Differentiation and diversity of subsets in group 1 innate lymphoid cells

Cyril Seillet$^{1,2}$ and Gabrielle T. Belz$^{1,2}$

$^1$Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Melbourne, Victoria 3052, Australia
$^2$Department of Medical Biology, University of Melbourne, Melbourne, Victoria 3010, Australia

Correspondence to: G. T. Belz; E-mail: belz@wehi.edu.au
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Abstract

NK cells were first identified in 1975 and represent the prototypical group 1 innate lymphoid cell (ILC). More recently, the discovery of new members of the ILC family has highlighted the complexity of this innate lymphoid lineage. Importantly, it has been recognized that different subsets exist within the group 1 ILC, which have potential roles in mediating immune protection and immunosurveillance, and in regulating tissue homeostasis and inflammation. Here, we review the developmental relationships between the different group 1 ILC, which have been identified to date and discuss how heterogeneity within this expanding family may have arisen.

Keywords: differentiation, immune protection, innate immunity, lymphocyte, transcription factors

Introduction

Group 1 innate lymphoid cells (ILC) are a heterogeneous group of cells that are broadly defined by their expression of the T-box transcription factor T-bet (encoded by \( Tbx21 \)) and their production of the signature effector cytokine, IFN-\( \gamma \). The prototypical group 1 ILC is the NK cell, which mediates early immune responses against viruses and cancerous cells. They have typically been identified by their expression of the NK-cell p46-related protein (\( \text{Nkp46; NCR} \)) and NK1.1. More recently, however, it has become evident that distinct populations within the group 1 ILC exist and it seems likely that this diversity is necessary for immune protection. Thus, a better definition of these populations is necessary to understand their molecular and cellular wiring, and contributions to immunity. This review provides an overview of the different populations within the group 1 ILC and highlights the questions that surround our understanding of their relationship to each other.

Subsets of group 1 ILC

At steady state, four subsets of group 1 ILC can be identified. These are (i) conventional NK cells (NK cells), (ii) ILC1, (iii) innate memory cells and (iv) ex-Ror\( \gamma \) (retinoic acid receptor-related orphan receptor \( \gamma \)t) ILC3 ILC1-like cells (Fig. 1).

NK cells

NK cells are conventional group 1 ILC that exert crucial early control of pathogen infections and malignancies (1). The early NK progenitors give rise to immature NK cells ([iNK cells]; CD3\( ^{–} \)CD122\( ^{+} \)NK1.1\( ^{–} \)CD49b\( ^{+} \)) that have not yet acquired effector functions such as cytotoxicity or the capacity to produce cytokines (e.g. IFN-\( \gamma \)). Fate-mapping of NK cells using Ncr1-YFP (\( \text{Nkp46}^{\text{Cre}}R26R^{\text{YFP}} \)) mice shows that NKp46 expression on NK cells is stable and indicates irreversible entry into the NK-cell lineage (2). Mature NK cells ([mNK cells]; CD3\( ^{–} \)CD122\( ^{–} \)NK1.1\( ^{–} \)CD49b\( ^{+} \)) have been characterized by their expression of Nkrg1c (NK1.1), CD49b (DX5) and Nkp46. Sequential expression of CD11b and CD27 delineates sequential steps in peripheral NK-cell maturation (3–6).

The NK cells also express multiple activating and inhibitory receptors of the NKG2 and Ly49 (KIR in humans) family (3, 7). Coordinate regulation of these receptors allows the cells to rapidly sense changes in the environment. NK cells can kill infected or transformed cells through cytolytic mechanisms (granzymes or perforin) or engagement of death receptors. In addition, they produce a range of chemokines and pro-inflammatory cytokines such as IFN-\( \gamma \) and express some chemokine receptors.

Classically, NK cells have been considered to represent a single lineage. The recent identification of a variety of different sub-populations within the liver, intestinal epithelium, uterus, skin and salivary glands has revealed the unprecedented complexity of the group 1 ILC and raised many questions on the relatedness of each of these subsets (8–12).

