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Editorial: **Natural killer cells "strike" a new cord**

By Stephen N. Waggoner[*,†] *and Domenico Mavilio*[‡,§,1]

*Center for Autoimmune Genomics and Etiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [†]Department of Pediatrics, University of Cincinnati Medical School, Cincinnati, Ohio, USA; [‡]Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy; and [§]Unit of Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Rozzano, Milan, Italy

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HSCT is a curative, therapeutic approach for hematologic malignancies, and CB represents a unique and effective graft source of HSC to be used in this procedure. As the first lymphocyte compartment reconstituted after myeloablative conditioning and HSCT, NK cells play a significant role in the HSCT engraftment and protection against the onset of GVHD. CB NK cells also display an impressive proliferation index after being infused in recipients where they are highly efficient in avoiding tumor relapse by inducing a GVL response [1]. CB is a potent and immediately available source of NK cells for immunotherapy considering the following: 1) that NK cells from CB are easy to collect and cryopreserve; 2) that CB has a reduced frequency of T cells compared with adult PB; and 3) that CB is characterized by the absence of specific receptor repertoires shaping NK cell effector functions. Indeed, CB NK cells exhibit a lower functional potential compared with their counterparts in PB and are generally considered immature, as they have not yet been exposed to various encounters, such as viruses or environmental factors. This so-called "naïve" status of CB NK cells is associated with distinctive phenotypic features, and it still remains unclear which aspects of the repertoire of these cells substantiate or detract from the successful use of CB NK cells in cell-based therapies against leukemia and other cancers.

During NK cell interaction with tumors or viral-infected cells, the absence or downmodulation of HLA leads to NK cell activation as a result of "missing-self" recognition. The effector functions of NK cells against "nonself" targets are associated with a dynamic interplay of signals delivered by large families of NKRs that are either aNKRs or iNKRs. These include KIRs, which are predominately inhibitory receptors specific for self-HLA molecules. NK cells are also controlled by HLA-reactive heterodimers of CD94 coupled with C-type lectin receptors, such as NKG2A and NKG2C, that provide inhibitory and activating signals, respectively. Other families of NKRs also provide instruction to NK cells, including the

Abbreviations: aNKR = activating NK cell receptor, CB = cord blood, GVHD = graft-versus-host disease, GVL = graft versus leukemia, HCMV = human CMV, HSCT = hematopoietic stem cell transplantation, ILT2 = Ig-like transcript 2, iNKR = inhibiting NK cell receptor, KIR = killer cell Ig-like receptor, NKR = NK cell receptor, PB = peripheral blood

1. Correspondence: Unit of Clinical and Experimental Immunology, Dept. of Medical Biotechnologies and Translational Medicine, University of Milan School of Medicine, Humanitas Clinical and Research Center, Via Alessandro Manzoni, 113, 20089 Rozzano, Milano, Italy. E-mail: domenico.mavilio@unimi.it

inhibitory ILT2, stimulatory natural cytotoxicity receptors (e.g., NKp44), a family of nectin-like proteins (e.g., CD226), and the antibody-binding receptor CD16 [2]. The binding of specific alleles of self-HLA with their putative iNKRs (i.e., iKIR and NKG2A/CD94) regulates the effector functions of mature NK cells through a process, variably termed education or arming or licensing [3]. Through this mechanism, self-tolerant NK cells are poised to recognize nonself cells. However, the absence of inhibitory signals is insufficient to trigger NK cell cytotoxicity, as this function requires additional signals from aNKRs. In the context of haploidentical HSCT, donor-derived alloreactive NK cells play a key role in inducing a GVL response. Therefore, the development of a repertoire of iNKRs, either matched or mismatched with their cognate HLA molecules, is a relevant process that determines the efficacy of NK cell alloreactivity in tumor immunotherapy. However, the precise role of HLA in shaping NK cell repertoire is still being debated.

In this issue of the *Journal of Leukocyte Biology*, Rettman et al. [4] provide new insights into the phenotypic and functional characteristics of CB NK cells. In particular, this study demonstrates that the phenotype of CB NK cells is similar to that of their counterparts in PB, thus suggesting that NK cell biology is structured and appears to be complete since the first periods of human life. Indeed, the authors confirmed that the expression of KIRs on CB NK cells is formed independently of the autologous HLA environment [5] and demonstrated for the first time that the KIR genotype regulates the repertoire of the CB NK cell. Specifically, the frequency of KIR2DL1^{pos} NK cells is shaped by genes for KIR2DL2 and KIR2DS1. Moreover and in line with previous findings [6], NKG2A, a potent iNKR that contributes to NK cell education, is expressed at highest levels on CB NK cells compared with their counterparts in PB. This might explain, at least in part, the absence of a correlation between KIR^{pos} CB NK cells and their degree of spontaneous degranulation, although low surface levels of aNKRs on CB NK cells have also been observed. Furthermore, low levels of CD16 on CB NK cells result in a reduced or absent antibody-dependent cell-mediated cytotoxicity. Altogether, these findings demonstrate that the repertoire of the NK cell is structured early in CB by genetic signals, which contrasts with the hypothesis that KIR expression is shaped by HLA expression [7].

