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True Detective: Unraveling Group 1 Innate Lymphocyte Heterogeneity

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Abstract

Innate lymphoid cells (ILCs) consist of a heterogeneous family of lymphocytes that regulate tissue homeostasis and can contribute to pathology in mice and humans. Mammalian group 1 ILCs are defined by the production of interferon (IFN)-γ and the functional dependence on the transcription factor T-bet. While recent studies demonstrate that group 1 ILCs consist of circulating mature NK cells and tissue-resident ILC1, the functional, phenotypic, and developmental properties that distinguish these two cell lineages are often confusing and difficult to dissect. In this review, we critically evaluate the current knowledge of mammalian group 1 ILC heterogeneity and propose new inclusive nomenclature to clarify the roles of ILC1 and NK cells during homeostasis and disease.

Group 1 ILC Identification and Functions

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 in mice, 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 mammalian ILCs can be further classified into group 1, 2, and 3 ILCs based on differential expression of transcription factors, cell surface markers, and effector cytokines [1]. Mouse group 1 ILCs, which include natural killer (NK)

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cells and ILC1, can be 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 [1]. 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 [1].

ILC1 are recently discovered tissue-resident sentinels that function to protect the host from bacterial and viral pathogens at initial sites of infection [2, 5, 6]. ILC1 rapidly produce IFN-γ following local dendritic cell activation and interleukin (IL)-12 production to limit viral replication and promote host survival prior to 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 [7, 8]. However, persistent inflammatory signals can also lead to unrestrained activation of group 1 ILCs during obesity and inflammatory bowel disease (IBD) [3, 9–12]. While mounting evidence suggests important roles for group 1 ILCs during host protection and pathology in mice and humans, gaps in evidence have inhibited the ability of recent studies to definitively distinguish between the roles of ILC1 and NK cells in these contexts, leading to varied nomenclature. In this review, we critically analyze the current characteristics that might distinguish different types of group 1 ILCs during homeostasis and inflammation, and use this framework to propose new inclusive nomenclature for mouse and human studies.

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 T cell Receptor (TCR) proteins [13]. 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, 14] (Table 1). Similarly, human Lin^-CD56^+ cells have been reported to be heterogeneous for the expression of transcriptions factors (EOMES and T- BET), cell surface markers (CD49a, CD56, CD16, NKp80, CXCR6, IL-7Rα, CD94, CD69, NKp44), and cytotoxic molecules (Perforin) [1, 14] (Table 2). Previous studies concluded that $CD56^+$ or $NK1.1^+$ cells in peripheral organs and blood in humans and mice, respectively, likely represented immature NK (iNK) cells due to alternative cell surface and transcription factor phenotypes relative to putative mature NK cells (mouse: $Lin^-NK1.1^+T\text{-}bet^+Eomes^+CD49b^+$; human: Lin^- $IL\text{-}7R\alpha^-CD56^{dim}CD16^+$) [15–19]. This hypothesis was supported by studies demonstrating that subsets of developing mouse NK cells could be distinguished based on CD27 and CD11b expression [20, 21]. Similarly, previous studies suggested that $CD56^{bright}CD16^-$ human NK cells in the blood might be immature precursors to $CD56^{dim}CD16^+$ mature NK cells because $CD56^{bright}$ NK cells could gain the transcriptional signature of $CD56^{dim}$ NK cells following cytokine activation *in vitro*, and GATA2-deficient patients show a marked defect in $CD56^{dim}$ NK cell numbers and function with complete loss of the $CD56^{bright}$ subset [16, 17]. 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, IL-7Rα$^+$T-bet$^+$Eomes$^-$NK1.1$^+$NKp46$^+$ "ILC1" in the small intestine were absent in T-bet$^{-/-}$ mice, similar to NK cells. In contrast, ILC1 in the small intestine of mice with a mesenchymal deletion of *Eomes* were present, whereas NK cells required Eomes expression for their development [5]. These experiments supported the presence of two developmentally distinct group 1 ILC subsets in the mouse small intestine. Subsequent lineage tracing experiments using Eomes reporter mice further suggested that adoptively transferred Eomes$^-$T-bet$^+$ group 1 ILCs did not gain Eomes expression during homeostasis, and could be considered a stable lineage distinct from Eomes$^+$T-bet$^+$ mature NK (mNK) cells, rather than an iNK cell [5, 22]. The concept of distinct group 1 ILCs in peripheral organs was further supported by the findings from **mouse parabiosis** experiments demonstrating that T-bet$^+$Eomes$^-$CD49b$^-$ group 1 ILCs (in addition to ILC2 and ILC3) robustly represented host parabiont-derived long-term tissue-resident cells, whereas circulating Eomes$^+$CD49b$^+$ mNK cells were equally derived from both host parabionts, in almost all organs tested [4]. Similarly, in one human study, a subset of donor liver CXCR6$^+$ group 1 ILCs was found to be maintained up to 13 years post liver transplant, while donor CXCR6$^-$ NK cells were absent, suggesting that a subset of long-term tissue-resident CXCR6$^+$ group 1 ILCs might be conserved in mammals [23]. Furthermore, mouse CD49b$^-$ Eomes$^-$ group 1 ILCs with a phenotype consistent with ILC1 in the liver exhibited higher expression of TRAIL than mNK cells at steady state; furthermore, these ILC1 produced higher percentages of tumor necrosis factor (TNF)-α and IFN-γ than mNK cells following activation with proinflammatory cytokines *ex vivo* [2, 15, 18, 22]. ILC1 in the small intestine and liver were observed to have poor *ex vivo* cytotoxicity to tumor targets, and expressed lower amounts of the cytotoxic molecules granzyme A/B and perforin at steady state compared to mNK cells [5, 22]. However, mouse peripheral ILC1 have been reported to express higher amounts of granzyme C and display higher cell surface expression of TRAIL compared to mNK cells, which can both promote cytotoxic function through induction of apoptosis in target cells [2, 22, 24–26]. Because of these observed functional differences in cytotoxic molecule expression between ILC1 and mNK cells in mice, it will be important for future studies to determine whether perforin-independent killing mechanisms can be used as definitive criteria to functionally separate ILC1 from NK cells across all mouse and human tissues. Thus, significant phenotypic and functional heterogeneity has been demonstrated in group 1 ILCs; yet, it is still unclear to what extent these individual pieces of evidence can be used in isolation to define group 1 ILC subsets.