ILC1

ILC1 are found at steady state in virtually all tissues but are enriched in the liver, uterus, skin, salivary glands and the gut (10). In contrast to NK cells, which express integrin \( \alpha 2 \)
CD49b), ILC1 express high levels of integrin α1 (CD49a) but lack integrin α2 and the T-box transcription factor eomesodermin (Eomes). At steady state, ILC1 in the liver and salivary gland also express the TNF family member, TNFSF10 (TRAIL), but this expression appears not to be maintained following infection or inflammation. Currently, it is unclear whether the salivary ILC are indeed ILC1 or NK cells as they share features with both cell types. A striking characteristic of liver ILC1, and at least some salivary ILC, is that they are ‘tissue-resident’ and do not recirculate but reside in organs where they are poised to respond to antigens and pathogens (8, 10, 11, 13).

NK cells and intestinal ILC1 rely on the transcription factor nuclear factor IL-3 (Nfil3); encoded by Nfil3) for their development while in general ILC1 bypass this requirement (8, 11). At first glance, this dependence or lack of dependence would seemingly delineate NK cells from ILC1. However, although salivary ILC develop independently of Nfil3, they also express CD49b and the transcription factor Eomes, both characteristics of NK cells, suggesting that some group 1 ILC subsets could be more closely related to NK cells (8). Salivary gland ILC also appear to be actively recruited into this site following mouse cytomegalovirus (MCMV) infection, implying they are not obligate tissue-resident cells and can be amplified by signals transduced by Ly49H and pro-inflammatory cytokines to allow them to be drawn into tissues (14). Nevertheless, ILC1 generally express low levels of markers normally found on mature NK cells such as CD11b, CD43 and KLRG1 and lack expression of Ly49 family receptors.

Resting ILC1 also express a pattern of chemokine receptors and adhesion molecules distinct from NK cells that are likely to be important in maintaining their tissue-resident state. This includes high expression of CXCR6, CD103, VLA1, CD69 and CD39 (Table 1) (8). In the gut, homing receptor expression by ILC1 and ILC3 is augmented by mucosal dendritic cells and tissue-specific expression of retinoic acid (15).

**Innate memory cells**

A major conundrum in the field currently is the recognition that some NK cells play an important part not only in early responses to pathogens but also in long-term immunity. In MCMV infection, similar to adaptive immune responses, NK

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**Fig. 1.** Model for mouse NK-cell and ILC1 development. Development of murine ILC, partitioned into different subgroups largely based on their cytokine production. Individual subsets depend on the expression of specific transcription factors that determine their terminal differentiation.
cells can expand substantially (~2.3-fold) and subsequently undergo significant contraction resulting in Ly49H+ cells residing in lymphoid and non-lymphoid tissues for many months. They replenish this long-lived compartment by self-renewal, and analogous to CD8+ T cells, they appear to be primed for rapid secondary expansion and effector function (16). This phenomenon, referred to as ‘innate memory’, reflects that ILC can exhibit some features previously attributed solely to adaptive immune cells. Indeed, NK cells have been shown to mediate antigen-specific responses in the context of contact hypersensitivity when adaptively transferred into recombinase-activating gene 2 (Rag2)-deficient (Rag2−/−) mice that lack T and B cells (17).

Strikingly, in response to contact hypersensitivity, only hepatic NK cells, and not splenic NK cells, could generate the virus-specific recall responses and this was dependent on CXCR6 (18). Extending from this, Lanier et al. (16) demonstrated that primed NK cells could be recruited into a secondary immune response following MCMV infection. Whether this reflects true memory or the responsiveness of long-lived primed NK cells is not entirely clear. However, the notion is raised that innate cells, like adaptive immune cells, can be imprinted by their experience. Precisely, how NK-cell memory forms is not yet clear. Understanding this pathway to memory for NK cells is further complicated as, although naive NK cells rely on Nfil3 for their development, antigen-experienced NK cells have no such requirement either for their maintenance or for their secondary expansion (19). This might reflect that naive and primed NK cells fundamentally differ in their transcriptional wiring or, alternately, that primed cells are at least partially aligned with programs utilized by ILC1 (Table 1).

Thus, despite tremendous recent efforts to characterize NK cells and ILC1, we still do not know how many subsets might exist in the group 1 ILC or how NK-cell memory might fit into this picture. In the case of NK cells, memory is characterized by their high proliferative potential, multipotency, rapid recall and maintenance of homeostatic turnover, similar to the definition applied to T cells. It is unclear whether these attributes reflect quantitative differences (altered potential) in the types of inflammatory signals experienced by naive NK cells during an immune response establishing different survival and recall potential, or whether memory NK cells emerge as a distinct lineage (fate commitment and progressive differentiation). Nevertheless, immune-experienced NK cells reflect a cell type with attributes akin to those described for adaptive immune cells.

