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Chapter Ten

The localization and migration of natural killer cells in health and disease

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As you set out for Ithaca hope your road is a long one, full of adventure, full of discovery.
Ithaca, by Konstantinos P. Kavafis

ABSTRACT

Natural killer (NK) cells comprise a finite lymphocyte lineage with distinctive gene expression patterns. The unique transcriptome of NK cells renders them capable of protecting the host from a vast array of disease states. Their undisputed importance in host protection is conferred by their ability to eliminate unhealthy cells. However, in order for NK cells to exert their effects, they need to be strategically located at the right places. Furthermore, within their anatomical microenvironment, NK cells must be able to freely move and receive stimuli that will direct their migration between different organs. This chapter will provide an overview of our current understanding of the localization of NK cell populations and their ability to migrate in response to homeostatic and pathological conditions.

KEY WORDS

Natural killer cells, Migration, Chemokines, Lymphoid tissue, Localization

Introduction

Cell migration occurs mainly due to the action of cellular chemo-attractants that are usually in the form of small proteins, collectively known as chemokines. Chemokines bind to specific receptors that are expressed by immune cells and can only exert their effects if the cell expresses the specific receptor. However, immune cells express an array of chemokine receptors at differing levels, depending on their activation status, which makes them multi-chemokine responders. Thus, the ability to react to a number of chemokines provides cells of immunological significance with the capacity to be recruited to wherever they are needed.

Controlled migration of immune cells is a fundamental process for the development, homeostasis and function of the immune system. B and T lymphocytes undergo continuous re-circulation via blood and lymph to secondary lymphoid tissues (spleen, lymph nodes...
and mucosal-associated lymphoid tissues) in response to chemokines expressed in fixed stromal cell populations that both attract and guide their specific interactions. For example, during lymph node (LN) organogenesis, lymphoid tissue inducer (LTi) cells populate the LN (Mebius, 2003) or spleen (Withers et al., 2007) allowing where they interact with stromal cells and activate them to produce B and T cell-specific chemokines. This attracts B and T lymphocytes to defined B and T zones and initiates the formation of secondary lymphoid organs. This example clearly demonstrates the need for controlled cell migration.

Natural killer (NK) cells develop in the bone marrow (BM) (Haller et al., 1977) and are not static but populate secondary and primary lymphoid organs. A unique feature of NK cells is their expression of activating and inhibitory receptors, which allow them to respond either when ligands for activating receptors are upregulated or when ligands for inhibitory receptors are downregulated (Yokoyama and Plougastel, 2003).

There have been significant advances in our understanding of NK cell migration. This was primarily achieved by the progress of in situ cell-tracking systems, real-time live microscopy and flow methods that allow identification of NK cells in lymphoid- and non-lymphoid organs. In this chapter, we will describe the anatomical location of different NK cell subsets and how they are recruited, with emphasis on chemokines and their receptors that are involved in mobilizing NK cells during health and disease.

**NK cells in secondary lymphoid organs**

While trafficking around the body, B and T cells perform necessary ‘pit-stops’ within secondary lymphoid organs, such as the LNs, the spleen, and the mucosal associated lymphoid tissue (MALT), where they scan for trapped antigen and engage in cognate interactions with accessory cells, such as dendritic cells (DCs). Within the lymphocyte rich areas, B and T cells respond to chemokine signals and segregate to distinct B follicles and T cell zones, respectively. This B/T segregation allows lymphocytes to move in an organized fashion (Bajenoff et al., 2006b) and to help find each other for the development of effective adaptive immune responses. NK cells are classically characterized as innate lymphocytes, implicating that they can be readily activated without the need for a high degree of organization in the surrounding lymphoid environment. However, there is compelling evidence that secondary lymphoid organs contain unique NK cell subsets with distinct phenotype and function. Here we describe NK cell subpopulations in the LN and the spleen. MALT will not be discussed in this section because there is little evidence that NK cells are directly associated with the lymphoid follicles found in such tissues, despite their undisputed presence and importance in the surrounding microenvironments. In later sections, however, we will discuss gut and lung NK cell populations.

**Lymph node NK cells**

Peripheral blood human NK cells can be largely subdivided into CD56dimCD16+ and CD56brightCD16–/dim (Benedict et al., 2001; Campbell et al., 2001). The former are highly cytotoxic, whereas the latter have lower natural cytotoxicity but produce an abundance of cytokines (Benedict et al., 2001). Early attempts to further characterize differences between the two subsets showed that only CD56bright cells expressed constitutively and at high levels the adhesion molecule L-selectin (Frey et al., 1998). Moreover, L-selectin expressing CD56bright NK cells bind to high endothelial venules (HEVs), which constitute the point of entry of lymphocytes into the LN (Frey et al., 1998). L-selectin, together with the chemokine ligand for CCR7, CCL21 facilitate cell transmigration within the LN through HEVs (Ansel et al., 1999; Warnock et al., 1998, 2000). Expression of L-selectin by peripheral CD56bright NK cells was later confirmed independently, and it was additionally shown that this population, and not CD56dim NK cells, expressed CCR7 and were capable of migrating towards CCL21 gradients (Campbell et al., 2001). It has also been shown that stimulation of human peripheral blood NK cells with interleukin-18 (IL-18) upregulates their expression of CCR7 (Mailliard et al., 2005). This provided evidence that NK cells might enter the LN, a specialized secondary lymphoid organ. Immunohistological and in situ hybridization screening of normal human LN tissue sections revealed that the T cell areas contained CD56+ cells that lacked expression of CD3 (Fehniger et al., 2003; Ferlazzo and Munz, 2004). Further analysis showed that NK cells in LN tissue were CD56brightCD16–, confirming the hypothesis that these cells, through their expression of CCR7 and L-selectin, could home to LNs. Importantly, CD56bright NK cells in the LN expressed different sets of activating and inhibitory receptors, and their low cytotoxicity could be restored upon stimulation with IL-2 (Ferlazzo and Munz, 2004), providing further evidence that NK cells in secondary lymphoid organs were distinct from those populations found in other locations. Endogenous IL-2 is believed to be provided by resident T cells (Fehniger et al., 2003; Ferlazzo and Munz, 2004), whereas DCs residing in the T cell areas play a role in promoting interferon-γ (IFN-γ) expression and proliferation of CD56bright NK cells through IL-12 and IL-15, respectively (Ferlazzo and Munz, 2004).
Following their discovery, two hypotheses were proposed for the derivation and localization of CD56\textsuperscript{bright} NK cells within the LN. Firstly, that CD56\textsuperscript{bright} cells developed in the BM and by virtue of their expression of L-selectin and CCR7 migrated to LN. The alternative hypothesis suggested that a precursor residing in the LN was giving rise to CD56\textsuperscript{bright} NK cells in response to stimuli. Whereas there is no evidence rejecting the first hypothesis, Caligiuri and co-workers have identified a CD34\textsuperscript{dim}CD45RA\textsuperscript{+}CD43\textsuperscript{bright} hematopoietic progenitor that develops in the BM and migrates to the LN, where it gives rise exclusively to CD56\textsuperscript{bright} NK cells in response to T cell produced IL-2 or IL-15 (Freud et al., 2005).