Arguing the Case for a Distinct ILC1 Lineage

Experimental Evidence defining Mouse ILC1

Unbiased whole-genome epigenetic and transcriptional signatures collectively suggest that mature mouse and human ILCs contain distinct regulomes (i.e. epigenetic landscapes controlled by chromatin modifications that regulate gene expression) that allow for their

functional and phenotypic distinction during homeostasis at the population and single-cell levels [27–30]. While these studies collectively concluded that ILC1 were a distinct lineage from NK cells based on transcriptional and epigenetic datasets, ILC1-associated markers such as CD90, CD69, CD49a, TRAIL, Ly6C, CD103, CD61, and IL-7Rα were subsequently found to be either heterogeneously expressed on peripheral ILC1, or also expressed by iNK or activated NK cells; thus, these were not suitable lineage defining markers (Figure 1) [2, 15, 31–34]. However, a recent study using *Eomes*-GFP reporter mice generated core transcriptional signatures of Eomes$^-$ ILC1 and Eomes$^+$ NK cells from 4 independent tissues [2]. The identified core ILC1 signature led to the discovery of the inhibitory receptor CD200r1 as a stable marker expressed by ILC1, but not NK cells, during homeostasis and murine cytomegalovirus (MCMV)-induced inflammation [2]. Long-term parabiosis experiments also revealed that ILC1 in peripheral organs, but not recirculating mNK or iNK cells, were tissue-resident during homeostasis and inflammation induced by MCMV or transient regulatory T (T_{reg}) cell ablation [2–4, 35]. These results suggest that NK lineage cells do not become long-term tissue-resident following inflammation, whereas ILC1 may be maintained in peripheral tissues through long-term self-renewal [2–4, 35]. Collectively, lineage tracing studies in mice using CD49a$^+$CD49b$^-$, CD200r1$^+$CD49b$^-$, or Eomes$^-$CD49b$^-$ ILC1 from the liver, fat, bone marrow, peritoneum, and gut have supported the hypothesis that ILC1 do not convert to NK cells during homeostasis or inflammation because they do not acquire cell surface or transcription factor expression phenotypes consistent with the NK lineage following adoptive transfer [2, 3, 22, 35–37]. Similarly, Eomes$^+$CD49b$^+$ or CD49a$^-$CD49b$^+$ mouse mNK cells from these tissues do not convert to an ILC1 phenotype *in vivo*, suggesting that ILC1 are not an end stage or activation state of NK cell development [2, 3, 22, 36, 37].

While the growing consensus from these studies is that NK cells and ILC1 are distinct and stable lineages of cells *in vivo*, a recent study reported that splenic CD49a$^-$CD49b$^+$ NK cells can gain CD49a expression and lose Eomes expression following adoptive transfer into *Rag2*$^{-/-}$ x *Il2rg*$^{-/-}$ (lymphocyte-deficient) mice [38]. This raises the possibility that in certain peripheral mouse tissues, such as the spleen, cells identified as ILC1 with a few phenotypic markers may represent a state of the NK cell lineage. However, other studies have shown that adoptive transfers of CD49b$^+$ splenic NK cells into wild type (WT) mice do not display a similar conversion of NK cells to an ILC1 phenotype during homeostasis or MCMV-induced inflammation [2, 22]. Furthermore, although mouse CD49a$^+$Ly49H$^+$ mNK cells activated during MCMV infection share CD49a expression with ILC1, they do not become tissue-resident in the lung, suggesting that CD49a is not sufficient to promote tissue-residency and should not be used as a marker to functionally define tissue-resident group 1 ILCs [39]. Consequently, there is no experimental evidence that demonstrates fate-mapped or lineage traced NK lineage cells becoming long-term tissue-resident cells, suggesting that ILC1 may not represent an early or end-stage of NK cell development. While these results collectively argue that ILC1 are a stable independent lineage of cells distinct from NK cells, it will be necessary in future studies to continue investigating the lineage stability of group 1 ILCs in other mouse tissues (i.e. salivary gland, uterus, lung, skin, tumors) through lineage tracing based on CD200r1, CD49b, and Eomes expression; this may allow to further validate group 1 ILC heterogeneity in those tissues during homeostasis and disease.

