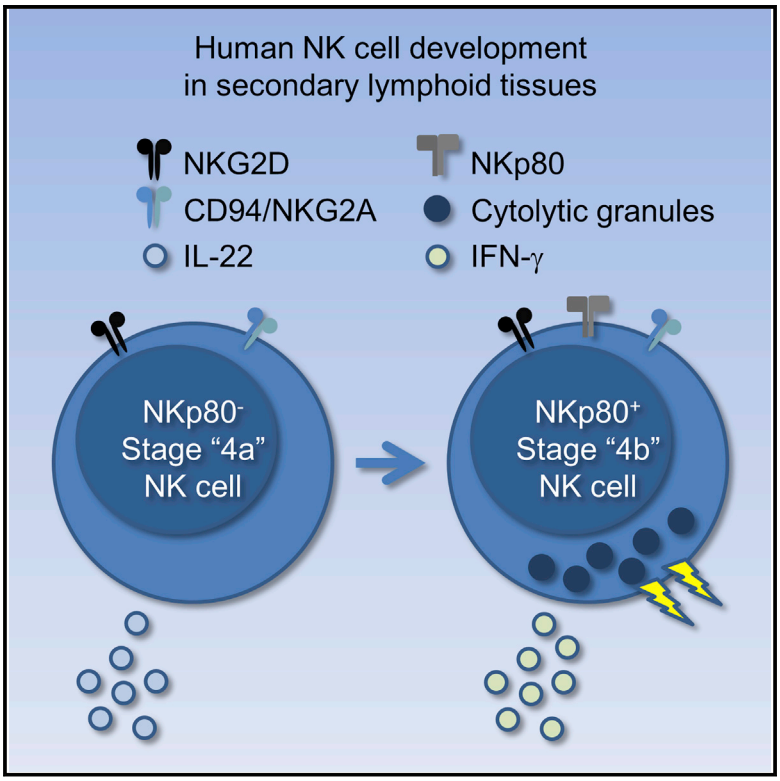
Article

Cell Reports

# NKp80 Defines a Critical Step during Human Natural Killer Cell Development

## Graphical Abstract




## Authors

Aharon G. Freud, Karen A. Keller, Steven D. Scoville, ..., Jianhua Yu, William E. Carson III, Michael A. Caligiuri

## Correspondence

aharon.freud@osumc.edu

## In Brief

Human natural killer (NK) cells have potent effector functions against cancer; how NK cells develop in humans is unclear. Freud et al. demonstrate that NKp80 expression marks functionally mature NK cells as they develop in secondary lymphoid tissues. These findings help define the pathway of human NK cell development.

## Highlights

- NKp80 is expressed on a subset of CD94$^{+}$ NK cells in secondary lymphoid tissues
- CD94$^{+}$NKp80$^{-}$ NK cells have ILC3-associated features
- CD94$^{+}$NKp80$^{+}$ NK cells produce IFN-γ and kill MHC class I$^{-}$ target cells
- CD94+NKp80$^{-}$ NK cells are precursors to CD94$^{+}$NKp80$^{+}$ NK cells

Freud et al., 2016, Cell Reports *16*, 379–391
July 12, 2016 
http://dx.doi.org/10.1016/j.celrep.2016.05.095

Cell Reports
Article



# NKp80 Defines a Critical Step during Human Natural Killer Cell Development

Aharon G. Freud,[1,7,*] Karen A. Keller,[1,7] Steven D. Scoville,[2,3,7] Bethany L. Mundy-Bosse,[4,7] Stephanie Cheng,[1,7] Youssef Youssef,[1,7] Tiffany Hughes,[4,7] Xiaoli Zhang,[5,7] Xiaokui Mo,[5,7] Pierluigi Porcu,[4,7] Robert A. Baiocchi,[4,7] Jianhua Yu,[4,7] William E. Carson III,[6,7] and Michael A. Caligiuri[4,7]
[1]Department of Pathology
[2]Medical Scientist Training Program
[3]Biomedical Sciences Graduate Program
[4]Division of Hematology, Department of Internal Medicine
[5]Center for Biostatistics
[6]Division of Surgical Oncology, Department of Surgery
[7]Comprehensive Cancer Center and The James Cancer Hospital and Solove Research Institute
The Ohio State University, Columbus, OH 43210, USA
*Correspondence: aharon.freud@osumc.edu
http://dx.doi.org/10.1016/j.celrep.2016.05.095

