

tribute to the unique pattern of gene expression already noted after the induction of T cell anergy¹⁵. Alternatively, anergy may ultimately represent a differentiation state that leads to loss of autocrine growth factor production and proliferation as a consequence of the new expression of many distinct counter-regulatory molecules (such as Cbl-b, Itch and Grail), with DGK being just one more factor needed to prevent anergy reversal and maintain the tolerant state. Regardless of which is true, this identification of DGK as an important mediator of clonal anergy now provides the basis for new therapeutic approaches to

the treatment of clinical autoimmunity that take advantage of diacylglycerol antagonism in T cells. Likewise, vaccines designed to promote diacylglycerol formation and limit the accumulation of DGK activity will have the greatest chance of eliciting a durable and protective response by antigen-specific T cells.

1. Lamb, J.R., Skidmore, B.J., Green, N., Chiller, J.M. & Feldmann, M. *J. Exp. Med.* **157**, 1434–1447 (1983).
2. Jenkins, M.K. & Schwartz, R.H. *J. Exp. Med.* **165**, 302–319 (1987).
3. Quill, H. & Schwartz, R.H. *J. Immunol.* **138**, 3704–3712 (1987).
4. Kaibuchi, K. *et al. J. Biol. Chem.* **258**, 6701–6704 (1983).

5. Zha, Y. *et al. Nat. Immunol.* **7**, 1166–1173 (2006).
6. Olenchok, B.A. *et al.* **7**, 1174–1181 (2006).
7. Fields, P.E., Gajewski, T.F. & Fitch, F.W. *Science* **271**, 1276–1278 (1996).
8. Li, W., Whaley, C.D., Mondino, A. & Mueller, D.L. *Science* **271**, 1272–1276 (1996).
9. Hickman, S.P., Yang, J., Thomas, R.M., Wells, A.D. & Turka, L.A. *J. Immunol.* **177**, 2186–2194 (2006).
10. Mueller, D.L., Jenkins, M.K. & Schwartz, R.H. *J. Immunology* **142**, 2617–2628 (1989).
11. Crespi, D. *et al. Eur. J. Immunol.* **32**, 2500–2509 (2002).
12. Powell, J.D., Lerner, C.G. & Schwartz, R.H. *J. Immunol.* **162**, 2775–2784 (1999).
13. Spitaler, M., Emslie, E., Wood, C.D. & Cantrell, D. *Immunity* **24**, 535–546 (2006).
14. Gozani, O. *et al. Cell* **114**, 99–111 (2003).
15. Macian, F. *et al. Cell* **109**, 719–731 (2002).

IL-7 and the thymus dictate the NK cell ‘labor market’

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The identification of a functionally distinct thymus-dependent lineage of mouse natural killer cells demonstrates the diversity of the natural killer cell population.

In contrast to the well established and complex mixture of sublineages and developmental states of B cells and T cells, natural killer (NK) cells have been thought to undergo a relatively simple differentiation program from a bone marrow progenitor into an effector cell¹. However, evidence has suggested that NK cells in the liver, lymph nodes, uterus and thymus are developmentally distinct, although in most cases neither the functional capability nor the pedigree of each population has been established. In this issue of *Nature Immunology*, Di Santo and colleagues firmly establish the existence of a developmentally and functionally distinct lineage of thymus-derived NK cells².

Many years ago, NK cell precursors were identified in the early fetal thymus³. Subsequent studies have shown that a few mature NK cells contain rearranged T cell receptor genes, suggesting a thymic origin⁴. In addition, thymic progenitors differentiate *in vitro* into NK cells. However, the extent and relevance of that thymic NK cell ‘potential’ has remained unclear. Di Santo and colleagues have resolved that issue by demonstrating that NK cells representing 0.05% of thymic cellularity can be identified by

their dependence on three factors: the thymus, interleukin 7 (IL-7) and the transcription factor GATA-3. Conveniently, surface expression of the IL-7 receptor (CD127) ‘marks’ this lineage both in the thymus and in peripheral organs. Having identified a marker for thymus-derived NK cells, the authors were able to ask two crucial questions: what do those NK cells look like? And what do they do?

