Dazed and Confused: NK Cells

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INTRODUCTION

Innate lymphoid cells (ILCs) are rapid producers of both proinflammatory and regulatory cytokines in response to local injury, inflammation, pathogen infection, or commensal microbiota perturbation (1). Because most ILCs have been shown to be tissue-resident during homeostasis (with the exception of circulating NK cells) in almost all organs analyzed, their ability to quickly respond to tissue stress and inflammation underpins their critical role in regulating tissue homeostasis and repair during infection or injury (2–4). Recent evidence has suggested that mature ILCs can be further classified into group 1, 2, and 3 ILCs based on different expression of transcription factors, cell surface markers, and effector cytokines (1). Mouse group 1 ILCs, which include natural killer (NK) cells and ILC1, were initially distinguished from other ILCs based on their constitutive expression of the transcription factor Tbx21 (T-bet), co-expression of activating receptors NKp46 and NK1.1, and production of interferon (IFN)-γ following activation (5). In humans, group 1 ILCs are harder to definitively differentiate from other ILCs due to the lack of lineage defining markers and reported functional plasticity amongst group 2 and group 3 ILCs (6).

ILC1 are recently discovered tissue-resident sentinels that function to protect the host from bacterial and viral pathogens at initial sites of infection (2, 7, 8). ILC1 rapidly produce IFN-γ following local dendritic cell activation and interleukin (IL)-12 production to limit viral replication and promote host survival before the recruitment of circulating lymphocytes into infected tissue (2). Unlike ILC1, NK cells can be recruited from the circulation into the parenchyma of infected or cancerous tissues where they display potent perforin-dependent cytotoxicity in addition to rapid IFN-γ production (9, 10). However, persistent inflammatory signals can also lead to unrestrained activation of group 1 ILCs during obesity and inflammatory bowel disease (IBD) (3, 11–14). While these studies suggest important roles for group 1 ILCs during host protection and pathology, gaps in evidence have inhibited the ability of recent studies to definitively distinguish between the roles of ILC1 and NK cells in these contexts.

GROUP 1 ILC PHENOTYPIC AND FUNCTIONAL HETEROGENEITY

NK cells, the founding member of ILCs, were initially defined based on the cell surface expression of NK1.1 in mouse or CD56 in human with the absence of cell surface expression of other lineage (Lin) defining markers including CD3, CD14, CD19, and TCR proteins (15). In subsequent mouse studies over the last 30 years, Lin−NK1.1+ cells were found to be heterogeneous for the expression of activating and inhibitory Ly49 receptors, cell surface integrins [α1β1 (CD49a), α2β1 (CD49b)], αEβ7 (CD103)], cell surface proteins (TRAIL, CD69, CD27, CD11b), transcription factors (Eomes), chemokine receptors (CXCR6), and cytokine receptors (IL-7Rα) in various organs (1, 16). Similarly, human Lin−CD56+ cells have been reported to be heterogeneous for the expression of transcription factors (EOMES and T-BET), cell surface markers (CD49a, CD56, CD16, Nkp80, CXCR6, IL-7Rα, CD94, CD69, Nkp44), and cytotoxic molecules (Perforin) (1, 16).
Early studies concluded that cells with an alternative cell surface or transcription factor phenotype from putative mature NK cells (mouse: Lin−NK1.1+T-bet+Eomes+CD49b+; human: Lin−IL-7Rα−CD56dimCD16+) in peripheral organs and blood likely represented immature NK (iNK) cells (17–21). This hypothesis is supported by studies demonstrating that subsets of developing mouse NK cells can be distinguished based on CD27 and CD11b expression (22, 23). Similarly, previous studies have suggested that CD56brightCD16− human NK cells in the blood may be immature precursors to CD56dimCD16+ mature NK cells (18, 19). However, whether other phenotypic differences observed in mouse and human group 1 ILCs are due to tissue-specific microenvironments, distinct lineages of cells, or developmental/activation states of NK cells is still under considerable debate and investigation.