## SUMMARY

**Human natural killer (NK) cells develop in secondary lymphoid tissues (SLTs) through distinct stages. We identified two SLT lineage (Lin)$^-$CD34$^-$CD117$^{+/-}$CD94$^+$CD16$^-$ "stage 4" subsets according to expression of the C-type lectin-like surface-activating receptor, NKp80: NKp80$^-$ (stage "4a") and NKp80$^+$ (stage "4b"). Whereas stage 4b cells expressed more of the transcription factors T-BET and EOMES, produced interferon-gamma, and were cytotoxic, stage 4a cells expressed more of the transcription factors RORγt and AHR and produced interleukin-22, similar to SLT Lin$^-$CD34$^-$CD117$^+$CD94$^-$CD16$^-$ "stage 3" cells, whose phenotype overlaps with that of group 3 innate lymphoid cells (ILC3s). Co-culture with dendritic cells or transplantation into immunodeficient mice produced mature NK cells from stage 3 and stage 4a populations. These data identify NKp80 as a marker of NK cell maturity in SLTs and support a model of human NK cell development through a stage 4a intermediate with ILC3-associated features.**

## INTRODUCTION

Natural killer (NK) cells are innate lymphoid cells (ILCs) that can kill pathogen-infected and malignant cells as well as modulate other components of the immune system by producing chemokines and cytokines. Numerous recent studies highlight the existence of other ILC populations, and collectively, all ILCs are now categorized into three groups according to their differential expression of surface antigens, transcription factors, and cytokines (Spits et al., 2013). NK cells represent a subtype of group 1 ILCs, and their distinguishing features include (1) expression of the transcription factors, T-BET and EOMES; (2) expression of major histocompatibility complex (MHC) class I molecule-binding receptors, CD94/NKG2 (considered specific for NK cells among ILCs; Spits et al., 2013) and killer immunoglobulin-like receptors (KIR); (3) production of interferon-gamma (IFN-γ); and (4) the ability to mediate perforin-dependent natural cytotoxicity (Caligiuri, 2008; Cortez et al., 2015). ILC1s represent the other group 1 ILC subtype (Spits et al., 2013). Whereas ILC1s similarly produce IFN-γ and express T-BET, recent mouse and human studies indicate that they comprise a lineage that is distinct from NK cells in that the former are non-cytolytic and lack expression of numerous NK-associated molecules, including EOMES, CD94, CD56, CD16, perforin, granzymes, and KIRs (Bernink et al., 2013, 2015; Fuchs et al., 2013; Klose et al., 2014).

Studies in both mice and humans indicate that NK cells can develop in multiple tissues, including bone marrow (BM), secondary lymphoid tissues (SLTs), the liver, the uterus, and the thymus (Yu et al., 2013). A five-stage model of human NK cell development within SLTs was proposed based on the differential expression of CD34, CD117, CD94, and CD16 among lineage (Lin)-negative cells (i.e., cells lacking T, B, dendritic cell [DC], and myelomonocytic-associated antigens): stage 1, CD34$^+$CD117$^-$CD94$^-$CD16$^-$; stage 2, CD34$^+$CD117$^+$CD94$^-$CD16$^-$; stage 3, CD34$^-$CD117$^+$CD94$^-$CD16$^-$; stage 4, CD34$^-$CD117$^{+/-}$CD94$^+$CD16$^-$; and stage 5, CD34$^-$CD117$^{+/-}$CD94$^{+/-}$CD16$^+$ (Freud et al., 2014). The original study characterizing these stages of development (Freud et al., 2006) showed that, in bulk cultures, CD34$^+$ stage 1 and stage 2 populations are capable of DC, T cell, and NK cell differentiation, whereas the CD34$^-$ stage 3 population can give rise to NK cells, but not to DCs or T cells. In addition, stage 3 cells lack the two hallmark functions of mature NK cells (i.e., IFN-γ production and perforin-dependent cytotoxicity) that are detected at stage 4 (CD94$^+$). Therefore, it was originally concluded that stage 3 cells are lineage-restricted NK cell precursors and that functional maturity is acquired at stage 4 (Freud and Caligiuri, 2006). This NK cell development model is supported by analysis of in-vitro-derived intermediates (Grzywacz et al., 2006).