Murine thymic NK cells: a case of identity

Victoria Male[1] and Hugh JM Brady[2]

1 Institute of Immunity and Transplantation, UCL Medical School, Rowland Hill Street, London, NW3 2PF, UK

2 Department of Life Sciences, Imperial College, London SW7 2AZ, UK

Address correspondence to:

Victoria Male
Institute of Immunity and Transplantation
UCL Medical School
Rowland Hill Street
London NW3 2PF, UK
Email: v.male@ucl.ac.uk

Hugh JM Brady
Department of Life Sciences
Imperial College
London SW7 2AZ, UK
Email: h.brady@imperial.ac.uk

Keywords: Thymus; Natural Killer cell; ILC1; transcription factor; Tbet; Nfil3; Id2; Ets1

See accompanying article by Gabrielli et al.

Abstract

Just over a decade ago, it was established that NK cells in the thymus do not follow precisely the same developmental pathway as conventional NK cells that develop in the bone marrow. Subsequently, it has emerged that NK cells are one branch of a family of innate lymphoid cells (ILCs). ILC1s and thymic NK cells have, however, sufficient similarities such that questions have been raised about how distinctive each cell type is from the other. In this issue of *European Journal of Immunology*, Gabrielli et al [Eur. J. Immunol. 2017. 47: xxx-xxx] make a detailed study of the transcription factor requirements of murine thymic NK cells. They provide a valuable insight into the distinctive identity of thymic NK cells with regard to Tbet, Nfil3, Id2 and Ets1. In addition, they clarify the nature of DX5 expression on NK cells and ILC-like cells in the murine thymus.

It has been more than a decade since Vosshenrich and colleagues first reported a thymic pathway of NK-cell development distinct from that in the bone marrow [1]. Unlike circulating NK cells, the thymic NK cells identified by Vosshenrich require IL-7 signalling and the transcription factor GATA3 for their development and can be identified by their expression of the IL-7 receptor component IL-7Rα (CD127).

More recently, it has emerged that NK cells form only one branch of a larger family of innate lymphoid cells (ILCs), which also include a number of $CD127^+$ GATA3-dependent non-NK "helper" ILC types [2]. ILCs are now divided into three subgroups by their cytokine production and transcription factor dependence: ILC1s produce IFN-γ and TNF-α and require Tbet, ILC2s produce IL-5 and IL-13 and require GATA3, and ILC3s produce IL-22 and require RORγt [3]. There is some dispute over whether NK cells should be considered ILC1s [4] or if, analogous to cytotoxic T cells and Th1 cells, the two should be considered distinct cell types [5]. Notwithstanding questions of lineage, the discovery of non-NK ILC1s has made it necessary to re-examine populations throughout the body that were previously regarded as NK cells in order to determine whether they are genuine NK cells or non-NK ILC1s. In this issue of *The European Journal of Immunology,* Gabrielli and colleagues tackle the question of whether murine thymic NK cells more closely resemble NK cells or non-NK ILC1s [6].

Previous investigations of murine thymic NK cells have defined them as being DX5 (CD49b)- positive [1, 7]. However, a number of recent studies have identified tissue-resident NK-cell subsets that are $DX5^-$, such as XXX and XXX [8-11] (Figure 1). Therefore, Gabrielli et al examined both $DX5^-$ and $DX5^+$ cells within the thymic Lin^- $CD122^+NK1.1^+$ population. They found that the $DX5^-$ cells are absent in $Rag1^{-/-}$ mice, suggesting that these cells are actually members of the T-cell lineage. Further, these murine thymic $DX5^-$ cells stain positive with PBS57-loaded CD1d tetramers, indicating that they are NKT cells, and not any kind of ILC [6].