**Table 1. Phenotype of different subsets of ILC1 found throughout the body**

<table>
<thead>
<tr>
<th>Marker</th>
<th>NK cells</th>
<th>Ex-Rorγt ILC</th>
<th>Hepatic ILC1</th>
<th>Salivary gland ILC1</th>
<th>Memory NK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface molecules</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK1.1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>NKp46</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CD49a</td>
<td>✓</td>
<td>x</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CD49b</td>
<td>✓</td>
<td>x</td>
<td>x</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>TRAIL</td>
<td>x</td>
<td>x</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CD122</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>IL-7R</td>
<td>x</td>
<td>✓</td>
<td>n.t.</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>CXCR6</td>
<td>x</td>
<td>n.t.</td>
<td>n.t.</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Transcription factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-bet</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Eomes</td>
<td>✓</td>
<td>x</td>
<td>x</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Nfil3</td>
<td>✓</td>
<td>n.t.</td>
<td>✓</td>
<td>n.t.</td>
<td>n.t.</td>
</tr>
<tr>
<td>Tcf7</td>
<td>x</td>
<td>n.t.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>n.t.</td>
</tr>
<tr>
<td>Rorγt</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Zbb32</td>
<td>✓</td>
<td>n.t.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>✓</td>
</tr>
<tr>
<td>Cytokines produced</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>TNF-α</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>n.t.</td>
<td>✓</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>✓</td>
<td>n.t.</td>
<td>✓</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>✓</td>
<td>n.t.</td>
<td>✓</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>Cytokine dependency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-7</td>
<td>x</td>
<td>✓</td>
<td>n.t.</td>
<td>✓</td>
<td>n.t.</td>
</tr>
<tr>
<td>IL-15</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>✓✓</td>
<td>n.t.</td>
<td>✓</td>
<td>n.t.</td>
<td>✓✓</td>
</tr>
<tr>
<td>Distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>Few</td>
<td>Throughout body</td>
<td>Few</td>
<td></td>
<td>Absent</td>
</tr>
<tr>
<td>Steady-state</td>
<td>Throughout body</td>
<td>Few</td>
<td>Liver-restricted tissue-resident</td>
<td>Few cells</td>
<td>Through body</td>
</tr>
<tr>
<td>Infection</td>
<td>Throughout body</td>
<td>Increased</td>
<td>Through body</td>
<td>Recruited, many cells (MCMV)</td>
<td>Through body</td>
</tr>
</tbody>
</table>

n.t., not tested.
instead depend on induction of T-bet expression through cytokine stimulation such as IL-12 and IL-18 during inflammation (21, 22).

These cells have been identified to be highly enriched particularly in human oral and intestinal mucosa where they express CD127 (IL-7Rα) but lack NK-cell markers and cytolytic effector molecules such as perforin and granzyme B (23). They also appear to be distinct from a second population of ILC1 found at this site, which express the αE integrin, CD103 (22, 23). The CD127+, but not the CD103+, ILC1 have recently been shown to be capable of reverting to RORγt+ ILC3 under the influence of IL-2, IL-23 and IL-1β, a process that is further enhanced by the presence of retinoic acid, suggesting that ILC are capable of rapidly adapting to changes in environmental cues, which appear to play a key role in determining the phenotype of the cells (22). Whether these cells should be formally classified as ILC1 or ILC3 is not settled. The capacity of some ILC to adopt different phenotypes dependent on environmental signals, or tissue-specific cues challenges the current relatively rigid nomenclature focused on three ILC subsets. It suggests that ILC form a dynamic cellular network rapidly adapting to changes in the environment that is not always neatly accommodated by the current schema.

**Differentiation of NK cells and ILC1**

Currently, the early regulation of transcriptional choices that give rise to NK cells and group 1 ILC subsets is poorly defined. All ILC arise from common lymphoid progenitors (CLP) that are restricted to a lymphoid fate (Fig. 1, Table 2). These progenitors are found in the liver during fetal development and in the bone marrow in the adult. Until recently, the NK-cell progenitor, or NKP (CD122+c-Kitlo/–Flt3–) markers

ILC1, ILC2, ILC3 - β-Sca1+Produce the NK-cell progenitor, or NKP (CD122+). Noteworthy, the major transition of developing ILC in the bone marrow is the conversion of the GMP-like CLP to the ILC progenitors (ILCP) (24–26), which act to regulate effector functions in the periphery (43–45). PLZF+ lymphoid progenitor.

