolysaccharide (LPS), Sigma (St. Louis, MO, USA), 25 μg i.v.] for the indicated times. Frozen sections of spleen were fixed with acetone and stained with anti-CD3, anti-CD19, (panel A, C) or anti-CD31 and goat anti-NKp46 antibodies (panel B, D), and goat anti-NKp46 antibodies, as described in *Fig.* 2. Sections were visualized by confocal microscopy.

C5a (103). The roles of these inflammatory mediators and of their receptors in NK cell recruitment *in vivo* remain to be thoroughly investigated.

Integrins

Upon activation by chemokines, NK cells interact firmly with the endothelium through integrins. The role of LFA1 (leukocyte function-associated antigen-1), as for other leukocytes, has been shown to be central in NK cell extravasation (104). Interestingly, NK cells sequentially express different integrins over development and maturation (43). To gain an insight into this phenomenon, we examined integrin expression at the mRNA level in sorted NK cells subsets, using microarrays. All NK cells express many transcripts of integrins involved in extravasation (*Table 1*). As reported previously, we found that CD49b and CD11b were acquired while CD51 was decreased upon NK cell maturation. Our data also show that CD11c and especially CD61 (β3 integrin) are decreased upon maturation. The significance of this observation is unclear, but different sets of integrins may contribute to the differential recruitment of NK cell subsets upon inflammation. Besides integrins, NK cell adhesion to the endothelium could also be mediated by CX_3CR1. Indeed, it was reported previously that CX_3CL1 mediates the rapid capture, integrin-independent firm adhesion, and activation of circulating leukocytes under flowthrough CX_3CR1 (105).

Recruitment of NK cells to the inflammatory liver

NK cells migrate to the liver in response to a variety of stimuli, such as injection of maleic anhydride divinyl ether (MVE-2), *Corynebacterium parvum* (106), poly(I:C) (89, 107, 108), MCMV (109), hepatitis B (110), or concanavalin-A (Con-A) (111). Concomitant to the increase in liver NK cells, NK cell counts decrease in the spleen and BM, suggesting that these organs serve as reservoirs of NK cells in case of inflammation (65). Various chemokine receptors may orchestrate this recruitment. CXCR3 is partly required in the mobilization of spleen NK cells, while CCR1 is required for the accumulation of NK cells in the liver (65). To gain an insight into the mechanism of spleen NK cell mobilization, we performed immunofluorescence experiments on frozen sections of spleens obtained from mice intravenously injected with lipopoly-

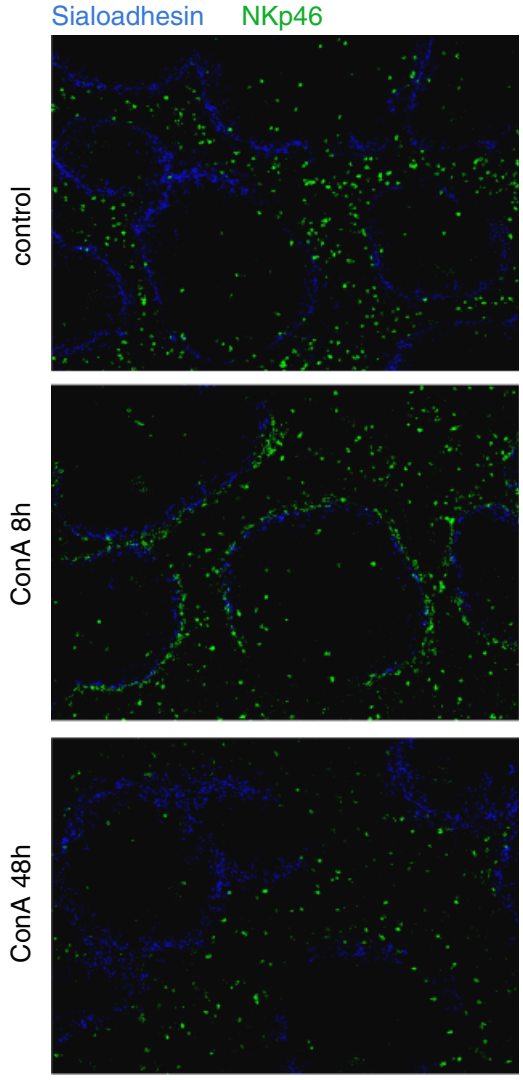

***Fig. 8.* Kinetics of natural killer (NK) cell response to Con-A injection.** C57BL/6 mice were treated with Con-A (300 μg) and sacrificed at the indicated times. Frozen sections of spleen were fixed with acetone and stained with anti-sialoadhesin and goat anti-NKp46 antibodies, as described in *Fig.* 2. Sections were visualized by confocal microscopy.

saccharide (LPS) or Con-A. A redistribution of splenic NK cells was observed in response to Con-A injection (*Fig.* 7).

NK cells appear to migrate from the red pulp to the marginal zone. This relocalization of NK cells was not observed when mice were treated with LPS, although this stimulus induces a massive redistribution of dendritic cells (*Fig. 7A* and data not shown). After Con-A injection, NK cells are thus found in close contact with marginal zone macrophages (*Fig.* 8). It will be of interest to test whether CXCR3 is involved in this process. This phenomenon is transient, as 48 h after Con-A injection, NK cell distribution in the spleen is normal (*Fig.* 8).

Recruitment of NK cells to the inflammatory LN

A massive recruitment of NK cells is observed in the draining LN in response to a footpad injection of *Leishmania major* (29), TLR 7/8 ligands, or LPS-activated dendritic cells (87). This recruitment is dependent on CXCR3 (87). CXCR3 ligands such as CXCL9 are expressed on the surface of inflammatory HEVs (112). Under inflammatory conditions, most NK cells are recruited preferentially to the T-cell zone of the LN, near HEVs, in close proximity to dendritic cells (29). NK cell recruitment is required for T-helper 1 cell polarization of naive T cells activated within the LN (87). In another system, it was found that CD62L was also required for the recruitment on the inflammatory LN in response to a subcutaneous injection of tumor cells (72).

Concluding remarks

The precise knowledge of the anatomy of the immune system is obviously critical for our understanding of immunity. In contrast to T, B, and dendritic cells, the trafficking of NK cells in normal and disease conditions is poorly characterized. Nevertheless, it appears that NK cells patrol lymphoid and non-lymphoid organs. At a steady state, NK cells are present at a high frequency in the circulation, ready to extravasate to tissues under inflammatory conditions. Besides the roles of $S1P_5$ and chemokine receptors, the set of molecules that govern NK cells trafficking *in vivo* remain to be identified. The dissection of the mechanisms that regulate NK cell migration may likely provide new perspectives for the manipulation of these cells for therapeutic purposes, as exemplified by the use of the S1P agonist, FTY720, as a T- and B-cell immunosuppressant.

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