Experimental Evidence defining Human ILC1

Although mounting evidence supports a stable mouse ILC1 lineage, the corollary evidence for a human ILC1 counterpart is currently lacking. While single cell sequencing studies have identified a rare human T-bet^{+} putative ILC1 population in human tonsils [40], they have also suggested that current gating strategies used to identify human ILC1 (Lin^{-}IL-7Rα^{+}NKp44^{-} CRTH2^{-}CD117^{-}) contain various contaminating cell types (i.e. T cells, NK cells, ILC3, and dendritic cells), and contain heterogeneous expression of both T-bet and Eomes in different tissues [40–43]. These results are likely due to the fact that there are currently no definitive markers to distinguish human ILC1 from NK cells, other ILCs, or T cells that may have undergone suboptimal TCR gene rearrangement [11, 44]. While recent unbiased multi-parameter mass cytometry studies could not find conclusive evidence for the existence of a putative human ILC1 population in human tissues (cord blood, tonsil, and colon), this study was also based on key assumptions that putative human ILC1 were CD56^{-} and IL-7Rα^{+}, and that certain clusters of tonsil and colon “NK” cells lacked perforin expression [42]. However, mouse studies have shown that peripheral ILC1 subsets express variable amounts of cell surface IL-7Rα (Table 1), and they do not depend on IL-7 signaling for their development because their numbers are not reduced in IL-7Rα$^{-/-}$ mice [45]. In support of this hypothesis, a population of CD56^{+}CD49a^{+}T-BET^{+}EOMES^{-} IL-7Rα^{-} group 1 ILCs have been identified in some healthy human liver samples; these cells express low amounts of perforin and might potentially represent a putative human ILC1, although further studies are warranted [46]. Thus, the current paradigm for phenotyping human ILCs may be too restrictive based on the Lin-CD56^{-}IL-7Rα^{+} phenotypic definition, and it is likely that human ILC1 may be better defined based on transcription factor staining or other stable markers of mature ILCs. It will be of interest to determine if CD200R1 expression can be used to separate human tissue ILCs from the NK cell lineage, as observed in mouse tissues and human peripheral blood [2, 47], in order to unveil a putative human ILC1 (Lin^{-} CD200R1^{+}T-BET^{+}EOMES^{-}).

Developmental and Activation States of Group 1 ILCs

A major issue in the ILC field is the complicated nomenclature used when identifying group 1 ILCs in various peripheral organs during homeostasis and inflammation. Various groups have described subsets consisting of “tissue-resident NK cells” (trNK), “intra-epithelial ILC1” (ieILC1), “conventional NK cells” (cNK), “helper ILC1” (ILC1hs), “cytotoxic killer ILC1” (ILC1ks), “mixed-ILC1s”, “intermediate ILC1” (intILC1), “mature NK cells” (mNK), and “immature NK cells” (iNK) with no clear consensus [2, 3, 10, 22, 26, 37, 38, 48, 49]. Even more problematic is the issue that the majority of these “subsets” have been defined as distinct from the NK cell lineage based on differences in either cell surface protein expression, developmental dependence on transcription factors, cytotoxic potential, or tissue-residency. However, whether these differences alone are sufficient to define subsets of group 1 ILCs as distinct lineages in the absence of lineage tracing studies remains poorly defined.

Phenotypes consistent with Immature and Activated Mouse NK cells

Collective reports have demonstrated that iNK cells in mouse bone marrow and periphery can express Ly49 receptors, CD49a, CD90, TRAIL, CD69, and Eomes, and lack CD49b expression [3, 19, 32, 34, 50]. Upon adoptive transfer into *Rag2*$^{-/-}$ x *Il2rg*$^{-/-}$ mice, iNK cells recovered in the spleen and adipose tissue can induce CD49b expression and retain Eomes expression [3]. Following adoptive transfer into MCMV-infected mice or in response to proinflammatory cytokines *ex vivo*, mNK cells can induce CD49a, Ly6C, CD69, TRAIL, and CD90 expression while also decreasing Eomes expression [2, 15, 31, 32]. These results suggest that iNK and mNK cell phenotypes can overlap with other reported group 1 ILC phenotypes based on these markers alone. Consistent with these findings, NK cells can repress Eomes and induce CD49a, TRAIL, and CD103 expression in response to TGFβ and IL-2 stimulation *ex vivo* [33, 38]. Moreover, a recent study also demonstrated that NK cells could repress Eomes expression while inducing CD49a and Ly6C cell surface expression stably following *toxoplasma gondii* infection in mice [51]. However, these cells accumulated in the blood and lung of infected mice in an *Eomes*-dependent manner, and were not found to be tissue-resident in the lung in mouse parabiosis experiments [51], suggesting that they could still represent an activation state of NK cells [2, 15, 31, 32]. These collective findings make the current dogma of utilizing CD49a, CD49b, and Eomes expression in Lin^{-}T-bet^{+}NK1.1^{+}NKp46^{+} cells insufficient to be able to distinguish between group 1 ILC subsets and activation or developmental states of NK cells. 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 functionally distinguish iNK cells from ILC1 without additional evidence. Thus, there is currently insufficient evidence to conclude that T-bet^{+} group 1 ILCs with a CD49a^{+}CD49b^{+}Eomes$^{+/lo}$NK1.1^{+} phenotype are either trNK cells or intILC1, because these cells could be activated NK cells in the tissue parenchyma following recruitment from circulation, although further evidence will be necessary to support this hypothesis. Furthermore, it is reasonable to speculate that CD49a^{+}CD49b^{-}Eomes^{+}NK1.1^{+} cells might not represent a transitional subset of group 1 ILC, but instead, might represent iNK cells in peripheral tissues, although extensive lineage tracing experiments will be necessary to robustly clarify these issues in the field.