**Developmental gene networks establishing NK-cell and ILC1 identity**

The development of different subsets of cells within a lymphocyte family depends intrinsically on transcription factors that sequentially restrict certain lineage choices to produce a specific subset. A number of transcription factors regulate the NK cell/ILC fate. Iδ2, E proteins, Ifnar1, Il6, Ifnl3 are among proteins known to drive early stages of NK-cell development (19, 29, 34–39). Other transcription factors such as T-bet, Eomes, Aiolos and Blimp-1 play roles in development and maturation (40–42). In addition, extrinsic factors such as cytokines influence these choices and play a critical role in the maintenance of mature subsets, linking extrinsic factors to internal mediators such as STAT1, STAT4, Zbtb32 and AhR, which act to regulate effector functions in the periphery.

**Id2**

Id2 is a member of the inhibitor of DNA-binding family of transcriptional repressors that work in concert with E-box proteins in the development of NK cells and ILC1.

**Table 2. Phenotype of early NK cell and ILC progenitors**

<table>
<thead>
<tr>
<th>Progenitor</th>
<th>Markers</th>
<th>Produce</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid progenitors</td>
<td>Sca1+c-Kit+Flt3+IL-7Rα+</td>
<td>NK cells, LTI, ILC1, ILC2, ILC3; adaptive immune lineages</td>
<td>(24)</td>
</tr>
<tr>
<td>αLP (CILP)</td>
<td>Sca1+c-Kit+Flt3+IL-7Rα+α4β7+ (includes CXCR6+ subset lacking T-cell potential)</td>
<td>NK cells, LTI, ILC1, ILC2, ILC3; adaptive immune lineages</td>
<td>(25–28)</td>
</tr>
<tr>
<td>Innate lymphoid cell progenitors</td>
<td>c-Kit+c-Kit+ID2+Sca1+IL-7Rα+α4β7+CD244+CD27+</td>
<td>LTI, ILC1, ILC2, ILC3</td>
<td>(29)</td>
</tr>
<tr>
<td>ILCP</td>
<td>Flt3 c-Kit+PLZF+IL-7Rα+α4β7+</td>
<td>ILC1, ILC2, ILC3</td>
<td>(30, 31)</td>
</tr>
</tbody>
</table>

αLP, α4β7+ lymphoid progenitor.
to broadly regulate cellular differentiation. They modulate transcription by forming heterodimers with E protein transcriptional activators (which include E2A, E2-2 and HEB) to limit their capacity to induce downstream transcription. Genetic ablation of Id2 in mice results in the loss of NK cells and the failure of lymph node and mucosal-associated lymphoid tissues to develop \(^{34, 46, 47}\). The CLP express little or no Id2 but expression is switched on early in differentiation at the prepro-NK stage/pre-NK stage, initiating commitment to the group 1 ILC family and restricting the development of B and T cells \(^{29, 32, 48}\).

**Tox1**

Tox1 is one of four members of the thymocyte selection-associated high-mobility group box protein family that binds DNA and has been shown to be required for the development of NK cells, ILC and T cells (particularly CD4\(^+\) T cells) \(^{49}\). Tox1\(^{-/-}\) mice have a number of defects including a significant block in T-cell development at the stage of positive selection, reduced peripheral NK cells, ILC and their precursors (loss of Lin-\(\alpha\)46\(^*\)CD127\(^*\)\(^{+}\)Flt3\(^*\)) together with LTi cells. This was accompanied by a failure to develop lymph nodes and reduced size and frequency of Peyer’s patches \(^{49–51}\). Strikingly, the phenotype observed in Tox1-deficient mice is similar to that of Id2-deficient mice, and the co-expression of both Tox–Tomato and Id2–GFP (Tomato and GFP are the fluorescent tags) suggests that these two factors operate similarly although over-expression of Id2 was not sufficient to rescue the Tox1-deficient phenotype \(^{51}\). Other Tox family members have not previously been implicated in NK/ILC development; however, in human NK cells, Tox2 has recently been shown to impact NK-cell development through a T-bet-driven pathway that drives maturation \(^{52}\).