Mouse LNs contain less NK cells compared to man. However, in vivo activation studies have shown that they can rapidly migrate into antigen-draining LNs (Martin-Fontecha et al., 2004). Unlike in humans, the migration of mouse NK cells to the LNs depends on the chemokine receptor CXCR3 and not CCR7, although L-selectin is required for NK cell entry into the LNs of both species (Adam et al., 2005; Martin-Fontecha et al., 2004). Thus, CXCR3-deficient NK cells poorly migrated into LNs, whereas CCR7-deficient did not show any migratory defect (Martin-Fontecha et al., 2004).

As described previously, CD56 expression distinguishes two human NK cell subsets. In mice, expression of CD27 gives a similar picture, though distinctly different functionally (Hayakawa and Smyth, 2006). Thus, CD27\textsuperscript{high} NK cells are very effective killers and produce high amounts of cytokines in response to activation. In contrast, CD27\textsuperscript{low} NK cells present with low cytotoxicity and cytokine secretion, which could be attributed to their increased expression of inhibitory receptors (Hayakawa and Smyth, 2006). Markedly, CD27\textsuperscript{high} and not CD27\textsuperscript{low} mouse NK cells express CXCR3, respond to its ligands, CXCL10 and CXCL11, and can be found residing in or recruited to the LN (Hayakawa and Smyth, 2006; Watt et al., 2008). Detailed fluorescence and intra-vital 2-photon microscopy showed that LN NK cells are motile, they are located both in the T zone and the medulla and actively engage in interactions with DCs and MHC-mismatched cells (Bajenoff et al., 2006a; Garrod et al., 2007). Furthermore, in response to infection, LN NK cells were redistributed, locally produced cytokines and productively interacted with infected cells (Bajenoff et al., 2006a). Collectively, the significance of these studies lies in the fact that, like the T cell, which continuously scans for antigen, the life of the NK cell within the LN is highly dynamic.

**Splenic NK cells**

The spleen is the prototype secondary lymphoid tissue found in all vertebrates with an adaptive immune system in contrast to LNs that are almost exclusive to mammals. In addition to adaptive immunity that develops in the lymphocyte-rich white pulp areas, the dark grey pulp of the spleen is a meshwork of sinusoïds lined by macrophages that filter the blood of dead or dying erythrocytes and phagocytose pathogens (Mebius and Kraal, 2005). The lymphoid compartment of the spleen, the white pulp (named after its white colour in appearance due to the presence of white blood cells) is embedded within the dark grey pulp. Lymphocytes enter through the central arteriole and marginal sinus and exit via blood veins in the dark grey pulp. Whereas CCR7 and CCL21 facilitate cell migration in the splenic dark grey pulp, L-selectin (which is important for mouse and human NK cell recruitment to the LN) is not necessary. Consequently, while characterizing differences in NK cells between blood and secondary lymphoid organs, it became apparent that the spleen contained a mix of populations that could be present in both blood and LN. Researchers studying human NK cells found that the spleen contained both CD56\textsuperscript{bright} and CD56\textsuperscript{dim} populations, although on average CD56\textsuperscript{dim} cells were more abundant. Furthermore, CD56\textsuperscript{bright} cells were more prevalent in the spleen than in blood, suggesting preferential recruitment to this organ (Ferlazzo and Munz, 2004). In accordance, mouse spleen contains both CD27\textsuperscript{bright} and CD27\textsuperscript{low} NK cells, whereas as mentioned before, the LN contains almost exclusively CD27\textsuperscript{high} NK cells.

Early work with human splenic tissue showed that NK cells localize mainly in the dark grey pulp and occasionally in the marginal zone of the spleen (Garni-Wagner et al., 1990; Kummer et al., 1995). An analogous picture is observed in the spleen of rodents (Andrews et al., 2001; Bekiaris et al., 2008; Brown et al., 2001; Gregoire et al., 2007, 2008; Salazar-Mather et al., 1996; van den Brink et al., 1991). In situ identification of mouse NK cells shows that although under naïve conditions their preferred location is the dark grey pulp, they can migrate to the marginal zone upon type I IFN signals (Salazar-Mather et al., 1996). In murine cytomegalovirus (MCMV) infection where NK cells play a crucial role in early defence, infection results in the specific migration of NK cells from the dark grey pulp to the marginal zone and the white pulp (Andrews et al., 2001; Bekiaris et al., 2008; Brown et al., 2001; Gregoire et al., 2008) (Figure 10.1). Whereas recruitment of NK cells to splenic dark grey pulp is independent of chemokine receptor signalling (Gregoire et al., 2008), migration from the dark grey pulp to the white pulp depends on CXCR3, both during MCMV infection (Bekiaris et al., 2008) and during activation of the IFNα/β pathway (Gregoire et al., 2008). Due to the heterogeneity of splenic NK cell populations, it remains to be elucidated whether a specific subset is more potent at migrating towards the lymphoid white pulp, where it can interact with T zone resident DCs and primed B and T cells.
Gut NK cells

The gut contains organized lymphoid structures called Peyer’s Patches (PPs) immediately under the epithelial surface of the lamina propria. Like the spleen and LN, PPs have distinct T and B cell zones and promote strong immune responses. An important feature of the gut is that lymphocytes, including NK cells, reside within the intraepithelial space and are thus termed intraepithelial lymphocytes (IELs).

Initial studies by Tagliabue et al. showed that human IELs as well as lamina propria lymphocytes were able to eliminate NK-sensitive targets but were non-cytotoxic against NK-insensitive targets (Tagliabue et al., 1981, 1982), providing early evidence that gut contained NK cells. Besides, the same investigators could detect cytotoxicity but not NK cell activity in PPs (Tagliabue et al., 1983). Independent reports also supported the presence of intraepithelial NK cells and suggested possible differences from peripheral NK cells, such as a higher sensitivity to IL-2 stimulation and the requirement for prolonged contact with the target to exert cytotoxicity (Gibson and Jewell, 1985; Mowat et al., 1983).

