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Development of innate lymphoid cells

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Innate lymphoid cells (ILCs) are a family of immune effector cells that have important roles in host defense, metabolic homeostasis and tissue repair but can also contribute to inflammatory diseases such as asthma and colitis. These cells can be categorized into three groups on the basis of the transcription factors that direct their function and the cytokines they produce, which parallel the effector functions of T lymphocytes. The hierarchy of cell-fate-restriction events that occur as common lymphoid progenitors become committed to each of the ILC lineages further underscores the relationship between these innate immune cells and T lymphocytes. In this Review we discuss the developmental program of ILCs and transcription factors that guide ILC lineage specification and commitment.

Innate lymphoid cells (ILCs) are a heterogeneous population of lymphocytes that respond rapidly to pathogen infection or tissue damage though the use of germline-encoded receptors. For much of the past 30 years, natural killer (NK) cells were the only known ILCs; however, in recent years, several new ILC populations have been identified, which has revealed a system of innate cells whose developmental requirements and functional responses parallel those of T lymphocytes. By this analogy, NK cells are the counterpart to cytotoxic $CD8^+$ T cells[1], and the more recently identified 'helper' ILCs are the counterparts to $CD4^+$ helper T cells[2] (**Fig. 1**). The group 1 ILCs include NK cells and ILC1s, which function in the immune response to intracellular pathogens such as viruses and intracellular bacteria[3]. NK cells are also able to kill many kinds of cancer cells, including some forms of leukemia[4]. NK cells might be useful as therapeutics in treating cancer, especially during bone-marrow transplantation, in which they have been shown to be effective in mediating graft versus leukemia[5]. Group 2 ILCs include ILC2s (natural helper cells, nuocytes and innate helper 2 cells) and multipotent progenitor type 2 cells, all of which produce cytokines characteristic of the T_H2 subset of helper T cells that promote the immune response to helminthes, as well as factors that help to repair epithelial barriers[6,7]. Group 2 ILCs might also serve an important pathogenic role in inflammatory lung diseases such as asthma[8]. Group 3 ILCs include lymphoid-tissue-inducer (LTi) cells, which are necessary for the development of lymph nodes, Peyer's patches and ectopic lymphoid structures[9], and natural-cytotoxicity-receptor-positive (NCR^+) and NCR^- ILC3s, which function in the immune response to bacteria[10]. ILC3s have a role in establishing a healthy gut microbial environment, but their dysregulated activity can contribute to colitis[11,12]. Despite the apparent segregation of ILCs into distinct lineages with unique functional responses, studies have revealed substantial heterogeneity in their function and plasticity within and between these lineages that may develop under pressure from external signals. The mechanisms underlying such plasticity and its relevance to the immune response are discussed in the accompanying Review by Spits and colleagues[13]. In addition to this naturally occurring plasticity, disruption of the transcriptional networks in ILCs has revealed a low barrier to lineage plasticity and might identify mechanisms by which the function of specific ILC subsets can be usurped to promote immune responses that are not a signature of their lineage. In this Review we will discuss the current understanding of the developmental trajectories of ILCs and the transcriptional circuitry that supports these programs, with an emphasis on data derived from mouse models.

Group 1 ILCs

NK cells and ILC1s share many features that underlie their classification as group 1 ILCs[14]. Both cell types are responsive to inflammatory cytokines such as interleukin 15 (IL-15), IL-12 and IL-18, and when activated they produce interferon-γ (IFN-γ) and tumor-necrosis factor, although the range of inflammatory mediators produced by ILC1s seems to be greater than that produced by NK cells[15]. Both cell types express the activating NK cell receptors NK1.1 and NKp46, but ILC1s mostly lack activating receptors of the Ly49 family and the inhibitory receptors of the Ly49 family that detect major histocompatibility complex class I molecules[15–17]. Thus, 'missing-self' recognition, a mechanism for the detection of virus-infected and cancer cells that have major histocompatibility complex class I and for the direct detection of virus-infected cells, appears to be largely the charge of NK cells, not of ILC1s[18–20]. Indeed, the cytotoxic effector program that characterizes mature NK cells, including expression of granzyme B and perforin, is absent from ILC1s, consistent with the analogy of $CD8^+$ T cells and $CD4^+$ T_H1 cells as the counterparts of NK cells and ILC1s, respectively[2,15]. Nonetheless, the possibility of some cytotoxic function for ILC1s has not been firmly excluded[15,17]. Moreover, analysis of ILC1s and NK cells from multiple tissues by gene-expression profiling has shown that the core of genes whose expression could be used to distinguish these two cell types is small, possibly due to the lack of clarity about the phenotypes and assignment of cells to the NK-cell or ILC1 lineage or because of their functional heterogeneity[17]. ILC1 are tissue-resident

