Natural killer cell distribution and trafficking in human tissues

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INTRODUCTION

Natural killer (NK) cells are a class of lymphocytes characterized by a wide spectrum of effector functions spanning from killing of target cells, mainly tumor and virally-infected cells, to the ability to influence various steps of the immune response, such as editing dendritic cells (DCs) and influencing emerging T helper response (Moretta et al., 2003; Ferlazzo, 2005; Fink et al., 2007; Vivier et al., 2011; Morandi et al., 2012). It is now clear that NK cells are not exclusively found in peripheral blood (PB) but populate different tissues and organs. Nonetheless, the true distribution of NK cells across the human body is less than clear. The task of depicting a detailed analysis of NK cell distribution is also complicated by the recent discovery of other innate populations of lymphocytes that share some characteristics (i.e., NKp46, CD56) with conventional NK cells (e.g., CD57, CD56) or expressed only by subsets (e.g., CD16). Furthermore, interpretation of studies performed with NK cells in the tissues is limited by methodological shortcomings, as earlier analyses often relied on the use of erroneous markers for the detection of NK cells (i.e., the use of markers then proved to be either not sufficiently specific for NK cells (e.g., CD57, CD56) or expressed only by subsets of NK cells (e.g., CD16)).

Few data are available regarding the recirculation of natural killer (NK) cells among human organs. Earlier studies have been often impaired by the use of markers then proved to be either not sufficiently specific for NK cells (e.g., CD57, CD56) or expressed only by subsets of NK cells (e.g., CD16). At the present, available data confirmed that human NK cells populate blood, lymphoid organs, lung, liver, uterus (during pregnancy), and gut. Several studies showed that NK cell homing appears to be subset-specific, as secondary lymphoid organs and probably several solid tissues are preferentially inhabited by CD56bright/CD16neg dull non-cytotoxic NK cells. Similar studies performed in the mouse model showed that lymph node and bone marrow are preferentially populated by CD11b+ NK cells while blood, spleen, and lung by CD27 dull NK cells. Therefore, an important topic to be addressed in the human system is the contribution of factors that regulate NK cell tissue homing and egress, such as chemotactic receptors or homeostatic mechanisms. Here, we review the current knowledge on NK cell distribution in peripheral tissues and, based on recent acquisitions, we propose our view regarding the recirculation of NK cells in the human body.

DISTRIBUTION OF NK CELLS IN HUMAN NON-LYMPHOID ORGANS

Information on NK cell distribution across human tissues was limited by methodological shortcomings, as earlier analyses often relied on the use of erroneous markers for the detection of NK cells (i.e., the use of markers then proved to be either not sufficiently specific for NK cells (e.g., CD57, CD56) or expressed only by subsets of NK cells (e.g., CD16)). Furthermore, interpretation of studies performed with NK cells in the tissues is complicated by the fact that most studies have not been able to distinguish bona fide NK cells from the growing population of innate lymphoid cells (ILCs). In fact, as cited before, one of the challenges in identifying tissue NK cells lies in the difficulty in discriminating these cells from other NKp46+ cells. These latter ILCs, both in human and mouse, are functionally distinct from conventional NK cells and have been referred to as NK-22 cells or ILC22 cells. They share with NK cells the expression of the natural cytotoxicity receptor Nkp46 and with lymphoid tissue-inducer (LTi) cells (another subset of ILCs) the expression of retinoic acid receptor-related orphan receptor γ (RORγt). Differently from conventional NK cells, Nkp46+ RORγt+ ILCs produce IL-22 and are non-cytotoxic. These Nkp46+ RORγt+ ILCs are abundantly represented in mucosal tissues, particularly of the gut and oropharynx (Spits and Di Santo, 2011).