All ILCs, including NK cells, require the transcription factors Nfil3 [7, 12-15] and Id2 [16, 17] for their development, but NK cells and non-NK ILC1 differ in their requirements for other transcription factors. In particular, circulating NK cells require Eomes but not Tbet for their development (Figure 1, top left) whereas non-NK ILC1s are Tbet-dependent but Eomes-independent [8, 10, 18] (Figure 1, bottom right). Therefore, one way to determine whether a particular population comprises NK cells or non-NK ILC1s is by examining their transcription factor requirements. $DX5^+$ thymic NK cells are already known to require GATA3 [1, 10] and Nfil3 [7, 19] for their development (Figure 1, top right). Gabrielli et al confirm that $DX5^+$ thymic NK cells are Nfil3-dependent and further examine their requirements for Tbet, Ets1 and Id2 [6].

Thymic NK cells are present in the Tbet knockout mouse and this, more than any other finding, suggests that they are NK cells, rather than non-NK ILC1. Although the authors have not been able to examine an Eomes-deficient mouse, they show that thymic NK cells do express Eomes, further supporting the idea that they are NK cells 2012 [8]. It is interesting to note that recent findings suggest that ILC1s may have Eomes-dependent plasticity. For example, ectopic expression of Eomes in murine NK precursors was shown to be sufficient to divert ILC1 development into the conventional NK-cell lineage [20]. In a murine conditional knockout of Ets1, thymic NK-cell numbers are reduced but those cells that are retained have a more mature phenotype than cells from wild-type mice. These are similar to findings on the requirement for Ets1 in the medullary pathway of NK-cell development [21]. Therefore, in terms of their requirements for Tbet and Ets1, and their expression of Eomes, thymic NK cells resemble conventional NK cells.

Thymic NK cells are present in normal numbers in a conditional knockout of Id2, although their phenotype is subtly altered. This is unexpected given that all ILCs, including NK cells, are thought to rely on Id2 [16, 17]. The finding that thymic NK cells do not require Id2 is also surprising because they are Ets1-dependent, and Ets1 has previously been shown to regulate Id2 [21]. These differences in transcription factor requirements between thymic and medullary pathways of NK-cell development may be due to differences in the cytokine milieu. Id2 is required for survival in response to IL-15

signalling [17] so one possibility is that thymic NK cells, which require IL-7 for their development, rely instead upon IL-7 signalling and are therefore less dependent upon Id2. It is equally intriguing that Gabrielli et al find that thymic NK cells require Nfil3 but not Id2 [6] while previous findings point to Nfil3 directing Id2 expression in both NK cells [13] and ILCs [15]. Another possibility for differing transcription factor requirements between thymic NK cells and medullary NK cells as well as ILCs could be the prevalence of Notch signalling [5]. The pivotal role of Notch signalling in ILC development is well established. Perhaps, canonical Notch signalling within the thymus can reduce the transcription factor requirements for thymic NK-cell development.

The development and functions of tissue-resident NK cells in a variety of organs, such as the liver, has recently been a focus of intense research interest. Whether tissue-resident NK cells are NK cells or non-NK ILC1s has itself been a matter of debate [22, 23] but the similarities between the phenotype of functions of thymic NK cells, as previously reported, and those of tissue-resident NK cells are striking (Figure 1). It has previously been suggested that thymic NK cells are in fact “failed” T cells [19, 24]. This may be the case, particularly in the light of their very small numbers in Rag-sufficient mice. However, the possibility that thymic NK cells represent a tissue-resident NK-cell subset specific to the thymus is an intriguing one. Tissue-resident NK cells have specialist physiological functions in their home organs: for example, uterine NK cells mediate the depth to which the placenta implants during pregnancy [25]. There are a number of potential roles for thymic NK cells in the modulation of thymopoiesis and the maintenance of thymic architecture. NK cells are known to have a role in dendritic cell (DC) maturation [26], so it may be worth exploring any potential for thymic NK cells influencing DCs role in thymic selection processes. It will be interesting, in the future, to investigate their involvement in these processes in Rag-sufficient mice. Perhaps this small subset of cells will have a wide-ranging impact on T-cell development and repertoire, and on the entire immune system.