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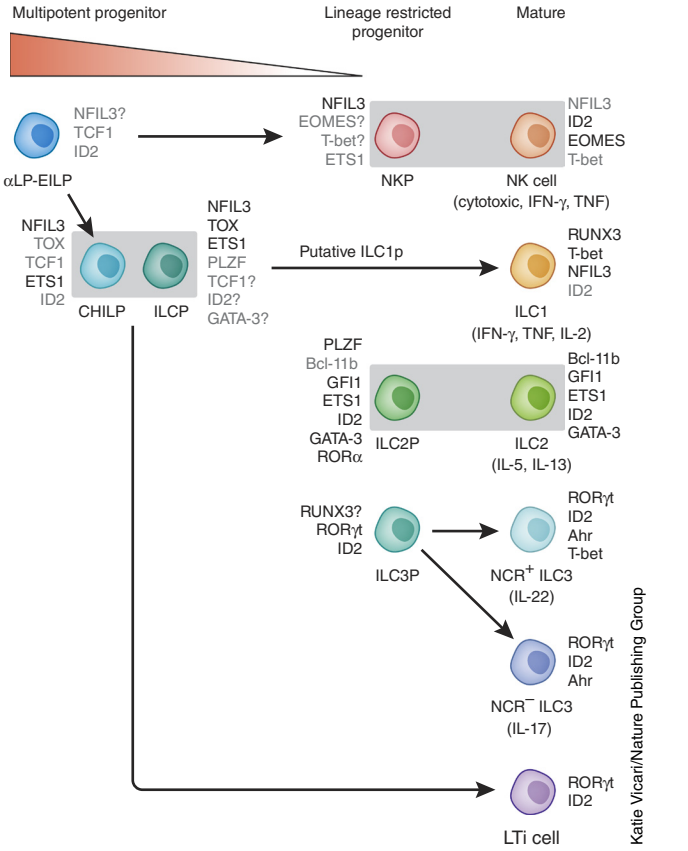

Figure 1 Transcriptional control of ILC development. Stages of ILC development from multipotent αLP-EILP to mature ILC, including specific transcription factors (left and right margins) and the stages at which they are expressed (bold indicates a transcription factor essential for the development of a cell type in Rag1-sufficient mice): left and middle, progenitors; right, mature ILCs and the signature cytokines they produce; arrows indicate precursor-progeny relationships.

cells, and a component of their gene-expression program reflects this property. Studies have revealed that some NK cells can acquire a tissue-resident gene-expression program that is similar to that of ILC1s[21–23], including a diminished cytotoxic effector program and altered cytokine production. Therefore, NK cells and ILC1s might not be easily distinguished solely on the basis of their gene-expression program, despite distinct developmental trajectories, as described below[24,25]. In this Review, we discuss conventional NK cells except where we specifically discuss tissue-resident NK cells.

The developmental pathway from specified NK cell progenitor to mature NK cell (mNK cell) is understood in more detail than that for ILC1s, in part because their existence has been known for a longer period of time and because intermediate cells in this pathway can be isolated from primary hematopoietic organs. However, earlier studies did not distinguish NK cells from ILC1s, a fact that might have led to inappropriate lineage assignments. ILC1s differ from NK cells in their expression of the cytokine receptor IL-7Rα (CD127), which marks all 'helper' ILCs, but they are functionally more akin to NK cells in that they require IL-15 rather than IL-7 for their maintenance[16,26], and both NK cells and ILC1 express CD122 (the β-chain of the receptor for IL-15 and IL-2)[27,28] (**Fig. 2**). The first NK-cell-lineage-restricted progenitor (NKP) was identified nearly 15 years ago in the basis of expression of CD122 in the absence of expression of NK1.1, the α_2 integrin CD49b (DX5) or receptors of the Ly49 family[27,28]. This NKP population is heterogeneous; however, a revised NK progenitor (rNKP) has been described that has a surface phenotype similar to that of common lymphoid progenitors (CLPs) but that lacks the receptor tyrosine kinase and growth-factor receptor Flt3 (CD135) and expresses CD122 and CD127 (ref. 29). These rNKPs show considerable enrichment for NK-cell-differentiation potential *in vitro* and *in vivo*, although studies have raised questions about whether this population, as well as a proposed precursor to rNKPs that lacks CD122 (called the 'pre-NKP'), is contaminated with or related to ILC1s[24,29]. Molecular characterization of the rNKP population and extensive analysis of NK-cell potential versus ILC potential is currently lacking, which leaves open the question of whether this cell is a lineage-restricted NK cell progenitor.