Finally, much of the studies performed to investigate human tissue NK cells were not technically able to distinguish between the two main NK cell subsets, i.e., CD56bright/CD16neg dull/KIRnull NK...
As a result, a lack of information about relative distribution of CD56dim and CD56bright NK cells still remains. Nonetheless, based on the available evidences, it seems that NK cells are present in healthy skin and gut, in the liver, in the lungs, and uterus during pregnancy. In addition, human NK cells were investigated also in other tissues such as the kidney (Schleypen et al., 2006), joints (Dalbeth et al., 2004), and breast (Fager et al., 2011) under pathophysiological conditions. In the normal intestinal mucosa, NK cells (Cella et al., 2009; Reynolds et al., 2011) are found predominantly as intraepithelial lymphocytes and within the lamina propria, but are rarely found to constitute approximately 90% of the lung NK cell infiltrating in this tissue (King et al., 1991; Bulmer et al., 2010). Although they are characterized by a CD56dimCD16neg phenotype, they differ from their blood counterpart in their expression of inhibitory receptors and the presence of high levels of lytic granules. Surprisingly, uterine NK cell rather than act as killers and/or drivers of inflammation, contribute to tissue building and remodeling and formation of new vessels by releasing high amounts of IL-8, VEGF, SCF-1-1, and IP-10 but low levels of IFNγ (Vasca et al., 2011). The presence of NK cells in the major human organs raises questions about how NK cells reach these peripheral organs.

**FACTORS THAT REGULATE HOMING AND TRAFFICKING OF HUMAN NK CELLS TO THE TISSUES**

The expression of chemokines by organ-specific cell types suggests that organ-intrinsic elements may be important in guiding NK cell homing during physiological and pathological conditions. Differences between the main human NK cell subsets include also the expression of chemokine receptors, as CD56dim and CD56bright NK cell subsets largely differ in their repertoires (Campbell et al., 2001). However, besides chemokine receptors, it has been demonstrated that NK cells can additionally migrate in response to factors that do not belong to the chemokine superfamily. This is the case of the proinflammatory protein chemerin and the sphingosine 1-phosphate (SIP) molecule that can both affect trafficking of NK cells during inflammation or steady-state conditions, respectively (Parolini et al., 2007; Walter et al., 2007). On the contrary, the chemotactic receptors expressed by NKp46+ROKyrt+ cells are largely unknown with the exception of the CCR6 receptor, which seems to promote leukocyte homing to the gut mucosa (Williams, 2006; Cella et al., 2009).

Collectively, the different chemokine receptor repertoires expressed by NK cell subsets may define entirely different routes of distribution for these cells. Nevertheless, the detailed migration patterns of NK cells have not yet been sufficiently characterized.

Furthermore, the presence of NK cells in many organs raises the question whether NK cells can exit the organs, or terminally reside in the tissues. The localization of NK cells in different tissues would suggest that: (i) they could migrate to various organs, then reside in the tissue taking on their peculiar activities, perhaps getting mobilized in the case of disease; alternatively, (ii) NK cells could re-circulate constantly through the tissues. This last hypothesis is supported by the finding that, 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 (Greig et al., 2007), thus suggesting that NK cells from one anatomical location are not restricted to that environment and can re-circulate between organs. Up to now, it is still unclear which of these alternatives might be more realistic. Based on the available data, it is likely that a combination of both scenarios could be possible. As described above for the gut, liver, and lung, different...
human solid tissues appear to be populated by different NK cell subsets (mainly by CD56bright NK cells with some exception) thus indicating that specific homing signals are important to drive localization of NK cells to the different tissues. However, the finding that human afferent lymph draining peripheral tissues contains a substantial number of NK cells (Montalto et al., 2010; and P. Carrega, personal observations) indicates that NK cells might even exit the organ and traffic through tissues in normal conditions.