NK cells are engaged in a lifelong mission of scrutiny and surveillance. They interact with all nucleated cells and assess, through inhibitory receptors in mice (the Ly49 family) and humans (killer cell immunoglobulin-like receptor), whether the correct self major histocompatibility complex profile is expressed. In addition, NK cells express receptors (such as NKG2D) that detect target cell ‘stress’.

In the bone marrow, expression of those and other NK receptors is acquired through a poorly understood process during an early stage of development¹ (Fig. 1). NK cell precursors develop in the absence of the common cytokine receptor γ -chain (which is essential for the transmission of IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 signals), ruling out the possibility of an obligatory function for those cytokines in NK cell commitment. NK cell development from that very early stage depends on IL-15, but IL-7, despite its ability to support NK cells in thymic progenitor cultures, has been considered unnecessary. After lineage commitment, NK cell precursors differentiate through several immature stages in which they sequentially acquire NK cell receptors and functional capacity. After upregulation of CD11b

and CD43, mature NK cells are exported via the blood to peripheral sites (spleen and liver), where they undergo further maturation¹. The tumor necrosis factor receptor family molecule CD27 has been used to discriminate two subsets of mature CD11b^{hi} NK cells in mice and humans; the CD27^{hi} subset has a lower threshold for activation, greater migratory capability and proliferative potential, and a mainly lymphoid tissue-restricted distribution⁵.

Notably, the thymus-derived NK cells identified by Di Santo and colleagues do not conform to that scheme and bear an unusual combination of phenotypic characteristics (CD11b^{lo}CD16⁻CD69^{hi}Ly49⁻) and functional attributes (weak cytotoxicity but strong production of interferon- γ , granulocyte-macrophage colony-stimulating factor and tumor necrosis factor). It seems that these IL-7-dependent thymus-derived NK cells have functions distinct from those of their bone marrow-derived counterparts. That is of particular importance, as human peripheral blood contains two distinct NK cell subsets distinguishable by differences in expression of CD56 and CD16 (ref. 6). Approximately 90% of human NK cells are CD56^{dim}CD16⁺, whereas approximately 10% of cells are CD56^{bright}CD16⁻. CD56^{bright}CD16⁻ NK cells have lower expression of killer cell immunoglobulin-like receptor and cytotoxic potential, but seem poised to efficiently secrete cytokines and home to lymph nodes. In contrast, CD56^{dim}CD16⁺ cells have more potent cytotoxic activity and may be recruited into inflamed tissues. Until now, evidence for such functional diversification of NK cells has not

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been obtained in mice. Notably, the two human NK cell subsets found in peripheral blood differ in CD127 expression: CD56^{bright}CD16⁻ human NK cells express CD127 and GATA-3, and CD56^{dim}CD16⁺ human NK cells lack CD127 and have low expression of GATA-3. The identification of this GATA-3- and CD127-dependent thymic NK cell developmental pathway may unite the studies of these two species and establish a distinct conserved 'division of labor' among NK cell subsets.

GATA-3 is expressed in hematopoietic and other tissues and regulates several distinct aspects of the differentiation of IL-7-independent NK cells, including their Ly49 receptor repertoire, expression of maturation markers, cytokine production and homing to the liver⁷. In early thymocyte precursors, CD127 is a Notch-dependent GATA-3 transcriptional target². Those data suggest that CD127⁺ NK cells are generated in the thymus from hematopoietic progenitors (most likely early T cell precursors) through a pathway involving Notch, GATA-3 and CD127 and support the idea of a close relationship between T cells and NK cells. However, they also raise several key questions. Are thymus-seeding cells 'bipotent'? If so, when does the bifurcation of T lineages and NK lineages occur, and do thymic NK cells contain rearranged T cell receptors? It also remains unclear what function Notch has, if any. IL-7-independent NK cells are present in mice with conditional knockout of Notch1 (ref. 8); it would be useful to determine if these mice also have thymus-derived NK cells.