Later analyses also identified NK cell activity in IELs (Kato et al., 1995), whereas IL-2 could induce the expression of the gut-homing integrin α4β7 (Baker and Wood, 1992; Berlin et al., 1993; Schweighoffer et al., 1993) on the surface of human blood NK cells (Perez-Villar et al., 1996) indicating that upon activation in the periphery, NK cells can gain the capacity to migrate to the gut. Furthermore, CCL21 was shown to be partly responsible for recruiting lymphocyte subsets, including NK cells, to PPs via a mechanism that involves binding of α4β7 to MAdCAM-1 (mucosal address in cell adhesion molecule-1) (Pachynski et al., 1998).

Intestinal epithelial cells are able to respond to infection and produce a number of cytokines. Among them, IL-15 has been shown to be a major activator of intraepithelial NK cells inducing potent perforin-mediated killing (Fukuyama et al., 2002). Thus, an attractive regulatory mechanism was proposed according to which infected epithelium could immediately and locally activate neighbouring NK cells through the production of IL-15 and quite possibly other inflammatory cytokines. Furthermore, the normal function and maintenance of intraepithelial NK cells also depended on normal expression of the surface phosphatase CD45 (Martin et al., 2001).

Recent evidence has identified potential roles of intraepithelial NK cells in both gut infections and autoimmune disease. Data from the mouse model of the helminth Trichinella spiralis show that infection is associated with IL-13-mediated gut pathology, and that the main IL-13-producing cell was the intraepithelial NK cell (Bienenstock and McDermott, 2005), linking thus infection-induced intestinal immunopathology with gut-resident NK cells. Furthermore, in the absence of protective Th2 immunity against infection with Trichuris muris, NK cells (and CD4 T cells) in the gut produce IL-13, which contributes to protection, in an IL-18-dependent mechanism (Fan et al., 2006).

In addition, intraepithelial NK cell have been associated with intestinal autoimmune disease, such as inflammatory bowel disease (IBD) and colitis. Mouse models have indicated that dysregulated immune responses to gut commensal bacteria can cause IBD-like disease (Keilbaugh et al., 2005). To this end, it was shown that colonization of the gut by commensal bacteria induces upregulation of IFNγ by NK cells (Keilbaugh et al., 2005). Interestingly, this only occurred in severe combined
immunodeficient and not in immunosufficient animals (Keilbaugh et al., 2005), suggesting that adaptive immune cells could negatively regulate NK cell activation. The opposite is also true. Thus, in a model of colitis, NK cells produce (1) the early IFN-γ that polarizes CD4 T cells towards the pathological Th1 phenotype and (2) the chemokine CXCL10, which attracts CXCR3⁺ cells, including more Th1 cells (Singh et al., 2008).

Exciting new research has identified unique gut NK cell subsets in the lamina propria as well as in cryptopatches (Luci et al., 2008; Sanos et al., 2008). These NK cells expressed low levels of CD122 and CD11b, but they were bright for CD27 and could be either NK1.1low or NK1.1high (Luci et al., 2008; Sanos et al., 2008). Functionally, they presented with almost undetectable cytotoxicity and very low capacity to produce IFN-γ, but they could secrete IL-22, which is involved in tissue repair. Interestingly, these NK cells expressed molecules related to LTi cells (Kim et al., 2003), including IL-7Rα, c-Kit, a number of tumor necrosis factor (TNF) ligands, and the transcription factor RORγt. Further research will characterize the importance of these NK cell subsets in health and disease.

Lung NK cells

Cytotoxicity assays from crude lymphocyte preparations of murine lung indicated the presence of NK cells (Puccetti et al., 1980). It was then further shown that the use of anti-NK cell depleting antibodies decreased lung cell cytotoxicity, whereas influenza infection increased cytotoxicity, providing evidence for resident NK cells in the mouse lung (Stein-Streilein et al., 1983). Further experiments with mice showed that lung NK cells can present with higher cytotoxic activity than blood and spleen populations (Wiltrout et al., 1985), while depletion of NK cells from the lung led to the increased susceptibility and mortality of mice infected with the influenza virus (Stein-Streilein and Guffee, 1986). In addition, human lung lymphocytes displayed cytotoxic activity (Bordignon et al., 1982), although resting lung NK cells were only capable of killing the target following stimulation (Robinson et al., 1984). It was also shown that alveolar macrophages could potently inhibit lung NK cell activity but had little effect on peripheral blood NK cells (Roth and Golub, 1989), suggesting that in the lung, NK cells might represent a different subtype that responds to different regulatory mechanisms.

In the mouse, NK cells can also be actively recruited to the lung following immunization (Fogler et al., 1996) and have been implicated in a vast array of bronchial disease models. Thus, in allergen-induced pulmonary airway inflammation, NK cells were found to induce the infiltration of both eosinophils and T cells (Korsgren et al., 1999). NK cells could also induce pulmonary inflammation during infection with Streptococcus pneumoniae (Kerr et al., 2005). Moreover, the cytokine IL-18 in combination with IL-2 is associated with lethal lung injury due to uncontrolled NK cell infiltration (Okamoto et al., 2002). IL-18 has also been shown to promote lung NK cell activity during influenza virus infection (Fan et al., 2006) as well as after intranasal challenge of herpes simplex virus (HSV)-1 (Reading et al., 2007). Infection with the intracellular pathogen Mycobacterium tuberculosis also results in local lung NK cell activation, however, their importance in clearing the infection is not clear yet (Feng et al., 2006; Junqueira-Kipnis et al., 2003). Nevertheless, lung NK cells appear to be very important for resistance against Staphylococcus aureus infection (Desanti et al., 2008).

In addition to controlling infection, NK cells in the lung present a very important line of defense against cancer. Thus, there is a direct correlation between the number of lung NK cells in patients with primary squamous cell adenocarcinoma: The higher the NK cell numbers, the better the survival (Villegas et al., 2002). Interestingly, when lung adenocarcinoma was deficient for MHC-I expression, the result was inhibition of killing this tumour but not other tumour cell lines or targets (Le Maux Chansac et al., 2005), suggesting that the tumour itself or its surrounding microenvironment can influence the functional capacity of NK cells.

NK cells in the lung therefore comprise a very important line of defense both against infection and malignancy.