NKPs have been reported to pass through an immature NK cell (iNK cell) stage en route to becoming mNK cells[27,28]. The iNK cell population expresses NK1.1 but does not yet express DX5. The iNK phenotype is similar to that described for ILC1s, and a fate-mapping study has assigned approximately 80% of iNK cells to the ILC1 lineage[24]. ILC1s can be distinguished from iNK cells by their expression of the integrins α_4, β_7 and α_1 (also known as CD49a or DX5a), CD127 and possibly the lymphocyte cytotoxic molecule TRAIL[3,15]. The iNK cell stage is extremely important in NK-cell development because it marks the onset of expression of the Ly49 receptors that are critical for NK cell missing-self recognition and for the detection of some invading pathogens, such as murine cytomegalovirus[28,30]. In humans, receptors of the KIR family are activated in a manner similar to that of Ly49 receptors and probably appear at an analogous stage of NK-cell differentiation[31]. Little is known about the mechanisms that control the onset of expression of the genes encoding the Ly49 receptors, and the identification of a highly purified population of cells undergoing receptor activation is critical for advancement in this area. Moreover, how NK cells are 'educated' to set their response threshold so that they can remain self-tolerant yet detect the loss of major histocompatibility complex class I, or become activated by engagement of activating NK-cell receptors, are major questions in NK-cell biology that are intricately linked to the timing with which Ly49 receptors are expressed[32,33]. Therefore, better definition and analysis of iNK cells is needed and will require exclusion of ILC1s from the NK1.1$^+$DX5$^-$ population.

Not all mature NK cells are functionally equivalent; they undergo a maturation process that can be tracked by the differential expression of the costimulatory receptor CD27 and the integrin α_M (CD11b)[34]. The most iNK-cell-proximal mNK cells, which are CD27$^+$CD11b$^-$, produce IFN-γ and tumor-necrosis factor when activated, but they are not yet efficient cytotoxic effector cells. These cells might be equivalent to the CD16$^-$CD56$^-$ subset of human NK cells[5]. Cytotoxic capacity improves with maturation and with upregulation of CD11b expression and an effector program similar to that of CD8$^+$ effector T cells[1,35]. The mNK cell population is critically dependent on IL-15, with high doses of IL-15 being required to activate the metabolic checkpoint kinase mTOR that is required for the generation of CD11b$^+$ cells and lower doses sustaining phosphorylation of the transcription factor STAT5 without mTOR activation[36]. In the absence of IL-15 signaling, the number of peripheral NK cells is severely compromised but, surprisingly, a few NK cells develop that are competent to respond to murine cytomegalovirus through activation of the receptor Ly49H and IL-12 (refs. 37,38).

Transcriptional programs for group 1 ILCs

The T-box transcription factors eomesodermin (Eomes) and T-bet regulate many of the phenotypic and functional properties of NK cells and ILC1s, such as their expression of CD122 and production of IFN-γ

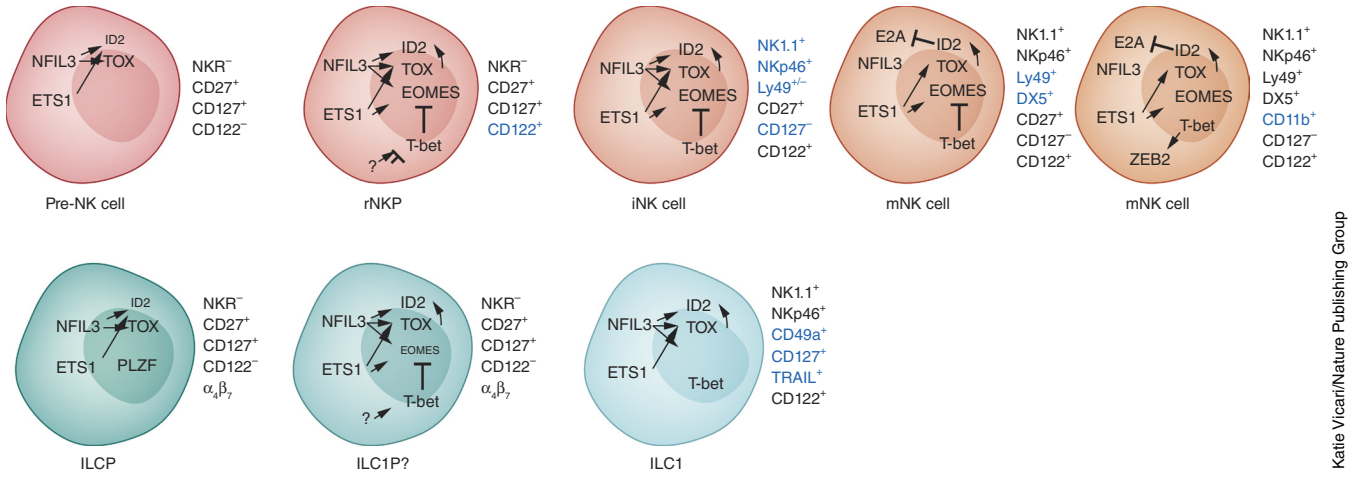

Figure 2 Comparison of the transcriptional networks that guide the development of NK cells and ILC1s from lineage-restricted progenitors. Cell-surface receptors (right margins) expressed on cells at various stages of development (blue font indicates surface receptors used to define each stage of development), as well as transcription factors (inside) known or hypothesized ('?') to be expressed at each stage (arrows indicate positive regulatory interactions; blunted arrows indicate repressive interactions; font size increases or decreases as expression changes during development).