NK cells in different organs may serve a variety of different functions. The bone marrow may be involved only in the initial steps of NK cell differentiation, and other sites may be required for final maturation. The identification of a lymph node-resident NK cell precursor in humans⁹ that apparently migrates from the bone marrow through the blood to the lymph node would be consistent with that possibility. The characterization of NK cell precursors and immature NK cells in the liver and spleen that are positive for the proapoptotic molecule TRAIL^{7,10,11} also provides additional evidence for organ-specific differentiation of NK cells. The discovery and characterization of a thymus-derived NK cell lineage is a key step forward².

Athymic mice and those with defects in the IL-7 signaling pathway have cytotoxic NK cells and are capable of rejecting tumors. However, given these new data from Di Santo and colleagues, it will now be necessary to extensively compare NK cell function in those mice with that of their wild-type littermates. It remains unclear whether the IL-7-dependent NK cells have a unique function in the thymus. Postulated functions include the modulation of thymopoiesis, maintenance of thymic archi-

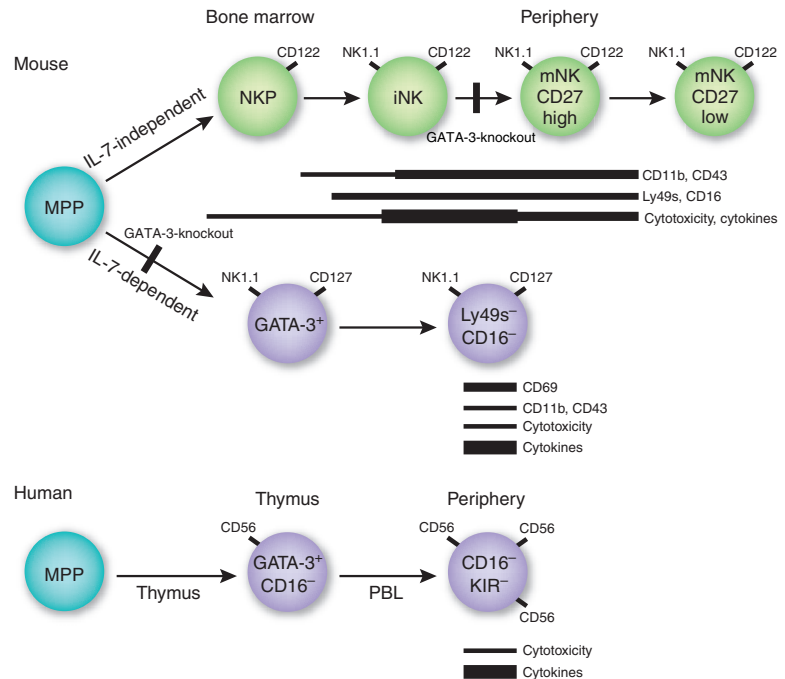


Figure 1 IL-7-dependent and IL-7-independent NK cell development. Stages and markers for mouse and human NK cells are included. Horizontal bars indicate the relative amount of a function or expression of a molecule. Short vertical bars indicate approximate points of the developmental block of GATA-3-deficient NK cells. MPP, multipotent progenitor; NKP, NK cell progenitor; iNK, immature NK cell; mNK, mature NK cell; PBL, peripheral blood leukocyte; KIR, killer cell immunoglobulin-like receptor.

itecture, and defense against thymocyte transformation and maintenance of the clonal size of T cell pools, among others. NK cells can modulate intrathymic T cell development through a perforin-mediated mechanism¹²; however, IL-7-dependent, thymus-derived NK cells are not noted for having high cytotoxic capacity². Once again, the topological organization of developing NK cells in generative organs (such as bone marrow and thymus) may both suggest their local functions and provide important clues to the mechanisms that control NK cell development.