Liver NK cells

One of the main NK cell reservoirs in humans and rodents is the liver. In fact, NK cells were first described in the rat liver, as large granular cells residing in the sinusoids and were named Pit cells (Doherty and O’Farrelly, 2000; Wisse et al., 1976). Their presence has been confirmed in both mouse (Doherty and O’Farrelly, 2000; Wiltrout et al., 1984) and human (Doherty and O’Farrelly, 2000; Doherty et al., 1999). NK cells comprise almost 30% and 20% of total hepatic lymphocytes in human (Doherty and O’Farrelly, 2000) or mouse, respectively (Smyth et al., 2001). Normal human liver contains approximately equal numbers of NK and T cells (Doherty and O’Farrelly, 2000; Doherty et al., 1999; Hata et al., 1991, 1992); however, during malignancy, NK cells can constitute more than 50% of total liver lymphocytes (Takii et al., 1994), suggesting an important role of NK cells in liver cancer. Hence, early experiments revealed that lack of NK cells is associated with significantly increased liver metastasis (Wiltrout et al., 1985). It has been shown that besides utilizing chemokines (discussed later), NK cells migrate and
enter the liver through interactions with vascular cell adhesion molecule-1 (VCAM-1) (Fogler et al., 1996, 1998), while NK cells that are deficient in their expression of the transcription factor GATA-3 have impaired capacity to migrate to the liver (Samson et al., 2003).

Besides the fact that the liver is enriched in NK cells, there is significant evidence showing that hepatic NK cells are functionally distinct from peripheral blood or splenic NK cells. Thus, murine hepatic but not splenic NK cells could lyse otherwise NK-insensitive target cell lines (Cohen et al., 1985) and be presented with a generalized increased activity (Magilavy et al., 1987; Wiltrout et al., 1985). Experiments using primary freshly isolated human NK cells from liver samples have also concluded that hepatic NK cells have a substantial increase in activation when compared to peripheral blood populations (Hata et al., 1991, 1992). It has been proposed that the differences in liver NK cell activity is due to the different microenvironment by comparison to blood and spleen, and especially due to the presence of liver-resident macrophages, the Kupffer cells (Alba et al., 2008; Vanderkerken et al., 1995). In agreement with this hypothesis, depletion of Kupffer cells results in reduced NK cell numbers, while culture of NK cells with media that were pre-conditioned with Kupffer cells showed increased cytolytic capacity (Vanderkerken et al., 1995). Furthermore, Kupffer cells isolated from human liver responded to Toll-like receptors (TLRs) and could induce the production of IFN-γ by NK cells in a contact-dependent manner (Alba et al., 2008). Interestingly, certain TLR ligands could stimulate Kupffer cells to secrete IL-10, which could in turn inhibit NK cell IFN-γ production (Alba et al., 2008). Therefore, Kupffer cells have an active and very important role in regulating NK cell activation in the liver.

The presence of NK cells in the liver indicates a role for anti-viral protection. Subsequently, it has been shown that NK cell depletion is associated with increased replication of MCMV, vaccinia virus and mouse hepatitis virus (Bukowski et al., 1983). Extensive in vivo mouse work has linked MCMV replication in the liver with NK cell activation and migration by type I IFNs and a family of chemokines (see Section CC chemokines). Strong associations between NK cells and hepatitis C virus (HCV) infection have also been reported. Accordingly, it has been shown that inhibitory NK receptors can confer relative resistance to HCV if their ability to inhibit is diminished (Khakoo et al., 2004). In addition, by comparison to healthy individuals, the numbers of NK cell populations in cirrhotic livers of chronic HCV-infected patients were decreased, whereas blood NK cell numbers were not affected (Kawarabayashi et al., 2000). On the other hand, peripheral NK cells from HCV-infected people were incapable of inducing the maturation of DCs because they produced higher levels than normal IL-10 and TGFβ, two important anti-inflammatory cytokines (Jinushi et al., 2004). These NK cells expressed increased levels of the inhibitory receptor complex CD94/NKG2A, which when blocked, resulted in reduction of both IL-10 and TGFβ and restoration of NK cell mediated DC maturation (Jinushi et al., 2004). It is apparent therefore that a liver-specific virus, HCV, is firstly actively controlled by NK cells and secondly can cause disturbances in NK cell subsets both locally and in the periphery.

In contrast to HCV causing liver NK cell numbers to drop (Kawarabayashi et al., 2000), infection with Plasmodium yoelii is followed by higher numbers of liver NK cells but reduced splenic NK numbers, which also lose their ability to protect (Roland et al., 2006) and further demonstrate the significant differences between NK cells in the liver and other organs.

NK cells have also been associated with protection against hepatitis B virus (HBV) (Webster et al., 2000), though a direct correlation is lacking. Nevertheless, there is evidence implicating hepatic NK cells in immune-mediated liver pathology that is common in HBV infection (Dunn et al., 2007). Liver NK cells uniquely express surface TRAIL (TNF-related apoptosis inducing ligand) (Smyth et al., 2001), which binds the TRAIL death-inducing receptors and induces apoptosis of target cells (Dunn et al., 2007). Thus, chronic HBV patients have elevated TRAIL expression on NK cells, whereas hepatocytes show induction of TRAIL receptors that renders them sensitive to NK killing, which in turn will cause liver damage (Dunn et al., 2007). Importantly, mouse studies have shown that a combination of TRAIL expression and lack of inhibitory receptors from liver NK cells makes them capable of syngeneic hepatocyte killing (Ochi et al., 2004), a property that is likely to contribute to liver injury. On the other hand, TRAIL-expressing hepatic NK cells can eliminate TRAIL-sensitive tumours, and as a result, they can protect against cancer metastasis into the liver, which is significantly increased when TRAIL is blocked (Smyth et al., 2001). In human liver NK cells, TRAIL has been reported to be expressed after IL-2 stimulation (Ishiyama et al., 2006).

The liver therefore contains phenotypically [TRAIL expression and lack of CD16 in humans (Hata et al., 1991)] and functionally distinct NK cell populations. The significance of this finding is that it suggests either specific recruitment of subpopulations of NK cells, or alternatively, modification of their phenotype and function by the local microenvironment in response to infection and malignancy.