ILC Phenotypes that are consistent with Immature and Activated Human NK cells

In the healthy state, mature human group 1 ILCs have been described as heterogeneous for cell surface expression of CD56, CD16, and NKp80 in peripheral tissues [52]. However, CD56 can be expressed on ILC progenitor populations and ILC3 in the tonsil [53], and it may be downregulated during activation, similarly to CD16 and NKp80 [54–56]. 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 stimulation with proinflammatory cytokines *ex vivo*, and during human immunodeficiency virus (HIV-1)-induced inflammation [54–56]. However, analysis of HLA-mismatched human liver transplant samples suggests that a subset of donor Lin^{-} CD56^{+}CXCR6^{+}EOMES^{hi}T-BET^{int} group 1 ILCs are present in the recipient host for up to 13 years following liver transplant

[23]. The observed long-term tissue-residency of these cells raises the question of whether this specific human group 1 ILC can be defined as a trNK cell, iNK cell, or ILC1. Because mouse iNK cells co-express Eomes and T-bet and are short-term tissue-resident [3], it is possible that these cells might represent a putative human iNK cell. In support of this hypothesis, T-bet-expression is lower in developing human and mouse iNK cells compared to mature NK cells, and T-BET is increased with the acquisition of killer immunoglobulin-like receptors (KIR) during human NK development [22, 57–59]. Similarly, while perforin expression in liver-resident $CXCR6^+$ NK cells is lower than $CXCR6^-$ liver NK cells, it is not absent, suggesting that these cells may represent a developmental state of the NK cell lineage [23, 60, 61]. Regardless, it remains to be further investigated whether liver-resident $CXCR6^+$ NK cells can develop into mature human $CXCR6^-$ NK cells using humanized mouse models to test the hypothesis that $CXCR6^+$ NK cells in the liver may be iNK cells. Understanding the key phenotypic differences between tissue-resident ILCs and developmental states of circulating NK cells in humans may better inform our knowledge of relevant cell types associated with disease, and increase our ability to develop novel candidate immunotherapies.

Mouse Group 1 ILC Development

Recent unbiased chromatin accessibility studies utilizing **ATAC** sequencing analysis 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 [62]. Similarly, mNK and iNK cells do not develop in mice that do not express Eomes at various stages in lymphocyte development [2, 18, 63], suggesting that Eomes may be the master transcription factor that defines NK cell lineage identity in mice. 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 when analyzing WT:*Tbx21*$^{-/-}$ and WT:*Nkp46*^{Cre} x *Eomes*$^{fl/fl}$ mixed bone marrow chimeric mice [2], arguing against tissue-specific transcription factor developmental requirements for mNK cells. While certain studies have observed that liver and gut mNK cell numbers are normal in the absence of T-bet [5, 6, 37], *Tbx21*$^{-/-}$ NK cells have been previously shown to display an immature phenotype, and are functionally deficient in trafficking from the bone marrow [3, 59, 64, 65]. Therefore, because *Tbx21* is required for optimal mature ILC1 and mNK development [2, 3, 66], *Tbx21*$^{-/-}$ mice cannot be used to study the contribution of mNK cells versus ILC1 in mouse models of disease, because both subsets are either absent, or functionally deficient due to developmental defects.

While recent studies have suggested that mature mouse ILC1 and NK cell development is perturbed in the absence of the transcription factors Id2 and Nfil3 [67, 68], certain reports have identified unique lineages of group 1 ILCs based on their development in *Nfil3*$^{-/-}$ mice; called “tissue-resident NK cells”, “salivary gland ILCs”, and “type 1 ILCs” [25, 33, 48]. However, cells with similar phenotypes to these identified lineages were also found to require *Nfil3* in a cell-intrinsic manner for their development when analyzing WT:*Nfil3*$^{-/-}$ mixed bone marrow chimeras [2, 69]; this has challenged the unique lineage identity of these cells. Because mNK cells can develop in an *Nfil3*-independent manner during MCMV-induced inflammation and aging in mice [33, 70], analysis of group 1 ILCs in *Nfil3*$^{-/-}$ mice