Perhaps the functions of IL-7-dependent NK cells are only manifested once they are exported to the periphery. Notably, those cells are prominent in the lymph node, the site of multiple interactions between NK cell and dendritic cells and the location where cytokines produced by activated NK cells reinforce T helper type 1 differentiation¹³. Whether those immune effects are mediated through or perhaps regulated by CD127⁺ or CD127⁻ NK cells (or both) remains to be determined. Notably, cell-cell interactions involving lymph node NK cells are undefined. Knowing the precise physical location of the IL-7-dependent and IL-7-independent NK cells relative to that of other cell types and under-

standing NK cell migration patterns before and after immunization should provide clues to any unique function of thymus-derived NK cells in the lymph node. It is likely that intravital imaging technology will be important in deciphering the steady-state and post-activation characteristics of these NK cell populations in the lymph node.

In humans, CD56^{bright} NK cells are the main NK cell producers of immunoregulatory cytokines, including interferon- γ , tumor necrosis factor, granulocyte-macrophage colony-stimulating factor, IL-10 and IL-13. In the CD56^{bright} population, CD8 is expressed by a discrete subset that produces T helper type 2 cytokines. The thymic dependence of mouse lymph node CD127⁺ NK cells suggests that human CD56^{bright}CD16⁻ NK cells, the main population found in the naive lymph node, may also be thymus-derived. To our knowledge, no studies of athymic or CD127-mutant humans (such as FoxN1 or CD127 mutants) have carefully analyzed whether the absence of a thymus affects CD56^{bright}CD16⁻ NK cells, but elucidation of that aspect will be of great interest. If the CD8⁺CD56^{bright} NK subset is a corollary of the IL-7-dependent, thymus-derived NK cell, then this newly identified mouse thymus-derived NK cell subset might

be expected to produce IL-10 and IL-13; however, those important functions have not yet been examined². CD127⁺ thymus-derived NK cells, like CD56^{bright} human NK cells, express the c-Kit receptor tyrosine kinase. However, notably, CD127⁺ thymus NK cells do not seem to constitutively express the high-affinity IL-2 receptor, as reported for CD56^{bright} human NK cells. Despite that finding, subtle differences between analogous subsets of NK cells in mice and humans would not be unexpected. Furthermore, the relationship between IL-7-dependent, thymus-derived NK cells and a subset of CD34^{dim} hematopoietic progenitor cells that seems to

reside in the lymph node and differentiate into CD56^{bright} NK cells⁹ remains to be established. Although many questions are still left to be answered, the demonstration of the existence of a distinct IL-7-dependent pathway of NK cell development in the thymus and the functional similarities of these cells to human CD56^{bright} NK cells provides a substantial step toward understanding how NK cells 'divide' the duties required for lifelong surveillance of self and protection against pathogens and malignancy.

1. Di Santo, J.P. *Annu. Rev. Immunol.* **24**, 257–286 (2006).

2. Vosshenrich, C.A.J. *et al. Nat. Immunol.* **7**, 1217–1224 (2006).
3. Rodewald, H.R. *et al. Cell* **69**, 139–150 (1992).
4. Veinotte, L.L., Greenwood, C.P., Mohammadi, N., Parachoniak, C.A. & Takei, F. *Blood* **107**, 2673–2679 (2006).
5. Hayakawa, Y. & Smyth, M.J. *J. Immunol.* **176**, 1517–1524 (2006).
6. Farag, S.S., VanDeusen, J.B., Fehniger, T.A. & Caligiuri, M.A. *Int. J. Hematol.* **78**, 7–17 (2003).
7. Samson, S.I. *et al. Immunity* **19**, 701–711 (2003).
8. Radtke, F. *et al. J. Exp. Med.* **191**, 1085–1094 (2000).
9. Freud, A.G. *et al. Immunity* **22**, 295–304 (2005).
10. Kim, S. *et al. Nat. Immunol.* **3**, 523–528 (2002).
11. Takeda, K. *et al. Blood* **105**, 2082–2089 (2005).
12. Schott, E., Bonasio, R. & Ploegh, H.L. *J. Exp. Med.* **198**, 1213–1224 (2003).
13. Martin-Fontecha, A. *et al. Nat. Immunol.* **5**, 1260–1265 (2004).