

Innate lymphoid cell development

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Abstract

Innate lymphoid cells (ILCs) are a heterogeneous family of lymphocytes that lack conventional antigen receptors and play roles in tissue homeostasis and immune responses. Based on their transcriptional and functional properties, ILCs are classified into three main groups: group 1 ILCs (ILC1s and NK cells), group 2 ILCs (ILC2s), and group 3 ILCs (ILC3s). The developmental origins of ILCs remain an area of active investigation. Multiple progenitor populations contribute to their differentiation, including common innate lymphoid progenitors (CILPs), ILC-restricted progenitors, and IL-18 receptor-positive progenitors. Recent studies suggest that ILCs can arise from diverse pathways beyond BM-derived progenitors, including extramedullary sites such as the fetal lung and thymus. Thymic-derived ILC2s have been identified, suggesting an alternative route of development that challenges the traditional BM-centric model. This commentary discusses the current understanding of ILC development, emphasizing the contributions of BM progenitors, tissue-resident precursors, and alternative developmental pathways. Unraveling the regulatory mechanisms governing ILC differentiation will provide critical insights into their roles in immune surveillance and tissue-specific immunity.

Introduction

Innate lymphoid cells (ILCs) are a diverse family of lymphocytes that lack conventional lineage markers and antigen receptors [1]. ILCs are divided into three groups based on their cytokine profiles and developmental regulators: group 1 ILCs (including NK cells and ILC1s) [2], group 2 ILCs (ILC2s) [3] and group 3 (ILC3s) [4,5]. Because of their capacity to produce large amounts of cytokines, ILC1, -2 and -3 are often referred to as helper-like ILCs.

NK cells and helper-like ILCs exhibit distinct developmental pathways. NK cells primarily develop from Lin[−]CD122⁺ NK progenitors in the bone marrow (BM) and circulate continuously [6]. In contrast, helper-like ILCs are mostly tissue resident cells that develop early in life and turn over slowly in adulthood [7]. ILCs and NK cells may play roles in tissue remodeling and immune defense. The replenishment of ILCs in some tissues suggests continuous recruitment throughout adult life [8], potentially driven by rare seeding of bone marrow committed ILC precursors (ILCPs) followed by local “ILC-poiesis.” These complementary mechanisms likely cooperate to sustain ILC homeostasis in peripheral tissues of adult organisms [7].

Multiple progenitor populations have been proposed for helper-like ILCs, including common innate lymphoid progenitors (CILPs), ILC-restricted progenitors, and IL-18 receptor-positive progenitors [9-11]. Additionally, there is evidence for a thymic-dependent pathway, particularly for ILC2s, though its role remains controversial [12,13]. The heterogeneity in progenitor populations and tissue-specific cues influencing ILCs differentiation add complexity to their developmental trajectory.

Bone marrow derived progenitors, such as CILP, play a key role in ILC development in stem cell transplantation. However, contribution of BM progenitors to ILC development during early

life remains unclear [14,15]. For instance, while lung ILC2s are predominantly tissue-resident, growing evidence suggest that a small fraction of ILC2s in the lung may develop in adulthood [16]. To what degree BM-derived progenitors contribute to the maintenance and replenishment of ILC populations in adulthood remains to be determined. We further discuss the complexity of ILC development involving multiple progenitor populations below.

ILC Development from BM ILCPs

ILCs, like T and B cells, arise from all lymphoid progenitors (ALPs), which include IL7R α -expressing lymphoid-primed multipotent progenitors (LMPPs) and common lymphoid progenitors (CLPs) [7]. Early innate lymphoid progenitors (EILPs) express key transcription factors Nfil3, Tcf-1, Id2, and PLZF [17,18]. EILPs initially retain limited potential for dendritic cell differentiation but later commit exclusively to NK cell and ILC lineages.

Committed EILPs give rise to ILCPs, which serve as precursors to helper ILC subsets while retaining NK potential. TCF-1 and ID2 transcription factors regulate the differentiation of EILPs into ILCPs [18]. The loss of ID2 or TCF-1 leads to the absence of ILCPs, highlighting their importance in ILC lineage commitment. Common helper-like innate lymphoid progenitors (CHILPs) emerge as intermediates, giving rise to both lymphoid tissue inducer (LTi) cells and ILCPs, which are restricted to ILC1, ILC2, and ILC3 and lack NK potential [19].