**GATA3**

GATA-binding protein 3 (GATA3) has been shown to be required for the development of multiple ILC lineages \(^{29, 53}\). Although no obvious defect in NK-cell numbers can be found in mice deficient in GATA3, it acts to regulate T-bet expression and IFN-\(\gamma\) production in addition to a number of homing molecules \(^{54}\). GATA3-deficient IL-7R\(^{α}\) progenitor cells failed to develop in the fetal liver and adult bone marrow, resulting in a loss of intestinal Eomes\(^+\) ILC1 and ILC3 (including LTi cells) \(^{29, 54, 55}\). However, the development of NK cells (Eomes\(^+\) ILC) does not appear to be affected, suggesting that GATA3 represents an important checkpoint in the lineage decisions between NK cells and ILC1.

**Nfil3**

Nfil3, also known as E4BP4, is a basic leucine-zipper-type transcription factor that is highly related to PAR family transcription factors \(^{56, 57}\). It has since been recognized that this factor is broadly expressed by multiple lymphocyte lineages and affects a number of biological processes including circadian rhythm and has a major role in the lineage determination in NK cells \(^{58, 59}\). Loss of Nfil3 impairs the development of NK-cell progenitors and intestinal IFN-\(\gamma\)-producing ILC1, ILC2 and ILC3, suggesting early involvement in the fate-decision process. Strikingly, it does not appear to be required for liver ILC1 or memory NK-cell survival \(^{19, 35, 36}\). The molecular targets of Nfil3 are not yet clear. It is proposed that Nfil3 might directly regulate Id2 and Eomes activation; however, Id2 expression is not altered when Nfil3 is blocked in NK cells \(^{11, 60, 61}\). Thus, it will be important to understand the connections between Nfil3 and other transcription factors in the regulation of ILC fate.

**Eomes and T-bet**

Eomes and T-bet are highly homologous members of the T-box transcription factor family and have been well defined as key drivers of immune-cell development and the development of effector functions, particularly in CD8\(^+\) T cells and NK cells \(^{41, 62–64}\). They are thought to exert their actions through both redundant and non-redundant actions in lineage fate decisions. Mice with compound deletion of T-bet and Eomes lack both mature NK cells and memory CD8\(^+\) T cells, which depend on these two factors for the induction of the IL-15\(\alpha\)-responsive element, CD122, that allows signaling via IL-15, a key survival factor for NK cells \(^{41, 62}\). T-bet-deficient mice also exhibit reduced numbers of mature NK cells in the peripheral organs that is thought to be brought about by the diminished expression of S1P5 and altered NK-cell trafficking \(^{65, 66}\). However, under naive conditions, these cells display increased proliferation and apoptosis, and reduced levels of maturation markers indicating altered regulation of the maturation process \(^{66, 67}\).

More recently, Eomes has been shown to be essential for the acquisition of inhibitory and activating receptors as deletion of this transcription factor resulted in the reversion to a TRAIL\(^+\) CD49b\(^−\) state \(^{41}\). NK cells express both T-bet and Eomes in peripheral cells, whereas the recently characterized liver-resident TRAIL\(^+\) cells lack Eomes expression \(^{10}\).

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**ILC \(^1\) subsets**

![Diagram](https://academic.oup.com/jimmunol/article-abstract/28/1/3/2614099/573x353)

**Salivary ILC1**

**Skin NK cells**

**Primed or ‘memory’ NK cells**

**Liver-resident ILC1**

**cNK cells**

**Fig. 2.** Inter-relationship between different ILC subsets. NK cells and liver-resident ILC1 exhibit relatively distinct phenotypic profiles. ILC and NK cells from other tissue sites overlap significantly with NK cells and liver-resident ILC1, blurring the distinction between different populations of cells.
Thus, Eomes can regulate the expression of several surface molecules associated with NK cell/ILC1 lineage identity and plays a role in the induction of group 1 ILC IFN-\(\gamma\), IL-2, and TNF-\(\alpha\) cytokine production (69).