**Thymic NK cells**

Early studies suggested that in vitro culture of human thymocytes resulted in the generation of NK-like cells
that could eliminate NK-sensitive targets (Blue et al., 1987), while culture with recombinant IL-2 generated CD56\textsuperscript{+} cells that lacked CD16 or CD3 and possessed NK cell activity (Ramsdell and Golub, 1987). Cells with NK cell activity and phenotype were also later described in mouse thymus, using animals that lacked all B and T cells (Garni-Wagner et al., 1990). In situ identification of NK cells in the adult murine thymus locates them both in the cortex and the medulla (Brown et al., 2001). Recent work in the mouse has provided substantial evidence that thymic NK cells constitute a unique population (Di Santo and Vosshenrich, 2006). Thus, NK cells in the thymus express high levels of IL-7R\textalpha and the transcription factor GATA-3, and, consequently, a deficiency in IL-7 or GATA-3 diminishes their production and homeostasis. Furthermore, they are negative for CD16, and low for CD11b and Ly49 receptors, but high for CD69, whereas functionally they resemble human CD56\textsuperscript{bright}CD16\textsuperscript{−} NK cells—that is decreased natural cytotoxicity but increased cytokine production. The authors additionally discovered that human CD56\textsuperscript{bright}CD16\textsuperscript{−} NK cells expressed more IL-7R\alpha and GATA-3, while the development of murine thymic NK cells completely depended on GATA-3 and IL-7 expression.

Moreover, human fetal thymus contained committed NK cell progenitors (Sanchez et al., 1993) as well as T/NK bi-potent progenitors (Ikawa et al., 1999; Sanchez et al., 1994) that can be identified by their expression of CD34 (Sanchez et al., 1994). Bi-potent T/NK progenitors in the thymus differentiate preferentially to the T lineage. NK cell differentiation depends on the availability of the cytokines IL-2 and IL-15 (Leclercq et al., 1996) and the expression of the transcription factor Id3 (Heemskerk et al., 1997). Thus, IL-15 (Leclercq et al., 1996) and Id3 (Heemskerk et al., 1997) are positive regulators of NK cell differentiation from thymic T/NK bi-potent progenitors. Surprisingly, the fetal murine thymus contains NK cells that express activating and inhibitory receptors and are capable of direct target lysis ex vivo (Carlyle et al., 1998).

Despite the fact that considerable work has elucidated the developmental requirements for thymic NK cells, their precise function during health and disease remains to be determined.

**NK cell migration**

All the preceding information suggests that the typical NK cell stems from the BM and then migrates to various organs where it rests until it encounters a danger signal (Table 10.1). This, however, is oversimplified. First, it is now well appreciated that NK cells undergo homeostatic proliferation. Thus, there is constant NK cell turnover (Jamieson et al., 2004), which is increased substantially in lymphopenic environments (Jamieson et al., 2004; Prlic et al., 2003). Importantly, NK cells express a vast array of chemokine receptors (Berahovich et al., 2006; Gregoire et al., 2007) and respond to chemokines (Inngjerdingen et al., 2001). An extensive amount of work has characterized important chemokine signatures that help NK cells to move in response to infectious, tumourogenic or inflammatory challenges.

**CC chemokines**

Chemokines contain one or two conserved NH\texttwo-terminal cysteines (C), which can be adjacent (CC chemokines) or separated by amino acids (CXC or CX\textthree-C chemokines; where X = any amino acid) (Cyster, 1999). Specialized cell membrane-expressed receptors can recognize and bind chemokines; however, a single chemokine can interact with more than one receptor. A receptor can only bind either a CC chemokine [CC-receptor(R)] or a CXC chemokine (CXCR), but never both. Thus, CC chemokines can bind one or more CCRs but not CXCRs, whereas the opposite stands for CXC chemokines.

Accordingly, the monocyte chemotactic proteins MCP-1 or CCL2, MCP-2 or CCL8, and MCP-3 or

| Table 10.1 | Anatomical location of mature NK cells |
|-------------|---------------------------------------|
| Organ       | Specific Location                     | Special features                                 |
| Lymph node  | Medulla & T zone                      | CD56\textsuperscript{bright} in human            |
|             |                                       | CD27\textsuperscript{low} in mouse               |
| Spleen      | Red pulp (marginal zone)              | Mixed populations                                |
|             |                                       | Migrate to white pulp after activation           |
| Gut         | Intraepithelial, lamina propria & cryptopatches | Low cytotoxicity & IFN-\gamma                     |
|             |                                       | Resemble LTi cells                               |
|             |                                       | Some make IL-22, and some can participate in Th2 responses |
| Lung        | Bronchoalveolar space, lung interstitium | Locally regulated by alveolar macrophages        |
| Liver       | Sinusoids                             | Express TRAIL                                    |
|             |                                       | CD16\textsuperscript{−} in human                 |
|             |                                       | Lower activation threshold                       |
|             |                                       | Regulated by Kupffer cells                       |
| Thymus      | Cortex & medulla                      | IL7R\alpha, GATA-3\textsuperscript{+}, CD16\textsuperscript{−}, CD11b/Ly49\textsuperscript{low} |
|             |                                       | Low cytotoxicity, high IFN-\gamma                |
|             |                                       | LTi, lymphoid tissue inducer cell                |

LTi, lymphoid tissue inducer cell
CCL7 share a common receptor, CCR2, which is expressed at a high level on IL-2 activated NK cells (Godiska et al., 1997; Polentarutti et al., 1997). Hence, all three chemokines are capable of inducing in vitro NK cell chemotaxis (Allavena et al., 1994). However, CCL2 was found to be the strongest NK cell chemo-attractant (Allavena et al., 1994; Maghazachi et al., 1994), and its contribution to NK cell migration has been more carefully characterized, in comparison to CCL7 or CCL8.

To experimentally test the in vivo importance of CCL2 in NK cell migration, tumour cells were engineered to express CCL2 and were then transplanted into mice (Nokihara et al., 2000). CCL2+ tumour cells were very inefficient in metastasizing to the lung compared to CCL2− tumours, and the recipients survived for longer. This however, was the case only in NK-sufficient but not in NK-depleted hosts, suggesting that CCL2 can induce NK cell-mediated eradication of metastatic tumours (Nokihara et al., 2000).

Aspergillosis can be a devastating fungal-induced disease in immunocompromised, especially neutropenic, patients (Mehrad et al., 1999; Morrison et al., 2003). Murine models have shown that infection with *Aspergillus fumigatus* results in increased levels of CCL2 in lung and serum and that depletion of NK cells correlates with increased susceptibility (Mehrad et al., 1999; Morrison et al., 2003). It was then demonstrated that neutralization of CCL2 firstly diminishes NK cell accumulation in the lungs of infected mice and secondly reduces the ability of fungal clearance, a phenotype that was replicated when CCR2 was absent from NK cells (Morrison et al., 2003). Therefore, there is evidence implicating the requirement for CCL2/CCR2-mediated recruitment of NK cells in the lung, both during tumour metastasis (Nokihara et al., 2000) and during fungal infection (Morrison et al., 2003).