Insight into these questions came shortly after the identification of Lin−IL-7Rα+ “helper” ILCs. Specifically, genetic evidence suggested that Tbx21-dependent IL-7Rα+“helper” Eomes+ NK1.1+Nkp46+ “ILC1” in the small intestine did not require Eomes for their development, whereas NK cells did require Eomes (7). A recent study further supported these initial data by using Eomes-GFP reporter mice to generate core transcriptional signatures of Eomes− ILC1 and Eomes+ NK cells from 4 independent tissues. The identified core ILC1 signature led to the discovery of the inhibitory receptor CD200r1 on CD56dimCD16+ ILC1 and Eomes+ NK cells from 4 independent tissues. The identified core ILC1 signature led to the discovery of the inhibitory receptor CD200r1 in almost all organs tested in mouse parabiosis experiments (1). Furthermore, adipose and small intestine iNK cells have also been found to be short-term (1 month), but not long term (4 months) tissue-resident in mouse parabiosis experiments (3), suggesting that short-term parabiosis (2 weeks–1 month) experiments are not sufficient to distinguish iNK cells from ILC1 without additional evidence. Thus, there is currently insufficient evidence to conclude that T-bet+ group 1 ILCs with the phenotype of CD49a+CD49b+Eomes+NK1.1+ are either tissue-resident NK (trNK) cells or transitional states of group 1 ILCs, because these cells may be activated NK cells in the tissue parenchyma following recruitment from circulation. Furthermore, CD49a+CD49b+Eomes+NK1.1+ cells may not represent a transitional subset of group 1 ILC, but instead may represent iNK cells in peripheral tissues, although further lineage tracing experiments will be necessary to clarify these issues in the field.

In the healthy state, mature human group 1 ILCs have been described to be heterogeneous for cell surface expression of CD56, CD16, and NKP80 in peripheral tissues (35). However, CD56 can be expressed on ILC progenitor populations and ILC3 in the tonsil (36), and may be downregulated during activation in a similar manner to CD16 and NKP80 (37–39). Thus, to date there are no known stable cell surface markers that can unequivocally distinguish between human mNK cells (or their developmental intermediates, which may be tissue-resident) and other proposed group 1 ILCs in inflamed human tissues, because activated mNK cells can lose expression of these cell surface markers during inflammation.

**Mouse Group 1 ILC Development**

Recent unbiased chromatin accessibility studies in mice suggest that NK cells can be defined epigenetically as a distinct ILC lineage through the enrichment of accessible T-bet and Eomes binding sites compared to other leukocytes (40). Similarly, mNK...
and iNK cells require Eomes for their development (2, 20, 41), suggesting that Eomes may be the master transcription factor that defines NK cell lineage identity in mice during homeostasis. In support of this hypothesis, mNK cells in the peritoneum, liver, spleen, salivary gland, and adipose tissue were all found to have a cell-intrinsic developmental requirement for Eomes and T-bet (2), arguing against tissue-specific transcription factor developmental requirements for mNK cells. While certain studies have observed that mNK cell numbers are normal in the absence of T-bet (7, 8, 42), it has been demonstrated previously that Tbx21−/− NK cells display an immature phenotype and are functionally deficient (3, 43–45). Therefore, because Tbx21 is required for optimal mature ILC1 and mNK development (2, 3, 46), Rag2−/− × Tbx21−/− mice are not a suitable model to test for the contributions of mature group 1 ILCs in vivo.