“IN VIVO” TRAFFICKING OF NATURAL KILLER CELLS THROUGH SECONDARY LYMPHOID ORGANS

Recent studies have demonstrated that a substantial number of NK cells are present in resting human LN and the large majority of these LN-NK cells are CD56bright (Fehninger et al., 2003; Ferlazzo et al., 2004b; Vossen et al., 2008; Ferlazzo and Munz, 2009), as might be predicted by their expression of CCRI7 and L-selectin (CD62L), which binds with high efficiency to physiologic L-selectin ligands on peripheral LN high endothelial venules (HEVs). Interestingly, HEVs may not represent the only route for NK cell entrance in the LN as we recently observed that NK cells are contained in afferent lymph accumulations following LN resection upon breast cancer surgery (Montalto et al., 2010; and P. Carrega, personal observations). This observation is supported by the findings that CD56+ lymphocytes were actually detected in human lymph draining normal skin (Hunger et al., 1999). It has also been proposed that LN may be sites for NK cell maturation. Like the bone marrow, LN can support the differentiation of NK cells from NK cell precursors through sequential maturation stages (Freed et al., 2003). Whatever their origin (developed “in situ” or migrated through blood and afferent lymphs), NK cells are present in the T cell areas of human normal donor LN (Ferlazzo et al., 2004a,b), indicating LN as potential sites for DC/NK cell crosstalk (Ferlazzo et al., 2004b). Moreover, by performing real-time observations, it has been shown that more than 90% of the NK cells in the T cell area were found to unambiguously remain in contact with the DCs for at least 25 min (Bajenoff et al., 2006a).

Recently, sequential events regulating the egress of NK cells from secondary lymphoid organs (SLO) have been described in the mouse model. Changes in responsiveness of the S1P receptors to its ligand (SIP, which concentration is high in peripheral fluids) seems to have a key role in allowing NK cell exit via lymphatics (Mayrol et al., 2011). However, whether this mechanism might also be effective in human has not yet been confirmed. Notably, the phenotype of NK cells exiting from LN by efferent lymph appears slightly different from that of NK cells found within these SLO. In particular, part of these cells express significant amounts of KIR and CD16, implying that CD56bright NK cells could acquire these molecules in the LN during inflammation and then circulate through the efferent lymph into PB as KIR+CD16+ NK cells (Romagnani et al., 2007). This notion is also supported by the finding that NK cells of highly reactive LN partially express KIRs and CD16 (Romagnani et al., 2007). Nevertheless, it cannot be excluded that CD16+ KIR- NK cells might reach LN because of local inflammation, as better discussed below.

ARE INFLAMED PERIPHERAL TISSUES SITES FOR NK CELL MATURATION?

It has been demonstrated that, upon activation, CD56bright NK cells could acquire the signature of CD56dim NK cells, i.e. perforin+, KIR+, CD16+, IL-7R+, c-kit+, CXCR3+, CCRI7+, CD62L+ (Ferlazzo et al., 2004b), whereas CD56dim CD16+ KIR+ NK cells substantially maintain their features of terminally differentiated cells (Romagnani et al., 2007). Even if this process was elegantly demonstrated in vitro, the sites and physiological conditions where this process may happen remain almost unknown in vivo. Nevertheless, several observations seem to support the hypothesis that similar steps of NK cell differentiation occur also in vivo. Indeed, it has been observed that non-reactive LN contain almost exclusively CD56bright KIR- CD16-bright NK cells, whereas a significant expression of KIR and CD16 is present in NK cells contained in highly inflamed LN and in the efferent lymph. Thus, it could be envisaged that in steady state or very early during an immune response, CD56bright, KIR- NK cells can be recruited into LN (Martin-Fonseca et al., 2004; Bajenoff et al., 2006b), whereas later on during inflammation mature NK cells leave LN and then circulate in PB to reach inflamed tissues (Figure 1). Although this hypothesis is very challenging, it cannot be excluded that the presence of KIR+CD16+ NK cells in inflamed LN (and in the effenter lymph) might be due to selective migration of this subset into LN. This latter theory is supported by some in vitro data, which showed that stimulation of human PB CD56dim cells NK cells with IL-18 can up-regulate their expression of CCRI7 (Mallioul et al., 2005). However, data derived from the investigation of tumor-infiltrating NK cells, support the hypothesis that, also in vivo, local activation could convert CD56bright/CD16dim NK cells into effector analogous to blood CD56dimCD16+ NK cells. Our observations in NK cells infiltrating lung cancers suggest that up-regulation of KIR on CD56dimCD16dim perforin+ NK cells can occur also in vivo, most likely because of a proinflammatory cytokine microenvironment due to local immune reactions at the tumor site (Carrega et al., 2008; and P. Carrega, unpublished observations). Therefore, we should consider that, besides normal distribution and homeostatic re-circulation, there are specific conditions and functions of NK cells in a specific tissue could also be influenced by in vivo differentiation due to local microenvironmental factors.