In addition to lung infections, CCL2 was found to be strongly expressed in the liver of mice that were infected with MCMV (Hokeness et al., 2005). Further experiments provided clear evidence that this CCL2 production was primarily coming from liver-resident leukocytes, and it was dependent on IFNo/3 (Hokeness et al., 2005). Significantly, mice lacking either CCL2 or CCR2 displayed deficient NK cell recruitment to the liver during MCMV infection (but not in uninfected mice), and this was associated with increased hepatic viral titres and exacerbated liver damage (Hokeness et al., 2005). Moreover, a model of adenoviral-based gene therapy against hepatocellular carcinoma showed that usage of recombinant adenovirus carrying CCL2 is more effective at tumour eradication via a mechanism involving the preferential recruitment of NK cells to the liver, as shown by increased NK cell infiltration and abrogation of protection when NK cells were depleted (Tsuchiyama et al., 2007).

Besides preventing infection or tumour formation, NK cells may also be the cause of disease in certain clinical settings. Thus, patients deficient in TAP2 (transporter associated with antigen presentation) can develop lethal lung granulomatous lesions due to increased and chronic NK cell accumulation (Moins-Teisserenc et al., 1999). To this end, it was recently shown that TAP2-deficient patients presented with increased levels of CCL2 in bronchoalveolar lavage samples, and their NK cells expressed unnaturally high levels of CCR2—and migrated towards gradients of CCL2—and the other MCP chemokines and CCR2 ligands, CCL7 and CCL8 (Hanna et al., 2005).

A crucial difference between CCL2 and CCL7 and CCL8 is that the latter two chemokines also interact with CCR5, which has the alternative ligand, CCL5. CCL5 or RANTES (regulated upon activation, normal T cell expressed and secreted) in turn can also interact with CCR1 and CCR3, and can be a potent NK cell chemo-attractant (Loetscher et al., 1996). Accordingly, injection of a CCL5-expressing thymoma cell line into syngeneic mice caused significant reduction in tumour growth and resulted in NK cell influx (Lavergne et al., 2004). However, the role of NK cells in this model remains obscure because mice lacking only B and T cells could not clear CCL5-expressing tumours, suggesting a more dominant role for lymphocytes other than NK cells (Lavergne et al., 2004). Furthermore, infection of CCR5−/− mice with HSV-1 results in decreased ability to control viral replication, which is associated with diminished mobilization of NK cells towards the spleen, brain stem and spinal chord (Thapa et al., 2007). Surprisingly, concanavalin A-mediated hepatitis in CCR5-deficient mice resulted in severe disease due to uncontrolled migration of NK cells to the liver (Ajuebor et al., 2007). It was then shown that in the liver of these animals, there were markedly elevated CCL5 levels, which actively attracted NK cells by a mechanism involving CCR1, the other receptor that recognizes CCL5 and is expressed on NK cells (Ajuebor et al., 2007). Besides the implications of the latter study in possible treatments against liver disease, a secondary outcome is that it presents a good example of the complex interactions between different chemokines with different receptors.

Therefore, the chemokines CCL3 (macrophage inflammatory protein-1 or MIP-1α) and CCL4 (MIP-1β) also bind CCR5, while CCL3 can interact with CCR1, and early on it was established that CCL3 is a more potent chemotactic molecule for NK cells (Maghazachi et al., 1994; Taub et al., 1995). Similar to CCL2, expression levels of CCL3 are markedly upregulated in the liver during the early stages of MCMV infection (Salazar-Mather et al., 1998). Hence, mice deficient in CCL3 displayed impaired NK cell recruitment to the liver, which was followed by decreased inflammatory foci but elevated viral replication (Salazar-Mather et al., 1998) and reduced host survival (Salazar-Mather et al., 2000). Interestingly, in the absence of CCL3, NK cell numbers decreased in
the livers of MCMV infected mice, and blood NK cell numbers increased significantly compared to uninfected animals (Salazar-Mather et al., 2000). Work from the same research group also demonstrated that CCL3 induction in the liver and subsequent NK cell accumulation depends on type I IFNs (Dalod et al., 2002), suggesting that CCL2 as well as CCL3 play an interconnecting role in NK cell recruitment towards the liver during CMV infection. In this infection setting, CCL2 is expressed earlier than CCL3 (Hokeness et al., 2005). This and the fact that CCL3−/− mice are able to produce CCL2 (Hokeness et al., 2005) depicts that the effects of these chemokines are synergistic but not redundant and would suggest that they act in sequence—that is CCL2 mediates the very early NK cell influx that needs sustained CCL3 production for efficient migration to the liver. It would be therefore of great interest to study NK cell migration in the absence of both CCL2 and CCL3 either by creating double-deficient animals or by antibody blockade of either chemokine in single-deficient mice.

The use of Klebsiella pneumoniae as an infection model showed that induced expression of CCL3 in the lung correlated very well with reduced bacteria replication and a significant increase of NK cell numbers in the lungs of infected mice (Zeng et al., 2003). Of interest is a study that showed that injection of rituximab (anti-CD20 antibody) induced the expression of CCL3 and this was required for efficient tumour cell eradication (Cittera et al., 2007). Subsequent experiments revealed the action of Rituximab and CCL3 could be reversed by depletion of leukocyte populations, including NK cells (Cittera et al., 2007). The diverse role of CCL3 is also evident by the observations that during pregnancy specialized placental cells can secrete CCL3 and attract maternal NK cells (Drake et al., 2001).

Phenotypic characterization both at the protein and transcript level provided evidence that NK cells can express the chemokine receptors CCR4 and CCR8 following activation with IL-2 (Inngjerdingen et al., 2000). Both of these receptors can induce the chemotaxis of NK cells through the action of CCL17, which binds both receptors; CCL1, which binds CCR8; and CCL22, which interacts with CCR4 (Godiska et al., 1997; Inngjerdingen et al., 2000, 2001).

It is apparent that CC chemokines and their receptors have a very important role to play in the biology of NK cells by inducing their migration and mobilization at situations of host evasion by means of inflammation, infection or malignancy.

**CXC chemokines**

Besides members of the CC chemokine family, the microenvironment that is created by inflammation, infection and malignancy induces the local production of a number of CXC chemokines, some of which have been shown to promote NK cell migration towards the affected tissue. Of these chemokines, CXCL9, CXCL10, and CXCL11 play a very prominent and often interchangeable role in NK cell mobilization by sharing CXCR3 as their binding counterpart (Weng et al., 1998) and by inducing strong NK cell chemotaxis (Maghazachi et al., 1997).