The transcription factors Id2 and Nfil3 have also been shown to be required for mature mouse ILC1 and NK cell development (47, 48). Certain studies have identified “tissue-resident NK cells,” “salivary gland ILCs,” and “type 1 ILCs” based on their developmental in the absence of Nfil3 (27, 33, 49). However, similar subsets have been also found to be Nfil3-dependent in a cell-intrinsic manner in other studies (2, 50). Because mNK cells can develop in an Nfil3-independent manner during virus-induced inflammation and aging (33, 51), analysis of Nfil3−/− mice is likely not sufficient to define group 1 ILC subsets due to these caveats. Previous studies have also utilized Zbtb16 fate-mapping studies and Id2 reporter mice to identify a common helper ILC precursor population that gives rise to all tissue-resident ILCs, but not mNK cells, to argue that ILC1 comprise a developmental lineage distinct from NK cells (7, 52, 53). However, a recent study using dual Zbtb16 and Id2 reporter mice demonstrated that both NK cells and ILC1 can develop from a Id2−/−Zbtb16+ shared precursor, suggesting that these transcription factors alone cannot be used to identify different group 1 ILC subsets during ontogeny (54). Instead, several studies have identified the transcription factor Zfp683 (Hobit) as highly expressed in peripheral ILC1 compared to mNK cells (2, 55, 56). Zfp683−/− mice display a loss of liver ILC1 but not other ILC populations (including ILC1 in other tissues) (2, 55), suggesting that mature liver ILC1 have a unique developmental pathway from other mouse ILCs. While developmental dependence on Eomes expression can be used to identify NK lineage cells in peripheral organs of mice, there is still no definitive evidence that a single transcription factor can define the development of other group 1 ILC subsets across all mouse tissues.

**DISCUSSION**

While collective evidence supports the hypothesis that mouse group 1 ILCs are composed of Eomes-dependent iNK and mNK cells, their activation or developmental states may be mistaken for novel subsets of group 1 ILCs. Eomes-independent ILC1 have been shown through single-cell sequencing, parabiosis, lineage tracing, and transcription factor deficient mouse experiments to be a distinct lineage of group 1 ILCs, and not a developmental or activation state of NK cells. In human tissues, there is currently no definitive evidence that can distinguish between developmental or activation states of group 1 ILCs during inflammation. Single cell sequencing studies will be needed to determine the extent of group 1 ILC heterogeneity in various peripheral tissues, and to identify stable markers that can distinguish between stable subsets of group 1 ILCs through lineage tracing in humanized mouse models.

**AUTHOR CONTRIBUTIONS**

The author confirms being the sole contributor of this work and has approved it for publication.

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**REFERENCES**

1. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells: 10 years on. Cell. (2018) 174:1054–66. doi: 10.1016/j.cell.2018.07.017
2. Weizman OE, Adams NM, Schuster IS, Krishna C, Pritykin Y, Lau C, et al. ILC1 confer early host protection at initial sites of viral infection. Cell. (2017) 171:795–808.e12. doi: 10.1016/j.cell.2017.09.052
3. O’Sullivan TE, Rapp M, Fan X, Weizman OE, Bhardwaj P, Adams NM, et al. Adipose-resident group 1 innate lymphoid cells promote obesity-associated insulin resistance. Immunity. (2016) 45:428–41. doi: 10.1016/j.immuni.2016.06.016
4. Gasteiger G, Fan X, Dikty S, Lee SY, Rudensky AY. Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. Science. (2015) 350:981–5. doi: 10.1126/science.aac9593
5. Vossenhirch CA, Di Santo JP. Developmental programming of natural killer and innate lymphoid cells. Curr Opin Immunol. (2013) 25:130–8. doi: 10.1016/j.coi.2013.02.002
6. Colonna M. Innate lymphoid cells: diversity, plasticity, and unique functions in immunity. Immunity. (2018) 48:1104–17. doi: 10.1016/j.immuni.2018.05.013
7. Klose CSN, Flach M, Möhle L, Rogell L, Hoyler T, Ebert K, et al. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. Cell. (2014) 157:340–56. doi: 10.1016/j.cell.2014.03.030
8. Abt MC, Lewis BB, Caballero S, Xiong H, Carter RA, Suisac B, et al. Innate immune defenses mediated by two ILC subsets are critical for protection against acute clostridium difficile infection. Cell Host Microbe. (2015) 18:27–37. doi: 10.1016/j.chom.2015.06.011
9. Hammer Q, Rückert T, Romagnani C. Natural killer cell specificity for viral infections. Nat Immunol. (2018) 19:800–8. doi: 10.1038/s41590-018-0163-6
10. Boudreau JE, Hsu KC. Natural killer cell education and the response to infection and cancer therapy: stay tuned. Trends Immunol. (2018) 39:222–39. doi: 10.1016/j.it.2017.12.001
11. Wensveen FM, Jelenčič V, Valentić S, Šestan M, Wensveen TT, Theurich S, et al. NK cells link obesity-induced adipose stress to inflammation and insulin resistance. Nat Immunol. (2015) 16:376–85. doi: 10.1038/ni.3120
Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nature {2013} 498:322–31. doi: 10.1038/nature12395