and the cytotoxic effector program. However, NK cells express both Eomes and T-bet, whereas ILC1 express only T-bet[15,17,39]. The timing of T-bet expression has been hypothesized to be a critical determinant of the NK cell–versus–ILC1 fate, because the concentration of T-bet is low in developing bone marrow NK cells despite its possible involvement in the regulation of the NK-cell effector program[15]. Moreover, raising T-bet expression in bone-marrow NK cells leads to repression of Eomes[15]. However, as discussed below, it is not clear that a common progenitor of both NK cells and ILC1s exists, and therefore while limiting the expression of T-bet might be necessary for NK cell development, it might not directly affect cell-fate 'choice', at least under physiological conditions. Nonetheless, Eomes is required for NK-cell development, and its ectopic expression in T-bet$^+$ cells might promote the acquisition of some NK-cell-like features in ILC1s[40]. Thus, the doses of T-bet and Eomes are an essential determinant of the phenotype and function of ILC1s and NK cells. Interestingly, T-bet and Eomes are highly related proteins and might even bind to the same DNA target sequence, yet they seem to have different functions in the development and effector maturation of NK cells. Eomes is expressed in all bone-marrow NK cells, but T-bet expression increases as mNK cells progress from a 'naive' state to activated effector state. During this transition, T-bet induces expression of the transcription factor ZEB2, which is required for activation of the NK-cell effector program in cooperation with T-bet[41]. Whether T-bet specifically is required for the induction of ZEB2 expression or whether the dose of T-box transcription factor is the critical determinant of ZEB2 expression has not been determined, but under physiological conditions, it is T-bet that drives this ZEB2-dependent maturation program.

Id2, an inhibitor of E-protein transcription factors, has a critical role in the development of all ILCs but was first identified as a factor for NK cells and LTi cells[42,43]. *Id2* mRNA is present in NKPs and rNKPs, and its expression increases during NK cell maturation, such that CD11b$^+$ mNK cells have the highest expression of *Id2* mRNA[34,44]. Given that E proteins are critical regulators of early T cell and B cell development, it was hypothesized that Id2 is essential for the specification of NK-cell progenitors from CLPs because it would prevent E-protein-mediated adaptive-lymphoid specification. However, careful analysis of *Id2*$^{-/-}$ mice has revealed that this is not the case; Id2 is not required until the mNK-cell stage, at which it might control the survival of IL-15-dependent cells[43,45]. Interestingly, *Id3* expression is higher in *Id2*$^{-/-}$ NKPs than in *Id2*$^{+/+}$ NKPs, which suggests that it can compensate for the loss of Id2 (ref. 43) and supports the hypothesis that the E-protein dose needs to be controlled for NK-cell development. Not surprisingly, many of the transcription factors that contribute to the development of mature NK cells affect the expression of *Eomes* or *Id2*. The transcription factors NFIL3, ETS1 and TOX have all been shown to control *Id2* transcription, and in their absence, the number of mNK cells is substantially reduced[44,46–49]. Interestingly, however, conditional deletion of *Nfil3* in immature NK cells through the use of *Ncr1*^{Cre}*Nfil3*$^{f/f}$ mice has revealed that NFIL3 is no longer required once mNK cells develop, despite a continued requirement for Id2 (ref. 50). Moreover, while ILC1s are dependent upon Id2, at least some ILC1s and tissue-resident NK cells can develop in the absence of NFIL3 (refs. 22,50–52). These findings indicate that NFIL3 is not always required for the expression of Id2, and they illustrate the context dependence of the transcriptional circuits that control the development and maturation of NK cells and ILC1s.

Group 2 ILCs

Group 2 ILCs become activated in response to the epithelial-cell-derived cytokines IL-33, IL-25 or TSLP[53]. These cells are non-cytotoxic and release mediators associated with a T_H2-response, such as IL-5, IL-6, IL-9 and IL-13, and their main function is in the response to helminthes. Adipose-tissue ILC2s serve a role in maintaining metabolic homeostasis by controlling the activation of eosinophils and the consequent development of alternatively activated macrophages, which promote appropriate insulin sensitivity and control obesity[54,55]. ILC2s can also influence whether adipocyte progenitors differentiate into white fat or beige fat and thereby affect thermal homeostasis[56]. At least three cell types have been defined that have innate T_H2-cell-like activity and are classified as ILC2s, although the degree to which these are distinct cell types remains to be clarified[57–59]. The multipotent progenitor type 2 cells also have T_H2-cytokine-producing ability, but these cells are also multipotent progenitors that have monocyte-macrophage- and granulocyte-differentiation potential and therefore may differ from ILC2s in their developmental trajectory[60]. ILC2s are 'preferentially' activated by IL-33 rather than IL-25, whereas multipotent progenitor type 2 cells respond more robustly to IL-25 (ref. 61). Interestingly, a lung-resident IL-25-responsive 'inflammatory' cell (the 'iILC2') has been described in mice that might

generate IL-33-responsive ILC2s *in vivo*[62]; however, the relationship of this cell type with the majority of ILC2s or multipotent progenitor type 2 cells is unclear. A lineage-restricted ILC2 progenitor (ILC2P) has been identified in the bone marrow on the basis of expression of the signature transcription factor GATA-3 and the ILC transcription factor Id2, along with many ILC2 markers such as ICOS, CD25 and IL-1RL1 (the receptor for IL-33)[63]. Only a fraction of ILC2Ps express the surface receptor KLRG1, a marker of mature and functional ILC2s in peripheral tissues[64].