Acknowledgements

Conflict of interest

The authors declare no financial or commercial conflict of interest.

References

1. **Vosshenrich, C.A., García-Ojeda, M.E., Samson-Villéger, S.I., Pasqualetto, V., Enault, L., Richard-Le Goff, O., Corcuff E. et al.,** A thymic pathway of mouse natural killer cell development characterized by expression of GATA-3 and CD127. Nat. Immunol. 2006. **7**: 1217-1224.
2. **Yagi. R., Zhong. C., Northrup, D.L., Yu, F., Bouladoux, N., Spencer, S., Hu, G. et al.,** The transcription factor GATA3 is critical for the development of all IL-7Rα-expressing innate lymphoid cells. *Immunity* 2014. 40: 378-388.
3. **Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J.P., Eberl, G., Koyasu, S. et al.,** Innate lymphoid cells--a proposal for uniform nomenclature. *Nat. Rev. Immunol*. 2013. **13**: 145-149.
4. **Serafini, N., Vosshenrich, C.A. and Di Santo, J.P.,** Transcriptional regulation of innate lymphoid cell fate. *Nat. Rev. Immunol.* 2015. **15**: 415-428.
5. **Diefenbach, A., Colonna, M. and Koyasu, S.** Development, differentiation, and diversity of innate lymphoid cells. *Immunity* 2014. **41**: 354-365.
6. **Gabrielli, S., Sun, M., Bell, A., Zook, E.C., dePooter, R.F., Zamai, L. and Kee B.L.** Murine thymic NK cells are distinct from ILC1s and have unique transcription factor requirements. *Eur. J. Immunol*. **47**: DOI 10.1002/eji.201646871View.
7. **Seillet, C., Rankin, L.C., Groom, J.R., Mielke, L.A., Tellier, J., Chopin, M., Huntington, N.D., et al.,** Nfil3 is required for the development of all innate lymphoid cell subsets. *J. Exp. Med*. 2014. **211**:1733-1740.
8. **Gordon, S.M., Chaix, J., Rupp, L.J., Wu, J., Madera, S., Sun, J.C., Lindsten, T. et al.,** The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity* 2012. **36**: 55-67.
9. **Daussy, C., Faure, F., Mayol, K., Viel, S., Gasteiger, G., Charrier, E., Bienvenu, J. et al.,** T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow. *J. Exp. Med.* 2014. **211**: 563-577.
10. **Sojka, D.K., Tian, Z. and Yokoyama, W.M.,** Tissue-resident natural killer cells and their potential diversity. *Semin. Immunol.* 2014. **26**: 127-131.
11. **Boulenouar, S., Doisne, J.M., Sferruzzi-Perri, A., Gaynor, L.M., Kieckbusch, J., Balmas, E., Yung, H.W. et al.,** The Residual Innate Lymphoid Cells in NFIL3-Deficient Mice Support Suboptimal Maternal Adaptations to Pregnancy. *Front. Immunol*. 2016. **7**: 43.
12. **Gascoyne, D.M., Long, E., Veiga-Fernandes, H., de Boer, J., Williams, O., Seddon, B., Coles, M. et al.,** The basic leucine zipper transcription factor E4BP4

is essential for natural killer cell development. *Nat. Immunol*. 2009. **10**: 1118-1124.