37. Hofmann C, Mortha A, Bui VL, Hernandez PP, Kiss EA, Hoyler T, et al. Regulated expression of nuclear receptor RORgammat+ in innate lymphocytes. Immunity {2017} 46:253–66. doi: 10.1016/j.immuni.2017.02.010

38. Castile J, Degenhardt H, Da Fonseca E, de Souza Calmon PB, Regnery RL, Gorga JC, et al. Conventional and non-conventional monocytes contribute to lung immunity during respiratory viral infection. J Immunol {2018} 199:1518–28. doi: 10.4049/jimmunol.1701757

39. Cevantes-Barragán L, Robinette ML, Bando JK, Wang Y, Geiger TL, et al. Transforming growth factor-beta signaling guides the differentiation of innate lymphoid cells in salivary glands. Immunity {2016} 44:1127–39. doi: 10.1016/j.immuni.2016.03.007

40. Gao Y, Souza-Fonseca-Guimaraes E, Bald T, Ng SS, Young A, Ngiow SF, et al. Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. Nature {2017} 547:1004–15. doi: 10.1038/nature22880

41. Takeda K, Czerniewicz K, Yoshida T, Kuroki T, Kohno K, Yoshida M, et al. Distinct responsiveness and migratory capacity. J Exp Med {2018} 225:1517–31. doi: 10.1084/jem.20180061

42. Zhou J, Peng H, Li K, Qu K, Wang B, Wu Y, et al. Liver-resident NK cells are long-lived and do not recirculate. J Immunol {2018} 199:392–403. doi: 10.4049/jimmunol.1761383

43. Jenne CN, Enders A, Rivera R, Watson SR, Bankovich AJ, Pereira JP, et al. T-bet regulates the terminal maturation and protection against intestinal pathogens. J Exp Med {2019} 216:1503–17. doi: 10.1084/jem.20180464

44. Satoh-Takayama N, Lesjean-Pottier S, Vieira P, Sawa S, Eberl G, Vosshenrich AJ, et al. Loss of canonical notch signaling affects multiple steps during Salmonella typhimurium infections. Proc Natl Acad Sci USA {2013} 110:2252–7. doi: 10.1073/pnas.1222047110

45. Chaves P, Zrizwi A, Wittmann L, Boukarabila H, Peitzsch C, Jacobsen SEW, et al. Loss of canonical notch signaling affects multiple steps in NK cell development in mice. J Immunol {2018} 201:3307–19. doi: 10.4049/jimmunol.1701675

46. Cuff AO, Male V. Conventional NK cells and ILC1 are partially ablated in mice lacking T-bet-deficient mice. J Immunol {2018} 201:3280–9. doi: 10.4049/jimmunol.1701299

47. Solka DK, Plougastel-Douglas B, Yang L, Pak-Witt-Mal M, Artyomov MN, Ivanova Y, et al. Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells. ELife {2014} 3:e01659. doi: 10.7554/eLife.01659

48. Bezman NA, Kim CC, Sun JC, Min-Oo G, Hendricks DW, Kamimura Y, et al. Molecular definition of the identity and activation of natural killer cells. Nat Immunol {2012} 13:1082–9. doi: 10.1038/ni.2395

49. Cortez VN, Varsabek-Meier A, Gobert D, de Souza Calmon PB, da Silva Neto S, Muñoz AM, et al. Contribution of Thy1+ NK cells to protection against malaria and dengue fever. J Immunol {2016} 196:1449–54. doi: 10.4049/jimmunol.1502396

50. Zhou J, Peng H, Li K, Qu K, Wang B, Wu Y, et al. Liver-resident NK cells control antiviral activity of hepatic T cells via the PD-1-PD-L1 axis. Immunity {2019} 50:403–17. doi: 10.1016/j.immuni.2018.12.024