Transcriptional programs of group 2 ILCs

The identification of ILC2Ps suggests that multipotent progenitor cells in the bone marrow can generate lineage-restricted ILC2s in this hematopoietic organ. Populations of ILC2Ps and ILC2s can be expanded in cultures containing stromal cells expressing ligands of the Notch family of receptors, supplemented with IL-2, IL-7 and the ligand for the stem-cell-factor-receptor c-Kit[16,65], or IL-7 and IL-33 (ref. 66). The *in vivo* development of ILC2s might also require Notch-derived signals[66], since Notch2 has a role in expanding a subset of fetal progenitor cells that give rise to these cells[67]. Notch signal strength might even divert early progenitors of T cells into the ILC2 lineage[68]. The generation of ILC2s requires the coordinated activity of multiple transcription factors, including the T_H2 signature factor GATA-3 (refs. 63,69,70), the T cell factor TCF1 (refs. 71,72) and Bcl-11b[73–75], RORα[66], ETS1 (ref. 76) and Id2 (refs. 57,77). Deletion of GATA-3 in mature ILC2s results in loss of the expression of many critical ILC2 genes, including those encoding the T_H2 cytokines IL-5 and IL-13, as well the gene encoding IL-1RL1 (ref. 63). ETS1 is required for the emergence of bone-marrow ILC2Ps and ILC2s in the mesenteric lymph node, but lung ILC2s are present in the absence of ETS1 (ref. 76). However, ETS1-deficient lung ILC2 populations fail to expand or produce cytokines after activation with IL-25 or IL-33, which suggests that ETS1 is required for both the development of ILC2s and the function of ILC2s. Bcl-11b expression is associated specifically with ILC2s, among the ILCs, in contrast to its broad expression in T cell subsets[73,74], and it is required for ILC2 development. Bcl-11b directly regulates the transcription factor Gfi1, and in its absence ILC2s have decreased expression of known targets of Gfi1, including *Gata3* and *Il1rl1* (refs. 73,78). Interestingly, Bcl-11b-deficient ILC2s can respond to IL-33, but they produce the ILC3 cytokine IL-17A rather than IL-13, which results in the recruitment of neutrophils rather than eosinophils to the lungs[73]. IL-17 is repressed by the Bcl-11b-dependent transcription factor Gfi1, but Bcl-11b directly represses multiple ILC3-associated genes, such as *Ahr* and *Rorc*[73,78]. Therefore, Bcl-11b seems to be a transcription factor critical for maintenance of fidelity to the ILC2 fate and does so at least in part by preventing ILC3 gene expression.

Group 3 ILCs

Group 3 ILCs are a heterogeneous population that includes $CCR6^+$ LTi cells and at least two subsets of ILC3s distinguished by their expression of the NK-cell receptor NKp46 in mice[79]. NCR^+ ILC3s and NCR^- ILC3s produce IL-22 or IL-17 when activated, which indicates that they are the innate counterparts to T_H22 cells and T_H17 cells, respectively, and they seem to have functions redundant with those of the corresponding T cells[80]. ILC3s have been studied most extensively in the intestinal mucosa, where they reside in cryptopatches and isolated lymphoid follicles, as well as in the lamina propria. The NCR^+ IL-22-producing subset has a role in maintaining a healthy gut microbiota and prevents colonization by pathogenic bacteria. ILC3s have been shown to be critical for the proliferation of intestinal stem cells, which replenish the epithelial barrier in the gut after damage induced by chemotherapy[81]. However, NCR^- IL-17-producing ILC3s can also produce the cytokine GM-CSF and can have a pathogenic role in colitis[11,12]. Thus, the balance of ILC3 function is critical for the maintenance of gut homeostasis.