is likely not sufficient to define distinct subsets because these cells could reflect artifacts that could escape a developmental dependence on *Nfil3* in the presence of inflammation (due to endemic pathogens present in mouse facilities)[33, 70]. Previous studies have also performed transcription factor lineage tracing experiments using Zbtb16 fate-mapping mice and Id2 reporter mice to identify a common helper ILC precursor population that can give rise to all tissue-resident ILCs, but not mNK cells, arguing that ILC1 comprise a developmental lineage that is distinct from NK cells [5, 71, 72]. However, a recent study using dual *Zbtb16* and *Id2* reporter mice demonstrated that both NK cells and ILC1 could develop from a $Id2^{+}Zbtb16^{+}$ shared precursor, suggesting that these transcription factors alone could not be used to identify different group 1 ILC subsets during ontogeny [73]. Instead, several studies have identified the transcription factor *Zfp683* (Hobit) as highly expressed in peripheral ILC1 compared to mNK cells [2, 74, 75]. Indeed, $Zfp683^{-/-}$ mice display loss of liver ILC1 but not other ILC populations (including ILC1 in other tissues) [2, 74], suggesting that mature liver ILC1 might undergo a developmental pathway that is unique from other mouse ILCs, warranting further investigation. Nonetheless, in humans, HOBIT expression is high in both blood and liver NK cells, and it is required for the development of mNK cells from $CD34^{+}$ cord blood progenitors [76, 77], indicating that the expression of HOBIT may not be useful to define heterogeneity within group 1 ILCs in human tissues. Thus, while several studies have claimed to identify novel subsets of group 1 ILCs based on developmental data from transcription factor deficient mice, several key caveats accompany these interpretations of experimental data. Consequently, even though developmental dependence on Eomes expression can be used to identify NK lineage cells in peripheral organs from mice, there is still no definitive evidence that a single transcription factor can define the development of other group 1 ILC subsets across all tissues in mouse and human.

Human Group 1 ILC Development

The developmental pathways through which ILC subsets are generated in humans are not as clearly defined as they are in mice. Nonetheless, accumulating data indicate that human ILCs can develop from $CD34^{+}$ hematopoietic progenitor cells, likely outside of the bone marrow and within peripheral tissues [78]. Recent studies also suggest that all ILCs can develop from $CD34^{+}CD45RA^{+}CD117^{+}$ common ILC progenitors (CILP) and their putative immediate progeny, $CD34^{-}CD45RA^{+}CD117^{+}$ common ILC precursors (ILCP), which are present in multiple peripheral tissues as well as in the circulation [53, 79]. NK cells likely derive directly from the $CD56^{+}$ fraction of ILCP, because $CD56^{+}$ ILCPs derived from human tonsils can develop into NK cells *in vitro* when cultured on OP9-DL1 stroma in the presence of Flt3 ligand and IL-7 [53]. Subsequently, human NK cell transcriptional and functional maturation may be associated with the step-wise acquisition of the surface markers CD94, NKp80, CD16, and CD57 such that terminally differentiated peripheral blood NK cells become $Lin^{-}CD56^{+}CD94^{+/-}$ $NKp80^{+}CD16^{+}CD57^{+}$ [52, 53]. Thus, given the current uncertainty in defining and distinguishing human ILC1 from NK cells, it is not yet known where and how human ILC1 derive from ILCP.

Group 1 ILC Functional Plasticity

Although a recent mouse study suggested that ILC lineage identity fate could be determined epigenetically during development in the bone marrow [28], recent studies have demonstrated that mouse and human ILCs can display unique transcriptional signatures when compared between several peripheral organs [41, 80]. These studies suggest that ILCs may be able to modify their transcriptional signatures in discrete tissue microenvironments following epigenetic imprinting during ontogeny. In support of this hypothesis, a number of studies have shown that ILC1-like populations (T-BET^{+}IFN-γ^{+}) can be derived from mouse and human ILC2 and ILC3 under inflammatory conditions *in vivo*, or following stimulation with proinflammatory cytokine stimulation *in vitro* (Figure 2) [11, 12, 81–85]. However, whether ILCs can undergo bona fide **transdifferentiation** into the ILC1 lineage is not well understood, because T-bet^{+}IFN-γ^{+} ILC2 populations in mice do not induce the expression of the activating receptor NK1.1, and human single cell mature ILC3 clones can display little plasticity towards an ILC1-like phenotype in cultures [79, 81, 82]. These results suggest that although ILC2 and ILC3 populations could transiently produce IFN-γ^{+} and induce T-bet, they may not undergo complete transdifferentiation to group 1 ILCs because they still lack the expression of key phenotypic markers that define group 1 ILCs (such as NK1.1 in mice). Future studies using single-cell transcriptional and epigenetic sequencing approaches will be needed to define whether ILC2 and ILC3 can undergo complete transdifferentiation into group 1 ILCs, in contrast to solely acquiring similar cytokine production profiles. Irrespective of these points, **Rorγt** fate-mapping studies in mice suggest that a subset of fate-map^{+} “ex-ILC3” Rorγt^{-} ILC1 that have expressed Rorγt during ontogeny exist in the gut of mice [5], and a putative ILC3-to-ILC1-like cell differentiation pathway has been proposed in the human intestine using single-cell RNA sequencing [86]. However, ILC1 development is not perturbed in Rorγt$^{-/-}$ mice or RORC-deficient patients [5, 79], suggesting that putative ILC3-to-ILC1 lineage plasticity might not necessarily contribute to significant ILC1 precursor frequency in the intestine during homeostasis in mammals. As such, the functional relevance of ex-ILC3, ILC1 populations during homeostasis or inflammation in mammals is still not well understood and warrants further investigation.