Another key progenitor stage, the α 4 β 7-expressing lymphoid progenitors (α LPs), also contributes to ILC differentiation [18]. These cells are subdivided into α LP1 and α LP2, with α LP1 serving as an intermediate between CLPs and α LP2. The differentiation potential of fetal versus adult α LP2s differs, influencing the composition of ILC subsets in different developmental stages. Notably, fetal α LPs efficiently generate LTi cells essential for lymphoid tissue formation, while adult counterparts are more restricted in their lineage potential [18].

In humans, multipotent ILC progenitors have been identified in peripheral blood and fetal liver, characterized by surface markers such as Lin[−] CD34⁺ CD127⁺ IL3RA⁺. These progenitors generate NK cells, ILC1s, and ILC2s, but not ILC3s. Lineage-biased precursors also exist in the adult BM, including ILC2-restricted progenitors and immature ILC1-like cells [20].

Recent single-cell analysis has revealed greater heterogeneity within BM ILC progenitors. These studies have identified distinct ILC3 subclusters, including fetal lymphoid tissue inducer (LTi) cells, adult CCR6⁺ LTi-like ILC3s, NKp46⁺ ILC3s, NKp46[−]CCR6[−] ILC3s (double-negative ILC3s), and T-bet⁺CCR6⁺ ILC3s, each guided by unique transcriptional programs.

Bcl11b⁺ ILC3 progenitors mark a branch point in ILC development, separating ILC3 differentiation from ILC1, ILC2, and NK cell pathways [21]. This branching highlights the adaptability of ILC progenitors to specific microenvironments and underscores the importance of local cues in shaping ILC identity. A subset of Bcl11b⁺Gata3^{lo} cells exhibited Rorc-Kat expression, indicating a propensity for ILC3 differentiation. These cells represent the earliest committed ILC3 progenitors in the bone marrow, suggesting that already-committed bone-marrow-derived ILC3 progenitors may populate mucosal tissues [22].

Key transcription factors such as T-bet and ROR γ t guide ILC3 differentiation. These subclusters share core ILC3 signatures, such as the production of IL-17 and IL-22, but differ in their developmental origins and functions [19,22]. Integrative analysis of scRNA-seq data suggests a model where ILC3 differentiation is governed by a balance of lineage-defining transcription factors and environmental cues. These findings underscore the importance of local cues in shaping ILC identity and suggest that modulating transcriptional regulators like T-bet or ROR γ t could offer therapeutic avenues for fine-tuning tissue-specific immune responses mediated by ILC3s, particularly in inflammatory diseases of the gut.

We have generated a ROR α lineage tracer mouse by crossing Rora-IRES-Cre mice with R26^{-flSTOP^{fl}}-EYFP mice. Since CHILPs (common innate lymphoid progenitors) in the BM express ROR α [11], ILCs derived from these progenitors are expected to be irreversibly marked by YFP (yellow fluorescent protein). Flow cytometry analyses showed that almost 90% of ILC2s are YFP⁺ whereas only 50% of ILC1s in the liver, and about 60% of ILC3s in the small intestine express YFP. Therefore, not all ILCs originate from ROR α ⁺ CHILPs, and alternative pathways of ILC development likely exist [11].

While BM-derived progenitors are traditionally considered the primary source of ILCs, the significance of peripheral tissue-resident ILCPs (committed ILC precursors) cannot be overstated. Peripheral tissues, including the liver, lymph nodes, lung, intestine, and skin, harbor dynamic populations of ILCPs characterized by high turnover rates and multi-lineage potential. These progenitors differ from their BM counterparts in their ability to recirculate through the blood, as demonstrated by parabiosis studies. This mobility allows them to seed distant tissues and respond rapidly to local inflammatory signals. The presence of ILCPs in peripheral tissues likely complements the limited replenishment capacity of tissue-resident ILCs in adulthood, ensuring sustained ILC homeostasis. Understanding the interplay between BM-derived and peripheral ILCPs is crucial for deciphering how ILC populations are maintained throughout life and how they contribute to tissue-specific immune responses.

ILCPs in Peripheral Tissues

Peripheral tissues, including the spleen, lymph nodes, liver, lung, intestine, and skin, harbor ILC progenitors. They are dynamic, characterized by high turnover rates and the capacity to differentiate into all major ILC subsets (ILC1, ILC2, and ILC3) in response to local environmental signals. Parabiosis studies showed that peripheral ILC progenitors are highly mobile, recirculating through the blood, which distinguishes them from the more stationary, tissue-resident mature ILCs [23,24].