Transcriptional programs for group 3 ILCs

Specification to the ILC3 lineage is regulated by the transcription factor RORγt[82]. In the embryo, retinoic acid (RA) derived from maternal vitamin A is critical for the development of LTi cells and secondary lymphoid tissues[83]. RA acts upstream of RORγt, which is directly regulated by multiple RA-responsive receptors. In the adult, RA has been linked to promotion of the expression of gut-tropic-homing receptor on progenitors of ILC1s and ILC3s but not on ILC2Ps[84]. RORγt regulates multiple genes encoding products critical for ILC3 function, including *Il17a* and *Il22*, which encode the signature cytokines of this lineage. RORγt also regulates *Ahr*, which encodes aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor that is essential for T_H17 cell function[85]. AHR, despite its broad expression, is required for the survival and function of ILC3s—in particular, the production of IL-22 by NCR^+ ILC3s[86]. The ligands for AHR can be derived from food, microflora or cells, and AHR is therefore a powerful sensor of the gut microenvironment. Id2 is also required for the generation of ILC3s, and its expression precedes that of RORγt[87]. Id2 represses the transcription factor E2A, and deletion of E2A is sufficient to restore LTi cells and many secondary lymphoid tissues in $Id2^{-/-}$ mice[43]. Id2 is also required in ILC3s after RORγt is expressed, since deletion of Id2 in $ROR\gamma t^+$ ILC3s through the use of $Rorc^{Cre}Id2^{f/f}$ mice results in a substantial reduction in gut IL-22 production, susceptibility to colonization by *Citrobacter rodentium*, and fewer IL-22–producing ILC3s[88]. Id2 affects early resistance to colonization by *C. rodentium* through a pathway involving AHR and the cytokine receptor IL-23R, which suggests that RORγt, AHR, and Id2 cooperatively control the function of mature ILC3s.

The transcription factor Runx3 functions in the generation $CD8^+$ T cells in part by repressing CD4 expression[89] and has been shown to control the activation of NK cells[90]. Runx3 is also required for the development of ILC1s and ILC3s but is not required for the generation of ILC2s[91]. Runx3 binds directly to *Rorc* (which encodes RORγt) and is required for optimal RORγt expression, which places Runx3 upstream of RORγt and suggests that it might be a factor critical for ILC3 specification. LTi cells are present in $Vav^{Cre}Runx3^{f/f}$ mice (which lack Runx3 in all hematopoietic cells), despite their reduced expression of RORγt. Runx3 is required for *Ahr* transcription, but it might regulate *Ahr* indirectly through regulation of RORγt[91]. GATA-3 is also essential for ILC3 development, and GATA-3 continues to have a critical role in mature ILC3s, in which it regulates expression of *Cd127* and controls the interplay between RORγt and T-bet in NCR^+ ILC3s[70,92,93].

Progenitors of ILCs

Over the past 3 years, many multipotent progenitor cells have been identified that are able to generate ILCs. Most ILCs, including NK cells, arise from CLPs or their lymphoid-primed multipotent progenitor (LMPP) precursors, which are the progenitors of adaptive lymphoid cells[94]. Indeed CLPs isolated from fetal liver and adult bone marrow are able to generate ILCs *in vitro* and *in vivo*[16,65,95]. Fate-mapping experiments with $Il7ra^{Cre}Rosa26^{Stop\text{-}flox\text{-}YFP}$ mice (which express yellow fluorescent protein (YFP) in the progeny of all $CD127^+$ cells) have revealed that NK cells are the progeny of $CD127^+$ cells, consistent with the hypothesis that LMPPs or CLPs are the source of these cells[96]. All helper ILCs (ILC1–ILC3) are $CD127^+$ and therefore should be labeled in $Il7ra^{Cre}Rosa26^{Stop\text{-}flox\text{-}YFP}$ mice regardless of their precursor origin.

However, a subset of ILCs and NK cells can be traced back to CLPs because they have a history of expression of the *Rag1* recombinase-encoding gene, as assessed in *Rag1*^{Cre}*Rosa26*$^{Stop\text{-}flox\text{-}tdRFP}$ fate-reporter mice (which express the red fluorescent protein tdRFP in the progeny of *Rag1*$^{+}$ cells)[97]. Nonetheless, greater than 50% of the ILCs examined were not labeled in *Rag1*^{Cre}*Rosa26*$^{Stop\text{-}flox\text{-}tdRFP}$ mice and therefore could have been generated from uncharacterized non-CLP progenitors or from LMPPs-CLPs that never expressed *Rag1*^{Cre}.