Intriguingly, the present study also describes a subset of NKG2C^{pos} NK cells that comprises >10% of the entire NK cell population within CB. The authors show that these cells proliferate at high degrees when cultured in vitro with HLA-E-expressing feeder cells and exhibit strong degranulation responses following the incubation with HLA-E-expressing targets. The expansion of PB NKG2C^{pos} NK cells is typically associated in adults with HCMV infection and represents an adaptive subset of NK cells that contributes to control HCMV reactivation [8]. In all CB tested in the present study, despite the absence or presence of a positive serology for HCMV, a significant fraction of NK cells constitutively expresses

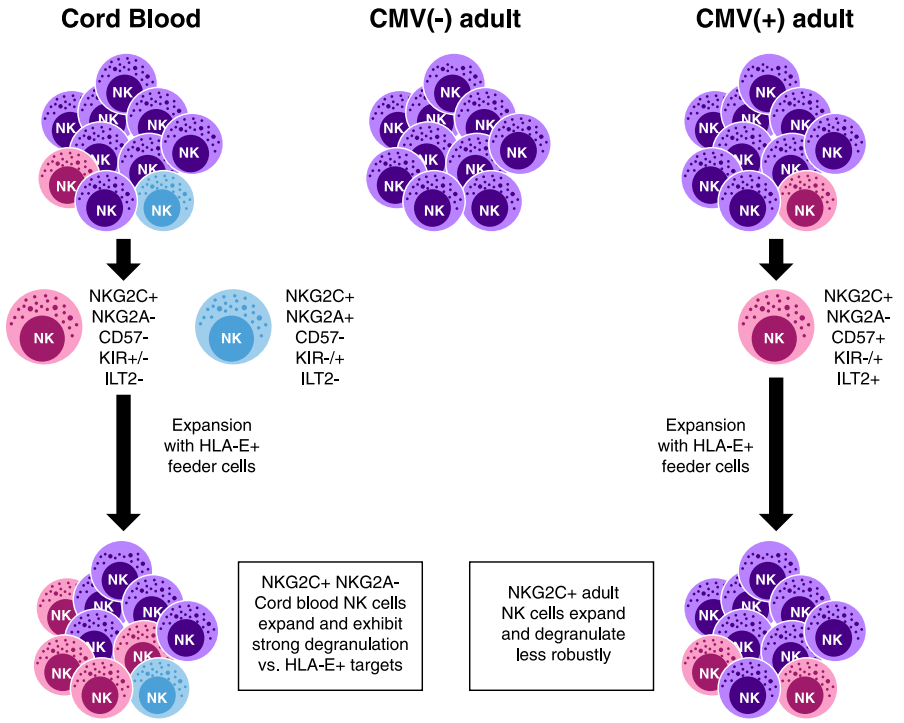

Figure 1. Unique proliferative and functional feature of NKG2C^{pos} NK cells in CB. (Upper) Representative NKR repertoire in CB NK cells compared with that of their PB counterparts from adult donors, seronegative or seropositive for HCMV. NKG2C^{pos}/NKG2A^{neg} NK cell subsets are labeled in red, whereas NKG2C^{pos}/NKG2A^{pos} are in blue. Differential expression of CD57 and ILT2 is noted, whereas the more heterogeneous expression of KIR is displayed as +/− (more often positive) or −/+ (more often negative). (Lower) The coculture with HLA-E expressing 221 feeder cells induces an expansion of the degranulating NKG2C^{pos}/NKG2A^{neg} NK cell subset but not of the NKG2C^{pos}/NKG2A^{pos} NK cell population. This phenomenon appears to occur selectively in CB rather than in the PB of adult healthy donors seropositive for HCMV.

TABLE 1. Critical questions to be addressed

1. What aspects of the CB environment contribute to NKG2C^{+} subset expansion?
2. How do KIR genes shape the NK cell repertoire in an HLA-independent fashion?
3. Will targeting of the highly expressed inhibitory NKG2A receptor increase the proliferative capacity and anti-leukemia potential of CB NK cells?
4. What factors contribute to specific changes in NK cell repertoire over the lifespan?
5. What are the contributions of CD57 and ILT2 to unique proliferative capacity of CB NKG2C^{+} NK cells?

NKG2C, thus suggesting that materno-fetal HCMV infection is unlikely accounting for this phenomenon. Moreover, whereas PB NKG2C^{pos} NK cells in adults typically do not coexpress NKG2A, the current study reports that the majority of NKG2C^{pos} CB NK cells is also NKG2A^{pos} and hypothesizes that NKG2A may inhibit cell degranulation and cytotoxicity. Moreover, NKG2C^{pos} CB NK cells have been shown not to express ILT2, an inhibitory receptor constitutively present on PB NKG2C^{pos} NK cells in HCMV seropositive adult patients [9]. Hence, it is tempting to speculate that the recent discoveries in regard to the unique immunosuppressive neonatal environment [10] and to the influence of maternal microchimeric cells [11] likely play a role in inducing the distinctive phenotypes and functions of CB NK cells. Nevertheless, Rettman et al. [4] also demonstrate that established protocols expanding NK cells in vitro to restore CB NK cell features favor the expansion of this unique NKG2C^{pos} NK cell subset. In this experimental setting, the coexpression of NKG2A provides strong inhibitory signals that constrain expansion and function of NKG2C^{pos} NK cells, which is consistent with previous reports with adult NK cells [12].

In summary, the experimental findings provided by this interesting study add important insight to our knowledge of the phenotype and function of CB NK cells (**Fig. 1**) and highlight complexities of their repertoire that likely contributes to the potent immune-therapeutic capacity of these cells to prevent leukemic relapse and GVHD. Further studies (**Table 1**) are needed to clarify the contributions of this unique population of CB NK cells in the clearance of malignant cells, as well as to ascertain the factors and mechanisms that induce the above-mentioned phenotypes and functions.

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