Several lines of evidence argue that NK cells and group 1 ILC subsets arise independently, including the lack of reliance of liver ILC1 on Nfil3 and Eomes and the differences in PLZF–GFP fate-mapping expression. Supporting this, TRAIL+CD49b+Eomes+ ILC1 arise in the mouse fetal liver and persist in this site in the adult (68, 69). Indeed, Eomes cells do not give rise to NK cells, and the repression of T-bet and the reciprocal expression of Eomes are essential for the development of NK cells, indicating that two mutually exclusive transcriptional programs exist to give rise to NK and liver ILC1 subsets. How these two transcription factors are balanced to guide the differentiation of both NK memory cells and ILC1 found at other sites that express both Eomes and T-bet is not yet known.

**Squaring the circles: how various phenotypes arise in different locations**

Fate-mapping has provided significant insights into the developmental pathway of NK cells and ILC subsets. Nevertheless,
how many distinct lineages there are in the ILC1 family remains unclear (Fig. 2). Initial efforts have focused on identification of different subsets through surface molecule expression, and more recently their dependence on various transcription factors. However, NK-cell and ILC1 subsets exhibit significantly overlapping features obscuring a clear explanation for how heterogeneity is generated (Fig. 2, Table 1). Both deterministic (integration of extrinsic and intrinsic signals) and stochastic (intrinsic cellular heterogeneity) factors are likely to play an important part in the generation of this diversity, but the extent to which each of these pathways contributes is not well defined.

In thinking about this problem, we propose a model that could reconcile the current findings (Fig. 3). In the first instance, each subset of group 1 ILC could represent a distinct lineage that is regulated by specific transcription factors leading to the development of independent fate outcomes. This appears to be the case for NK cells and liver-resident ILC1 where T-bet+Eomes- cells are not immature NK cells that simply acquire Eomes on maturation (69). However, it fails to fully explain the emergence of ILC1 that either express both T-bet and Eomes, as found in the salivary gland, or whose development does not depend on Nfil3 as is required by NK cells.

One possibility is that low-grade stimulation or other environmental effects might influence the phenotype of ILC1 subtypes, as even in the germ-free setting, food antigens could stimulate developing cells. We propose that a small fraction of NK cells at steady state, such as CD8+ T cells, exhibit a primed phenotype and may thus be found to co-exist with naive cells. This primed phenotype could be attributable to various antigenic stimuli, but such activation in NK cells can also arise from antigen-independent pathways such as through cytokine stimulation during low-grade inflammation (70). This raises the possibility that steady-state NK cells (T-bet-Eomes-) that co-exist with T-bet+Eomes- ILC1 found in the liver (16, 71), and steady-state salivary gland ILC1, also known as salivary gland NK cells (8, 14), represent differentially localized members of the same subset.

During infection, massive recruitment can occur offering an explanation for a small fraction of apparent ‘tissue-resident’ ILC1 in the salivary gland at steady state, but a significant influx of these cells during MCMV infection. Thus, salivary gland ILC1 may not all be genuine ‘tissue-resident’ cells (14) but some could arise in response to a TGF-β gradient, a factor that has been shown to be essential for the retention of CD8+ T cells in tissues (72, 73), providing a model in which the extrinsic cues of specific tissue microenvironments determine the phenotype of the cells. The co-existence of T-bet+Eomes-Nfil3-dependent NK cells and T-bet+Eomes-Nfil3-independent ILC1 in the liver, however, suggests that there are true lineages and that other phenotypes may reflect the intrinsic capacity to rapidly adapt to local signals within the tissues and environment.

Concluding remarks

We propose that there are key transcriptional checkpoint switches that drive core molecular programs of commitment to the ILC1, ILC2 and ILC3 lineages. Extrinsic signals, for example, from different tissue niches, overlay this primary developmental map to influence the final phenotype of emerging cells. This may create the possibility for unappreciated plasticity where the homeostatic balance of different subsets is orchestrated by tissue-derived cues and may ultimately reshape the nomenclature used to describe these different ILC1 and NK-cell subsets. As yet, little information is available on the degree to which environmental and stochastic processes might influence these pathways. Altogether, this knowledge provides a new framework in which to test both the relationships between different ILC subsets and their specific roles in immune protection, and to delineate new concepts for the homeostatic maintenance of ILC populations in maintaining the balance between immune protection and pathology, particularly in relation to the development of chronic inflammatory diseases.

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References

10 ILC1 subsets


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