Similarly, recent studies have suggested that the transcription factor Smad4 could prevent the conversion of mouse NK cells to an ILC1-like phenotype during homeostasis when analyzing the peripheral organs of *Nkp46*^{Cre} x *Smad4*$^{fl/fl}$ mice [75]. Specifically, NK1.1^{+} cells derived from *Nkp46*^{Cre} x *Smad4*$^{fl/fl}$ mice exhibited higher expression of certain ILC1-associated genes and increased cell surface expression of CD49a relative to WT[75]. However, Smad4-deficient NK cells were also found to express NK lineage-associated markers CD49b and Ly49H [75], and another recent study has demonstrated that non-canonical TGF-β signaling through Smad4 is required for optimal *Gzmb* expression and functional maturation of peripheral NK cells [87]. Thus, whether Smad4 is required for terminal maturation or suppression of bona fide NK-to-ILC1 conversion will require further investigation. Given these collective findings, it is likely that in some inflammatory settings, such as inflammation in the airways or intestine, ILC2 and ILC3 exhibit functional plasticity by inducing T-bet expression and production of IFN-γ [81–85]. Accordingly, current evidence collectively supports the hypothesis that tissue-resident ILC1 are likely not derived from other ILCs during homeostasis or inflammation through lineage plasticity [5, 79].

Indeed, it will be of interest to show the physiological importance of ILC2/3-to-ILC1 functional plasticity during homeostasis and disease, since functional redundancy of these subsets might exist within NK cell and ILC1 populations. A better understanding of the key cell types that contribute to host tissue protection or pathology may lead to improved candidate targeted therapies in a variety of human diseases.

Concluding Remarks

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. Single-cell sequencing, parabiosis, lineage tracing, and transcription factor deficient mouse experiments have suggested that *Eomes*-independent mouse ILC1 represent a distinct lineage of group 1 ILCs, and not a developmental or activation state of NK cells. Thus, we propose inclusive nomenclature for mouse group 1 ILCs based on current experimental evidence from a meta-analysis of several studies: mature NK cells (mNK: Lin^{-} $NK1.1^{+}NKp46^{+}T\text{-}bet^{+}Eomes^{hi}CD49b^{+}CD200r1^{-}$), immature NK cells (iNK: Lin^{-} $NK1.1^{+}NKp46^{+}T\text{-}bet^{+}Eomes^{hi}CD49b^{-}CD200r1^{-}$), ILC1 ($Lin^{-}$ $NK1.1^{+}NKp46^{+}T\text{-}bet^{+}Eomes^{-}$ $CD49b^{-}CD200r1^{+}$), and effector NK cells (Lin^{-} $NK1.1^{+}NKp46^{+}T\text{-}bet^{+}Eomes^{lo}CD49b^{+}CD49a^{+}CD200r1^{-}$). In human tissues, there is currently no definitive evidence that can actually 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 (see outstanding questions box). Collectively, studies from the past 40 years have unveiled the complex phenotypic and functional diversity in what were first identified as NK cells. Charting the constellation of group 1 ILC heterogeneity might better inform the roles of these cells during homeostasis and disease and ideally contribute to the development of new putative treatment strategies for inflammatory pathologies, such as cancer and colitis.

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Glossary

Perforin	a cytotoxic protein which destroys target cells by creating pore-like lesions in their membranes
Mouse Parabiosis	the surgical joining of two congenically distinct mouse skin flaps to chimerize blood vessels and blood flow through both mice
ATAC	assay for transposase-accessible chromatin using sequencing

Transdifferentiation	a process by which a mature somatic cell transforms into another mature somatic cell without undergoing an intermediate pluripotent or progenitor cell type
Rorγt	retinoic acid related orphan receptor gamma t is a nuclear receptor that is expressed selectively by lymphocytes

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Outstanding Questions Box:

- Can mature mouse NK cells become resident in certain tissue microenvironments? Previous studies have suggested that a population of tissue-resident NK cells exist in mammals based on cell surface phenotypes. However, these studies have not shown NK cells directly becoming tissue-resident in functional experiments. Discerning whether circulating NK cells can become tissue-resident during inflammation could lead to novel immunotherapeutic approaches in infectious diseases and cancer.
- What markers differentiate between human ILC1 and NK cells? Because there are no definitive markers that can distinguish ILC from NK cells in humans, it will be important for future studies to identify stable markers that are not altered during inflammation. Understanding these key phenotypic differences may lead to a better understanding of the roles of these cells during host protection and pathology.
- Do mammalian group 1 ILCs develop from distinct common progenitor cells? Current evidence suggests that all ILCs share a committed progenitor population. However, whether there is a developmental bifurcation of the NK and ILC1 lineages at the precursor level during development remains to be further assessed. Investigating the pathways that lead to distinct NK and ILC1 lineages could inform how tissue-residency programs are imprinted in lymphocytes during development.
- What is the physiologic significance of ILC plasticity in mammals? ILC2 and ILC3 have been shown to acquire functional attributes of ILC1 during inflammation in mammals; however, the functional relevance of ILC plasticity has yet to be demonstrated *in vivo.* Determining the significance of ILC plasticity in mammals could inform treatment strategies during chronic tissue-inflammation.

Highlights:

- Group 1 ILCs play an important role in host protection against viruses and intracellular bacteria in mice, but may also contribute to disease progression during obesity and inflammatory bowel disease (IBD) in humans.
- Group 1 ILCs display marked phenotypic and functional heterogeneity in mouse and human peripheral organs, making their distinction difficult during homeostasis and inflammation
- While developmental dependence on Eomes expression can be used to define the natural killer (NK) cell lineage during homeostasis in mice, no single transcription factor can delineate between type 1 innate lymphoid cells (ILC1) and NK cell development in all peripheral organs in mammals.
- CD200r1 can be used to identify ILC1 from NK cells during homeostasis and inflammation in mice, but no definitive markers currently exist to define human ILC1.

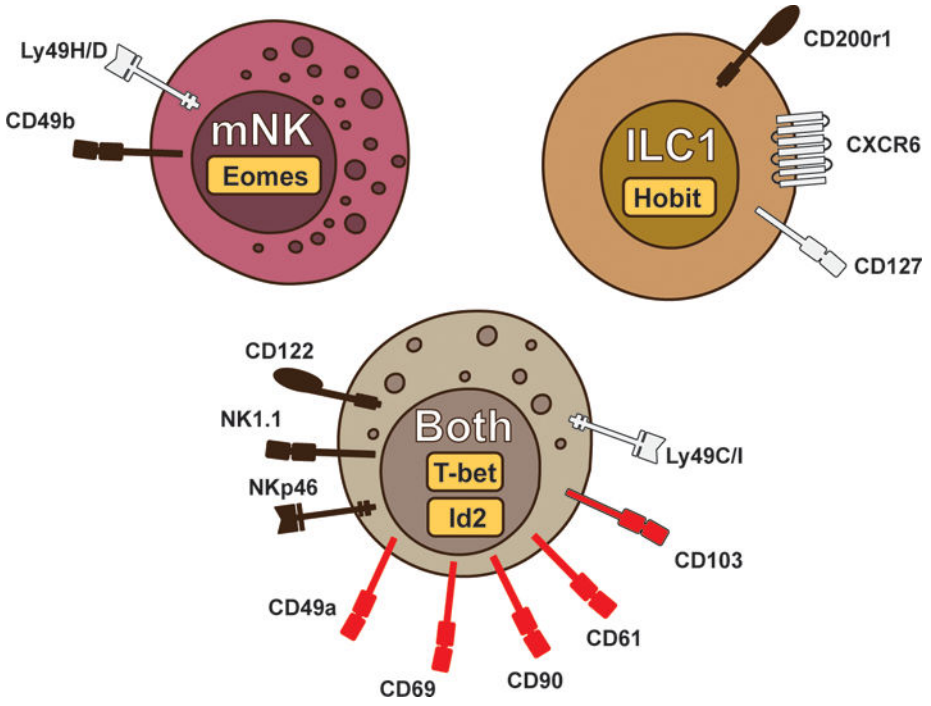

Figure 1. Shared and Distinct Mouse Group 1 ILC Phenotypic Markers.

Mouse ILC1 and NK cells share the expression of certain cell surface markers and transcription factors across peripheral organs that cannot be used to define these cell lineages (during homeostasis and inflammation). Certain cell surface markers are also not homogenously expressed on all peripheral NK and ILC1. However, previous studies have identified stable surface markers of group 1 ILCs that do not change during inflammation (shown in black). Surface markers that may lose or gain expression during states of activation or inflammation, or are heterogeneously expressed in peripheral ILC1 are shown in red. Stable but heterogeneous (not expressed on all peripheral NK or ILC1) surface markers are shown in light grey.