Despite the possibility of some alternative ILC developmental programs, multipotent but progressively restricted progenitors of NK cells and ILC have been identified, which has revealed a developmental hierarchy from CLPs to lineage-restricted cells that partially mirrors that of T cell development[25,94]. Two populations of cells, the α-lymphoid progenitors (αLPs) and the early innate lymphoid progenitor (EILPs), both of which are phenotypically related to CLPs, can generate NK cells and all helper ILCs but lack the potential to differentiate into B lymphocytes or T lymphocytes[98,99]. The αLP population differs from CLPs by its expression of the integrin $\alpha_4\beta_7$ and the chemokine receptor CXCR6 and the absence of Flt3. The generation of αLPs requires NFIL3, whose expression initiates in CLPs under the control of IL-7 signaling[99,100]. NFIL3 regulates, at least in part, expression of the transcription factor TOX, which is required for the development of NK cells and ILCs[100]. NFIL3 also promotes the expression of Id2, probably by acting together with ETS1, whose expression is also initiated in LMPPs-CLPs and their immediate progeny[44,46,48]. Single-cell gene-expression profiles of fetal liver αLPs at embryonic day 15 (E15) has revealed a lack of transcripts encoding most lineage-associated transcription factors and cytokines and low but detectable expression of Id2 in some of the cells[101], consistent with the expression of an *Id2*^{GFP} reporter (expression of green fluorescent protein (GFP) from *Id2*) in only a subset of these cells[99]. The transcriptome of EILPs is also very much related to that of CLPs but differs from that of CLPs and αLPs by its lack of CD127 expression[98]. EILPs have been identified through the use of a *Tcf7*^{GFP} reporter (with expression of TCF-1 (encoded by *Tcf7*) linked to GFP), and they express transcripts of *Nfil3* and *Id2*; however, Id2 is not required for development of this progenitor cell[98]. EILPs do not express mRNA encoding any ILC-lineage-specifying transcription factor, such as GATA-3, RORγt, T-bet or Eomes. Whether EILPs represent a distinct IL-7-independent pathway for innate lymphoid differentiation or whether the progeny of CLPs transiently downregulate CD127 expression en route to becoming more-mature ILC progenitors remains to be determined. Fate mapping of EILPs through the use of Cre recombinase expressed from the gene encoding CD127 (*Il7ra*^{Cre}) might reveal whether they are derived from the LMPP or CLP pathway or whether they represent an alternative pathway for the generation of ILCs.

Restriction of EILPs or αLPs to specific ILC lineages seems to be a progressive process, with the cells first losing NK-cell-developmental potential, followed by the loss of LTi-cell potential and, finally, restriction to the ILC1, ILC2 or ILC3 subset (**Fig. 1**). Multipotent progenitors with each of these restricted developmental options have been identified. A common helper ILC progenitor (CHILP) has been identified that lacks expression of Flt3 and CD25 but expresses IL-7Rα and $\alpha_4\beta_7$ and differs from αLPs in that it expresses *Id2* (ref. 16). CHILPs give rise to all ILCs, including LTi cells, but they fail to generate NK cells. CHILPs are present in adult bone marrow and fetal liver and, as a population, they do not have substantial expression of GATA-3, T-bet, RORγt or RORα[16,101]. However, the CHILP population is heterogeneous and includes a subset of cells that express PLZF, a transcription factor that has been associated with NKT cells[65]. PLZF^{+} CHILPs are a more-differentiated subset of CHILPs that express GATA-3 and have lost the ability to generate LTi cells, hence their designation as a 'helper ILC progenitor' (ILCP). Single-cell gene-expression profiling of fetal ILCPs has revealed co-expression of mRNA encoding transcription factors and cytokines associated with ILC1s, ILC2s and ILC3s, a process referred to as 'lineage priming', which in multipotent progenitors is associated with precursors undergoing lineage-fate specification[101]. Although PLZF marks the ILCP population, it is not essential for the generation of these cells or for the differentiation of most helper ILCs, with the exception of ILC2s and liver ILC1s[65]. Two factors are known to be essential for the generation of CHILPs: NFIL3, which probably acts at the EILP or αLP stage; and ETS1 (refs. 76,100). Interestingly, ETS1 is not absolutely required for the development of CHILPs; instead, the fitness of these cells is compromised in the absence of ETS1, such that they cannot survive in the presence of adaptive lymphoid cells. This compromised competitiveness of ETS1-deficient CHILPs suggests that CHILPs and some adaptive lymphoid cells might occupy the same limiting bone-marrow niche. Indeed, competition between regulatory T cells and NK cells or other ILCs for IL-2 has been reported[102,103]. TOX deficiency also affects the fitness of CHILPs, and the few CHILPs present in *Tox*$^{-/-}$ mice have lower expression of GATA-3, TCF1 and PLZF than that of wild-type CHILPs, which suggests that TOX is essential for development of ILCPs[47]. GATA-3 might also affect ILCPs, since all of the downstream progeny of these cells are dependent on GATA-3 expression[70]. The mechanisms that control the induction of these specific programs and the loss of alternative lineage potential are just starting to be revealed and probably involve many of the same factors that regulate the differentiation of T cells into the effector fate. For example, the interplay of various proteins of the STAT family, which are activated in response to cytokine signaling, as well as their effect on chromatin structure, might be a critical factor for differentiation of the innate lymphoid lineages[2,104]. However, key differences between the innate-lymphoid-cell-differentiation program and T cell–differentiation program exist, such as the requirement for NFIL3 and Id2.