13. **Male, V., Nisoli, I., Kostrzewski, T., Allan, D.S., Carlyle, J.R., Lord, G.M., Wack, A. et al.,** The transcription factor E4bp4/Nfil3 controls commitment to the NK lineage and directly regulates Eomes and Id2 expression. *J. Exp. Med*. 2014. **211**: 635-642.
14. **Geiger, T.L., Abt, M.C., Gasteiger, G., Firth, M.A., O'Connor, M.H., Geary, C.D., O'Sullivan, T.E. et al.,** Nfil3 is crucial for development of innate lymphoid cells and host protection against intestinal pathogens. *J. Exp. Med*. 2014. **211**: 1723-1731.
15. **Xu W, Domingues RG, Fonseca-Pereira D, Ferreira M, Ribeiro H, Lopez-Lastra S, Motomura Y, et al.,** NFIL3 orchestrates the emergence of common helper innate lymphoid cell precursors. *Cell Rep*. 2015. **10**: 2043-2054.
16. **Satoh-Takayama, N., Lesjean-Pottier, S., Vieira, P., Sawa, S., Eberl, G., Vosshenrich, C.A. and Di Santo, J.P.,** IL-7 and IL-15 independently program the differentiation of intestinal CD3-NKp46+ cell subsets from Id2-dependent precursors. *J. Exp. Med.* 2010. **207**: 273-280.
17. **Delconte RB, Shi W, Sathe P, Ushiki T, Seillet C, Minnich M, Kolesnik TB, et al.,** The Helix-Loop-Helix Protein ID2 Governs NK Cell Fate by Tuning Their Sensitivity to Interleukin-15. *Immunity* 2016. **44**: 103-115.
18. **Klose, C.S., Flach, M., Möhle, L., Rogell, L., Hoyler, T., Ebert, K., Fabiunke, C., et al.,** Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell* 2014. **157**: 340-356.
19. **Crotta, S., Gkioka, A., Male, V., Duarte, J.H., Davidson, S., Nisoli, I., Brady, H.J. et al.,** The transcription factor E4BP4 is not required for extramedullary pathways of NK cell development. *J. Immunol*. 2014. **192**: 2677-2688.
20. **Pikovskaya, O., Chaix, J., Rothman, N.J., Collins, A., Chen, Y.H., Scipioni, A.M., Vivier, E. et al.,** Eomesodermin Is Sufficient To Direct Type 1 Innate Lymphocyte Development into the Conventional NK Lineage. *J. Immunol*. 2016. **196**:1449-1454.
21. **Ramirez, K., Chandler, K.J., Spaulding, C., Zandi, S., Sigvardsson, M., Graves, B.J. and Kee, B.L.,** Gene deregulation and chronic activation in natural killer cells deficient in the transcription factor ETS1. *Immunity* 2012. **36**: 921-932.
22. **Peng, H. and Tian, Z.** Re-examining the origin and function of liver-resident NK cells. *Trends Immunol*. 2015. **36**: 293-299.
23. **Tang, L., Peng, H., Zhou, J., Chen, Y., Wei, H., Sun, R., Yokoyama, W.M. et al.,** Differential phenotypic and functional properties of liver-resident NK cells and mucosal ILC1s. *J. Autoimmun*. 2016. **67**: 29-35.

24. **Vargas, C.L., Poursine-Laurent, J., Yang, L. and Yokoyama, W.M.** Development of thymic NK cells from double negative 1 thymocyte precursors. *Blood* 2011. **118**: 3570-3578.
25. **Moffett, A. and Colucci, F.,** Uterine NK cells: active regulators at the maternal-fetal interface. *J. Clin. Invest*. 2014. **124**: 1872-1879.
26. **Ferlazzo, G. and Morandi, B.,** Cross-Talks between Natural Killer Cells and Distinct Subsets of Dendritic Cells. *Front. Immunol*. 2014. **5**: 159.
27. **Samson, S.I., Richard, O., Tavian, M., Ranson, T., Vosshenrich, C.A., Colucci, F., Buer, J. et al.,** GATA-3 promotes maturation, IFN--gamma production, and liver-specific homing of NK cells. *Immunity* 2003. **19**:701-711.

See accompanying article:

http://dx.doi.org/10.1002/eji.201646871

Figure Legend

Figure 1. Transcription factor requirements and phenotype of thymic NK cells [1, 6, 7, 19] liver-resident NK cells [8-10, 17, 23, 27] and small intestine lamina propria (siLP) ILC1 [2, 7, 14, 18, 23] compared to conventional NK cells. Arrow width represents the relative ability of the cells to perform the indicated functions.