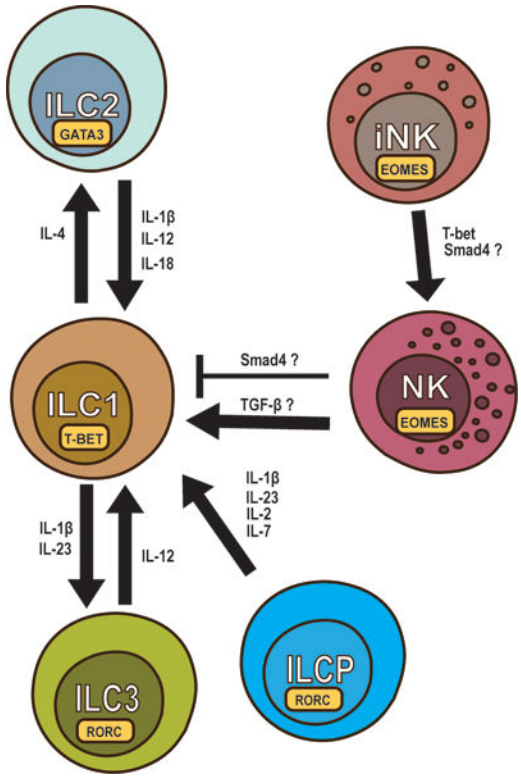

Figure 2. Group 1 ILC Plasticity in Mice and Humans.

Mature mammalian ILCs can be defined as distinct lineages through expression of the transcription factors T-bet, Eomes, Rorγt, and GATA3 at steady state. However, several studies have suggested that mature ILCs or ILC precursors found in tissues and the circulation can differentiate into mature ILC1 during inflammatory settings *in vivo* and *in vitro*. Here, we show a graphical illustration of the current factors and cytokines from the literature that may induce differentiation of indicated cell lineages into ILC1-like cells or NK cells. Arrows denote differentiation. Question marks denote differentiation pathways that are still not definitive.

Table 1:

Reported Phenotypes of Mouse Group 1 ILCs Across Different Organs

NK cell	CD49a	CD49b	CD200R	CD69	CD90	CD61	Eomes	TRAIL	CXCR6	IL-15R	IL-7R	Ly49H	Ly49C/I
Thymus	−	+	x	+	x	x	+	x	x	x	+/−	x	−
BM	+/−	+/−	-	-	+/−	x	+	x	−	+	−	+/−	+/−
Spleen	+i	+	−	-, +i	-, +i	-, +i	+, −i	−, +i	−	+	−	+/−	+/−
Liver	−, +i	+	−	−	+/−	-	+, -i	-, +i	−	+	−	+/−	+/−
Lung	x	+	−	x	x	x	+	x	x	+	x	+/−	+/−
Fat	−	+	x	-	+/−	x	+	−	x	+	−	+/−	+/−
Gut	x	+	x	x	x	x	+	x	x	+	−	+/−	+
Skin	x	x	x	−	x	x	+	x	x	x	−	x	x
Salivary Gland	+/−	+	−	+/−	x	x	+	-, +i	x	+	-	+/−	x
Uterus	+/−	+	x	+/−	x	x	+	+/−	-	+	−	+/−	x
PC	−	+	−	-	x	-	+	−	x	+	-	+/−	+/−
ILC1	**CD49a**	**CD49b**	**CD200r**	**CD69**	**CD90**	**CD61**	**Eomes**	**Trail**	**Cxcr6**	**IL-15R**	**IL-7R**	**Ly49H**	**Ly49C/I**
Thymus	+	−	x	x	x	x	−	x	x	+	+	x	x
BM	+	−	+	x	x	x	−	x	x	+	+	x	x
Spleen	+/−	-	+	+/−	+/−	x	−	+, +i	x	+	+/−	−	x
Liver	+	−	+	+	+/−	+	−	+, +i	+	+	−	−	+/−
Lung	x	−	+	x	x	+	−	x	x	+	x	x	x
Fat	+/−	−	+	+/−	+	+	−	x	x	+	−	−	+/−
Gut	+/−	-	+	+	+	+	-	x	x	+	+	−	+/−
Skin	x	x	x	+	x	x	−	x	x	x	−	x	x
Salivary Gland	+/−	−	+	+	x	+	-	+, +i	x	+	x	-	x
Uterus	+	-	x	+/−	x	x	−	+	+	+	−	x	x
PC	+/−	-	+	−	x	+	-	x	x	+	+	-	x

Denotes + (positive); – (negative); +/– (intermediate or heterogeneous); +i (increased during inflammation; –i (decreased during inflammation); x (not determined).

Table 2:

Reported Phenotypes of Human Group 1 ILCs and other CD56-expressing ILCs

	CD56	IL-7Rα	CD94	NKp80	CD16	CD69	CXCR6	CD49a	NKp44	CD103	Perforin	EOMES	T-BET	HOBIT
ILC1	-	+	-	x	-	+/−	x	x	-	-	-	-	+	x
ieILC1	+	-	+/−	x	+/−	+	+	+	+	+	+/−	+	+	+
trNK	+	-	+	x	dim/-	+	+	+/−	+/−	+/−	+	+	+	x
iNK	+	+	+	-	-	x	x	x	+/−	x	+/−	-	-	x
PB $CD56^{bright}$ NK	+	+	+	+	-	-	-	-	-	-	+	+	+	-
PB $CD56^{dim}$ NK	+	-	+/−	+	+	-	-	-	-	-	++	+	+	-
ILC3	+/−	+	-	-	-	+	-	-	+	-	-	-	-	-
ILCP	+/−	+	-	-	-	+/−	x	x	-	-	-	-	-	x

Denotes + (positive); – (negative); +/– (intermediate or heterogeneous); x (not determined).