Fetal versus adult innate lymphoid progenitors

The differentiation of CLPs into lineage-restricted ILCs seems to follow a similar trajectory in the embryo and the adult. That is, EILPs-αLPs, CHILPs and ILCPs can be identified in primary hematopoietic organs in both embryos and adults, and these cells have similar phenotypes and developmental options. However, a published study has revealed that the majority of peripheral helper ILCs are tissue-resident cells, which raises the possibility that many ILCs are derived from early waves of hematopoiesis. Studies of parabiotic mice have demonstrated that there is minimal replacement of tissue helper ILCs by circulating hematopoietic cells over the course of 4–6 weeks (ref. 105). There are minor exceptions to this rule; inflammation promotes the population expansion of tissue-resident lung ILC2s but also allows the recruitment of a small population of donor-derived ILC2s (as measured by the proportion of chimerism). While 4–6 weeks is only about 10% of a mouse's lifespan, these experiments raise the possibility that adult hematopoietic-compartment-derived ILCs might serve as a safeguard in situations of stress rather than functioning as the source of tissue helper ILCs. In contrast to the helper ILC lineages, NK cells are equilibrated rapidly in parabiotic mice, which indicates that NK cells move freely from blood to tissue and are renewed continuously from adult hematopoietic progenitor cells, a finding consistent with published studies[106]. Tissue-resident NK cells, which are most convincingly demonstrated in the salivary glands[22], act like helper ILCs in these parabiotic mice, which indicates that they are truly tissue resident and are distinct from conventional NK cells[105].

The slow turnover of helper ILCs in parabiotic mice raises the possibility that many peripheral ILCs arise during fetal or neonatal life. Which fetal progenitor cells seed peripheral tissues to generate tissue-resident ILCs? A cell-type-restricted helper ILC progenitor has been identified in the intestinal tissue of fetal mice at E15.5 that seems to arise from lineage-marker-negative progenitors that are already present in the intestine by E13.5, at nearly the same time that CLPs arise in the fetal liver[107]. In this study, *Arg1*^{YFP} reporter mice (which express YFP from the gene encoding arginase 1) were used to track potential progenitors of ILC2s, which were the only arginase-1-expressing ILCs in the adult mice[107,108]. *Arg1*$^{YFP+}$ fetal ILC progenitors isolated from the intestinal track at E15.5 gave rise to all helper ILC lineages *in vitro*; however, the majority of these cells appeared to be lineage restricted and gave rise to only one lineage. A small subset of cells that lacked lineage markers gave rise to more than one ILC lineage, and cells with this phenotype were the majority of intestinal *Arg1*$^{YFP+}$ ILCs at E13.5. Thus, multipotent or lineage-restricted progenitors but not highly differentiated ILC progenitors might seed peripheral tissues very early in embryonic development, perhaps even arising concomitant with CLPs. These ILC progenitors could maintain ILCs through population expansion in the absence of any catastrophic events such as chemotherapy, which would lead to the loss of these cells. This situation might parallel that of other tissue-resident cells such as macrophages, some of which develop from yolk-sac progenitor cells before definitive hematopoiesis[109]. Therefore, while adult bone-marrow progenitor cells have the ability to generate ILCs, there is an open question about whether and under what conditions these cells contribute substantially to the number of peripheral ILCs.

Conclusions

The past 8 years have witnessed an explosion in the identification of the ILC types in both mice and humans, which has revealed an innate developmental program with striking parallel to that of T lymphocytes. Controversy still exists over the identity of some cells, such as tissue-resident NK cells and ILC1s. It might be useful to consider whether this particular distinction should be based on developmental history, such as whether the cells arise from ILCPs or NKPs, or on function, such as whether the cells have cytotoxic potential or produce specific cytokines, or whether they are tissue-resident cells[25]. Many essential transcriptional regulators of the innate-lymphoid-cell-differentiation program have also been identified. However, the molecular basis of lineage restriction remains unclear. How do EILPs-αLPs, CHILPs or ILCPs lose the ability to differentiate into adaptive lymphocytes, NK cells or LTi cells, respectively? How do lineage-specific gene-expression programs become established? Full appreciation of the stability of specific lineage fates is also lacking. NCR$^+$ ILC3s can differentiate into ILC1s ('ex-ILC3s') under some conditions, and decreased expression of single transcription factors can affect lineage function and possibly lineage fate, as has been observed in Bcl-11b- and Gfi1-deficient ILC2s[73,78]. Can the function of these lineage-restricted cells be diverted under any physiological condition to protect against invading pathogens? While understanding of the program of ILC development has been emerging, there is an unresolved question about when ILC progenitors seed peripheral tissues and whether the tissue-seeding cells or the cells that maintain ILCs are multipotent or lineage restricted. Moreover, although multipotent ILC progenitors can be identified in the bone marrow, it is possible that tissues harbor multipotent cells that differentiate into specific ILC lineages, as has been proposed in humans[110]. Indeed, no intermediates in the ILC1 or ILC3 pathway have been identified in the bone marrow, possibly because this is not the site in which these cells differentiate. Identifying the intermediates in these developmental programs and their site of differentiation will allow greater insight into how to control these cells in situations of disease or for therapeutic purposes. The next few years will probably be exciting ones for ILC research as the answer to these questions unfold.

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