51. Townsend MJ, Weinmann AS, Matsuda JL, Salomon R, Farnham PJ, Biron CA, et al. T-bet regulates the terminal maturation and homeostasis of NK and NKG2D+ NKT cells. Immunity {2004} 20:477–94. doi: 10.1016/j.immuni.2004.08.010

52. Jenne CN, Enders A, Rivera R, Watson SR, Bankovich AJ, Pereira JP, et al. T-bet-dependent SIPS expression in NK cells promotes egress from lymph nodes and bone marrow. J Exp Med {2009} 206:2469–81. doi: 10.1084/jem.20090525

53. Malaisé M, Riviera J, Renner P, Eggenhofer E, Sabet-Baktach M, Lantow M, et al. KLRG1+ NK cells protect T-bet-deficient mice from pulmonary metastatic colorectal carcinoma. J Immunol {2014} 192:1954–61. doi: 10.4049/jimmunol.1300876

54. Cuff AO, Male V. Conventional NK cells and ILC1s are partially ablated in the livers of Ncr1+ (cIfN)Tbx21 Δ/Δ mice. Wellcome Open Res {2017} 2:39. doi: 10.12688/wellcomeopenres.11741.1

55. Geiger TL, Abt MC, Gasteiger G, Firth MA, O’Connor MH, Geary CD, et al. Nfil3 is crucial for development of innate lymphoid cells and host protection against intestinal pathogens. J Exp Med {2014} 211:1723–31. doi: 10.1084/jem.20140212

56. Satoh-Takayama N, Lesjean-Pottier S, Vieira P, Dhabar R, Biron CA, et al. IL-7 and IL-15 independently program the differentiation of intestinal CD3+NKP46+ cell subsets from Id2-dependent precursors. J Exp Med {2010} 207:273–80. doi: 10.1084/jem.20090229

57. Solka DK, Plougastel-Douglas B, Yang L, Pak-Witt-Mal M, Artyomov MN, Ivanova Y, et al. Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells. ELife {2014} 3:e01659. doi: 10.7554/eLife.01659
50. Erick TK, Anderson CK, Reilly EC, Wands JR, Brossay L. NFiL3 expression distinguishes tissue-resident NK cells and conventional NK-like cells in the mouse submandibular glands. *J Immunol.* (2016) 197:2485–91. doi: 10.4049/jimmunol.1601099

51. Firth MA, Madera S, Beaulieu AM, Gasteiger G, Castillo EF, Schluns KS, et al. Nfil3-independent lineage maintenance and antiviral response of natural killer cells. *J Exp Med.* (2013) 210:2981–90. doi: 10.1084/jem.20130417

52. Constantinides MG, Gudjonson H, McDonald BD, Ishizuka IE, Verhoef PA, Dinner AR, et al. PLZF expression maps the early stages of ILC1 lineage development. *Proc Natl Acad Sci USA.* (2015) 112:5123–8. doi: 10.1073/pnas.1423244112

53. Constantinides MG, McDonald BD, Verhoef PA, Bendelac A. A committed precursor to innate lymphoid cells. *Nature.* (2014) 508:397–401. doi: 10.1038/nature13047

54. Xu W, Cherrier DE, Chea S, Vossenrich C, Serafini N, Petit M, et al. An Id2(RFP)-reporter mouse redefines innate lymphoid cell precursor potentials. *Immunity.* (2019) 50:1054–68.e3. doi: 10.1016/j.immuni.2019.02.022

55. Mackay IK, Minnich M, Kragten NA, Liao Y, Nota B, Seillet C, et al. Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. *Science.* (2016) 352:459–63. doi: 10.1126/science.aad2035

56. Cortez VS, Ulland TK, Cervantes-Barragan L, Bando JK, Robinette ML, Wang Q, et al. SMAD4 impedes the conversion of NK cells into ILC1-like cells by curtailing non-canonical TGF-beta signaling. *Nat Immunol.* (2017) 18:995–1003. doi: 10.1038/ni.3809

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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