CONCLUDING REMARKS

Recent studies have significantly increased our knowledge on NK cell distribution in some human peripheral tissues, such as SLO and uterus. However, information on NK cells presence in most human solid tissues, as well as on how NK cell redistribute during pathological processes, is still largely lacking. Investigations in this field are now highly required in view of novel attempts of NK cell-based adoptive immunotherapies in both cancer and organ transplantation settings.

The few earlier data on NK cells infiltrating human peripheral tissues have often been obtained using markers then proved to be either not sufficiently specific for NK cells or expressed only by subsets of NK cells. In addition, nowadays, we appreciate that, in solid tissues, classical NK must be distinguished from other RORγ+ ILCs, which, despite functionally distinct, share
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FIGURE 1 | Hypothesis of NK cell subset recirculation in human body.

After their generation in the bone marrow, CD56bright/perforin− NK cells colonize most human tissues according to their chemokine receptor repertoire. They can eventually acquire cytolytic functions during immune response in inflamed tissues and act locally as innate effector cells.

Alternatively, as depicted, they can reach secondary lymphoid organs via afferent lymph, acquire perforins and cytolytic functions during immune reactions occurring in lymphoid organs and then recirculate in peripheral blood for exerting their protective activities in inflamed tissues.

several conventional NK cell hallmarks. On the basis of what we learned in the last years regarding innate lymphocytes, some previous correlations between NK cell infiltration and clinical status, such as cancer prognosis, should now probably be reconsidered and further dissected.

Apart from these newly described RORγt+ NKp46+ cells, the most recent analyses on human tissue NK cells have shown that perforinlow/neg, non-cytotoxic, NKp46+ cells represent in our body a population of cells much larger than previously realized. Accumulating evidences are indicating that, in addition to SLO, also many other human solid tissues, including some cancer histotypes, might predominantly host the CD56bright/KIRneg/perforinneg NK cell subset rather than the cytotoxic perforin+ counterpart, which is conversely largely dominant in PB.

We have recently identified NKp46+ cells within human afferent lymph and further analyses are currently performed in order to confirm and extend these preliminary results. Nonetheless, also in consideration of previous studies on developmental relationships of NK cell subsets and on the distribution of NK cell subsets in SLO, inflamed tissues, and efferent lymph (Ferlazzo et al., 2004b; Romagnani et al., 2007), we are now prone to hypothesize that CD56bright non-cytotoxic NK cells, similarly to naive T cells, could re-circulate through afferent lymph from peripheral tissues to SLO. Here, they could achieve or not their final maturation (depending on whether or not an immune reaction will occur inside the LN) and then re-circulate via venous blood vessels or efferent lymph to PB (Figure 1).

Thus, reactive SLO might be the sites for NK cell final maturation, acquisition of cytotoxic properties and tolerance to self (KIR expression). Complete maturation of NK cells is indeed accompanied by the switching of the chemokine receptor repertoire, which would drive blood re-circulating cytotoxic NK cells toward their targets in inflamed pathological tissues. Alternatively, NK
cells could also achieve their final differentiation steps directly in inflamed tissues, where tissue resident non-cytotoxic CD56bright NK cells might differentiate into perforin"-positive final effector NK cells during the local immune response against invading pathogens.

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