Previously we have discussed that CXCR3 binding chemokines can promote the active recruitment of NK cells from the dark grey to the white pulp of the spleen during infectious stimuli (Bekiaris et al., 2008; Gregoire et al., 2008), as well as their recruitment to LNs (Martin-Fonseca et al., 2004). The study of Leishmania major in mice showed that resistant but not susceptible strains expressed high levels of CXCL10 in infected LNs while injection of recombinant CXCL10 in infected mice increased LN NK cell activity (Vester et al., 1999). In addition to L. major, infection of DCs with M. tuberculosis renders them chemo-attractant for NK cells (Lande et al., 2003). This chemotactic property of DCs towards NK cells was attributed to their production of CXCL9 and CXCL10, and it was further shown that induction of CXCL10 depended on type I IFN (Lande et al., 2003).

The classical type I IFN-producing cells, the plasmacytoid DCs (pDCs), can also attract NK cells following their infection with HSV utilizing a mechanism involving the production of CXCL10 (Megjugorac et al., 2004). Interestingly, there was some evidence suggesting that IFNα could act on pDCs in an autocrine fashion to promote CXCL10 expression (Megjugorac et al., 2004). Ocular infection with HSV-1 in the absence of CXCR3 is associated with a significant reduction in NK cell recruitment and an elevation in the viral titre as marked in NK-depleted mice (Alba et al., 2008), providing evidence for the active participation of NK cells in ocular protection. Moreover, when either CXCL9- or CXCL10-deficient mice were infected with genital HSV-2, they presented with acute reduction in NK cell numbers from the brain and spinal chord, which was associated with elevated viral replication and impaired survival (Thapa et al., 2008).

In addition, infection of mice with mouse coronavirus can result in 100% mortality and very high titres in the brain unless the virus is engineered to express CXCL10 (Trifilo et al., 2004). Hence, CXCL10-expressing coronavirus induced substantial NK cell infiltration in the brain with subsequent resolution of infection and host survival (Trifilo et al., 2004).

Therefore, there is a very tight association between recruitment of NK cells in pathogen-infected tissues and the expression of CXCR3 ligands. A similar association has also been described for tumour- and inflammation-induced cell infiltration. Thus, tumours from
CXCR3−/− mice have reduced NK cell numbers, while CXCR3−/− NK cells present with impaired capacity to infiltrate tumours when adoptively transferred into wild type recipients (Wendel et al., 2008). Besides recruitment, CXCL10 can directly stimulate NK cell tumour killing as well as induce B7-H1 on the surface, which acts to activate anti-tumour T cells and thus confer long-term protection (Saudemont et al., 2005).

Furthermore, a mouse model of non-infectious pulmonary fibrosis showed that in CXCR3 deficiency, there is significant acceleration of disease on one hand and significant decrease in NK and CD8 T cell accumulation in the lungs on the other (Jiang et al., 2004). Importantly, the investigators found that CXCR3−/− mice had very reduced NK cell numbers in lung, liver and blood, and went on to demonstrate that early IFNγ production by lung cells is responsible for limiting lung fibrosis (Jiang et al., 2004). Besides, CD56brightCD16− NK cells that can be identified in and isolated from psoriatic human skin express CXCR3 and are able to migrate towards CXCL10 gradients (Ottaviani et al., 2006), suggesting a role of NK cells in psoriasis. Of importance is the observation that human uterine NK cells express high levels of CXCL8 (Burke et al., 2008). CXCL8 cells, which have been previously activated with reovirus damage and resolution of infection or malignancy.

It is worth noting, for example, a study that provided evidence that the potent NK-activating cytokines IL-2 and IL-12 reduced membrane CXCR3 levels and hence chemotaxis towards CXCL10 (Hodge et al., 2002). It follows that generalized NK cell activation might not necessarily lead to CXCR3 responsiveness. Moreover, our group has also observed downregulation of CXCR3 from NK cells during MCMV infection, and we attributed this to receptor/ligand downmodulation (Bekiari et al., 2008). However, it is very likely that expression of CXCR3 is regulated actively through the action of cytokines or other immunological mediators in order to maintain a balance between excessive NK-mediated damage and resolution of infection or malignancy.

In addition to CXCR3 ligands, other members of the CXC family of chemokines are involved in the mobilization of NK cells. It has been recently shown that mast cells, which have been previously activated with reovirus express high levels of CXCL8 (Burke et al., 2008). CXCL8 can bind either to CXCR1 or CXCR2, and its production from mast cells is sufficient to attract NK cells mainly via interactions with CXCR1 (Burke et al., 2008). Similarly, the chemokine CXCL14 has also been found to induce the chemotaxis of NK cells (Starnes et al., 2006). It is of interest that while most tissues can express CXCL14, a number of tumour cells fail to do so, indicating that down-regulation of CXCL14 might be a mechanism to prevent NK cell recruitment (Starnes et al., 2006).

CXCL12, also known as SDF-1α (stromal cell derived factor-1α) interacts with CXCR4, which is expressed on mouse (Bernardini et al., 2008) as well as human (Beider et al., 2003) NK cells. It has been shown that adoptive transfer of human NK cells into immunodeficient mice results in their migration towards both the spleen and the BM. However, only migration to the BM depended on CXCR4 (Beider et al., 2003). Thus, CXCR4 and CXCL12 appear to regulate homing of NK cells to the BM. CXCL12 is expressed by BM endothelium, which also expresses the adhesion molecule VCAM-1, the ligand for which, the integrin α4β1 or VLA-4 (very late antigen-4) is expressed on NK cells (Franitza et al., 2004). There is evidence demonstrating that CXCL12 induces the firm adhesion of NK cells on VCAM-1+ cells through interactions with VLA-4, while in vivo blocking of VLA-4 prevents NK cell recruitment to the BM (Franitza et al., 2004).

In the mouse, CXCR4 is expressed by blood and splenic NK cells as well as by BM NK populations irrespective of whether they are at the progenitor, immature or mature stage of their development (Bernardini et al., 2008). However, BM mature NK cells show the highest responsiveness to CXCL12, and when CXCR4 signalling is blocked, BM NK cell numbers decrease, whereas blood and splenic NK numbers increase accordingly, suggesting therefore that the CXCR4/CXCL12 interactions can promote retention of NK cells in the BM (Bernardini et al., 2008).

An important question however, is what regulates the exit of NK cells from the BM. Recent evidence suggests that BM emigration is not regulated by a chemokine/chemokine receptor pair but by the sphingosine 1-phosphate receptor 5 (S1P5) (Gregoire et al., 2007). Thus, mice deficient in S1P5 present with decreased NK numbers in blood, spleen and lung but increased numbers in the BM (Gregoire et al., 2007), i.e. the opposite of CXCR4 blockade.

In addition, CXCL12 is expressed by endothelial cells of adenoids (a MALT site), while adenoid NK cells express high levels of CXCR4, and in vitro, they can migrate towards CXCL12 gradients, suggesting that homing of NK cells to adenoids may be partly controlled by CXCL12/CXCR4 dependent migration (Mizrahi et al., 2007). Moreover, there is evidence to suggest that CD56brightCD16− human decidua NK cells express...
CXCR4, and respond and migrate towards CXCL12-expressing trophoblasts (Beider et al., 2003; Infante-Duarte et al., 2005).

Taken together, there is strong experimental evidence that links directly the mobilization of NK cells during disease and steady-state conditions with chemokines of the CXC family, particularly with CXCR3 and CXCR4 ligands. Further research will elucidate the exact mechanisms by which these and other chemokine systems organize how NK cells move throughout the body, a process that is essential for immune surveillance and protection against infection.

**CX3CL1, fractalkine**

Fractalkine was identified as a unique chemokine containing a three amino acid motif between the two cysteines (C-x-x-xC, CX3CL1) and a mucin-like domain (Bazan et al., 1997). In addition, CX3CL1 can exist as a soluble form inducing chemo-attraction and as a membrane-bound form promoting the firm adhesion of cells on endothelium (Bazan et al., 1997). Both of these functions require the presence of its specific receptor, CX3CR1, which is expressed at high levels on NK cells (Imai et al., 1997). Thus, CX3CL1 expression by endothelial cells can promote the adhesion of and subsequent lysis by NK cells (Yoneda et al., 2000).

Experiments to assess its role on NK cell biology showed a strong correlation between CX3CL1 and anti-tumour responses. Hence, when CX3CL1-expressing lymphoma cell lines were injected into mice, tumour growth was suppressed due to the recruitment of NK cells that controlled the tumour via production of IFNγ and perforin (Lavergne et al., 2003). Similar experiments utilizing a lung carcinoma cell line also concluded that CX3CL1 is required for NK cell recruitment at the site of tumour growth, leading thus to its eradication (Guo et al., 2003). Infiltration of NK cells associated with tumour clearance was also observed when an adenoviral vector expressing CX3CL1 was used to deliver the chemokine at the tumour site (Banks et al., 2005). Furthermore, mice deficient for CX3CR1 were substantially repressed in their ability to recruit NK cells in the lung both in the presence of cancer and under homeostatic conditions (Giroux et al., 2007).

In addition to promoting their migration, CX3CR1 is also involved in IFNγ production by NK cells. Thus, when CX3CR1 expression is lost, NK cells present with reduced capacity to secrete IFNγ (Giroux et al., 2007), while IFNγ production is induced in vitro by human peripheral blood NK cells upon incubation with CX3CL1 (Yoneda et al., 2003). Moreover, CX3CL1 expressed by mature DCs is involved in DC-induced NK cell activation (Pallandre et al., 2008). The effects of CX3CL1 can be modulated by IL-15 since it has been shown to downregulate the expression of CX3CR1 by NK cells (Sechler et al., 2004).

Therefore, CX3CL1 and its receptor CX3CR1 regulate the migration of NK cells to tumour sites as well as their effector function.

**Do NK cells traffic?**

From the preceding information, it becomes apparent that there is an avalanche of information regarding the migration of NK cells during disease states. We saw, for example, how CC and CXC chemokines regulate the mobilization of NK cells towards various organs that are affected by infection or cancer (Table 10.2). Similarly, chemokine/chemokine receptor codes localize NK cells in the organs around the body, while in the absence of infection, CXCR4 appears to regulate recruitment to and retention in the BM.

The fact that NK cells are seen in many organs would suggest two alternatives:

1. They leave the BM and migrate to various organs where they take on certain activities and phenotype

### Table 10.2 Examples of chemokines required for NK cell migration

| Chemokine | Receptor | NK migratory capacity |
|-----------|----------|-----------------------|
| CCL2      | CCR2     | Lung (malignancy, fungal infection, chronic inflammation) |
| CCL3      | CCR1, CCR5 | Lung (bacterial infection, inflammation) Liver (viral infection) Placenta (pregnancy) |
| CCL5      | CCR1, CCR3, CCR5 | Spleen (viral infection) Liver (viral infection) |
| CCL21     | CCR7     | Lymph node (homeostasis) |
| CXCL9     | CXCR3    | Splenic white pulp (viral infection) Lymph node (homeostasis) |
| CXCL10    |          | Eye & brain (viral infection) Various tumour sites Lung (inflammation) Skin (psoriasis) Endometrium (pregnancy) |
| CXCL11    |          |                      |
| CXCL12    | CXCR4    | Bone marrow (homeostasis) Adenoids (homeostasis) Trophoblasts (pregnancy) |
| CX3CL1    | CX3CR1   | Various tumour sites  |
imprinted by the local environment. They then can get mobilized again during disease that causes alterations in chemokine gradients.

2. NK cells are constantly ‘on the go’ trafficking around tissues.

Unfortunately, it is still uncertain which of these alternatives is true, or whether there is a mixture of both scenarios. Work from Vivier and colleagues suggest the latter. Thus, when transferred into a naïve syngeneic host, spleen-derived murine NK cells were found in all the organs where NK cells localize and at the same proportions as host populations (Gregoire et al., 2007). This suggests that NK cells from one anatomical location are not restricted to that environment and can re-circulate between organs. It is possible, however, that due to the direct connection between spleen and blood, splenic NK cells contain recent BM emigrants that have the capacity to migrate everywhere. It would be of interest to repeat the preceding transfer experiments with NK cells from different organs, as there is still the possibility that a certain microenvironment can imprint changes on the NK cell that affect its ability to traffic. It is also plausible that while certain NK cell subsets traffic continuously, some other subsets do not or do so during host evasion by pathogens, cancer or inflammation. The determination and characterization of these possible functions will be seminal in furthering our understanding of NK cell biology.

Concluding remarks

NK cells develop in the BM, which they exit using specific molecular interactions. Exit from the BM is followed by localization to a number of tissues, including secondary lymphoid organs. Within each tissue, NK cells often acquire unique function and phenotype that is regulated by the local microenvironment. Their localization is primarily directed by the action of chemokines and therefore is in tight association with the activation status of the organism. Changes in chemokine expression during disease results in further NK cell mobilization and allows them to protect the host from infection and malignancy. Thus, from their time of production until their end, NK cells travel exhaustively over long distances and visit places that influence their already dynamic life. The future promises to uncover a lot more truths about their nature.

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