IL-18R α ⁺ progenitors are found in both the BM and peripheral tissues. In the BM, IL-7R α ⁺IL-18R α ⁺ ILC progenitors have demonstrated multi-lineage potential, capable of giving rise to ILC1s, ILC2s, ILC3s, and even NK cells [25]. Lin[−]Thy1⁺CD127⁺IL-18R α ⁺ progenitors in the lung, which are phenotypically similar to the BM counterparts, are enriched in neonatal lungs, implying a critical role for the neonatal lung in early ILC2 development. Interestingly, these progenitors display low expression of GATA3 and CD25, and minimal production of ILC2-related cytokines, indicating an immature state. While they proliferate upon stimulation with IL-18 or papain *in vivo*, they fail to express key effector cytokines like IL-5 or IL-13 [11,26].

This highlights the need for further signals from the lung microenvironment to drive full ILC2 maturation. Moreover, circulating IL-18R α +PD1+PLZF+Tcf7+ progenitors, originating from the BM or inflamed tissues, can migrate to the lung during infection and contribute to the generation of effector ILC2 subsets [27]. This raises questions about the relative contributions of *in situ* differentiation versus recruitment from other sites in shaping the ILC2 landscape during inflammation.

Recent studies have illuminated the presence and characteristics of ILCPs in the fetal intestine, revealing their significance in early immune system development. A pivotal discovery was the identification of Arginase 1 (Arg1)-expressing fetal transitional ILC precursors in the mouse intestine, detected as early as embryonic day 13.5 (E13.5). These precursors exhibit the remarkable ability to differentiate into all ILCs indicating that the fetal intestine serves as a vital site for extramedullary ILC development. This finding challenges the traditional view that the fetal liver is the primary source of ILC progenitors [28].

Further investigations have revealed a heterogeneity among ILC progenitors in the fetal intestine. Single-cell RNA sequencing analyses have identified progenitors with CLP-like signatures, as well as IL-7R α + α 4 β 7+PLZF+ ILCPs. This complexity highlights a layered ontogeny of ILCs within this tissue [28]. In humans, studies of fetal tissues have similarly uncovered CD34+ lymphoid progenitors in the fetal intestine, which express CD127, suggesting their potential to give rise to ILCs [29,30].

These findings collectively underscore the critical role of the fetal intestine in shaping the early immune landscape, paving the way for future research into the mechanisms underlying ILC differentiation and their implications for immune health.

Recent research has revealed that the thymus plays a significant role in generating ILC2s [7,31]. Studies indicate that ILC2s can arise from early thymic progenitors (ETPs) and immature thymocytes. Using proximal Lck-Cre reporter mice, it has been indicated that a significant proportion of lung ILC2s may originate from the thymus. This is supported by the finding that ectopic Id1 expression leads to a massive overproduction of ILC2s that depends on the thymus [32,33].

A key finding is that some lung ILC2s undergo TCR γ locus recombination, a process typically associated with T cell development, further supporting this novel perspective on ILC2 development [31,32]. This, along with the presence of out-of-frame gene rearrangements, suggests that ILC2s may be “salvaged” from neonatal thymocytes that fail to mature into $\gamma\delta$ T cells. The thymic microenvironment supports ILC2 development. ILC2s are located within the medulla of the thymus, and the thymus produces cytokines including IL-25 and IL-33, which are essential for activating ILC2 progenitors [32,34].

Moreover, thymus-derived ILC2s can exit the organ, circulate through the blood, and home to peripheral tissues, particularly the lung [35]. These findings collectively highlight the plasticity of lymphoid progenitors and their ability to adapt to distinct microenvironments during development. The thymic pathway of ILC2 development complements the bone marrow-derived pathway, expanding our understanding of ILC2 ontogeny and the role of the thymus in innate immune cell development.

A large proportion of ILCs in the bone marrow belong to the ILC2 subset. Evidence suggests that ILCs can develop neonatally from thymic precursors and subsequently become tissue-resident. The remarkably slow turnover of these cells argues against their function as a precursor pool for peripheral ILC populations. Studies have shown that the self-renewal and maintenance of ILCs are facilitated by a pre-existing pool of tissue-resident innate lymphoid cell precursors (ILCPs), particularly under inflammatory conditions.

The thymic pathway may serve as a reservoir for replenishing ILC2 populations in peripheral tissues during inflammation or injury, complementing the more localized development observed in other pathways. Immature IL-18 receptor-expressing BM ILCPs contribute to the seeding of BM-derived ILC2s in the lung, where they give rise to the full phenotypic spectrum of ILC2s.

Recent research also has shown TCR γ gene rearrangement in CD127+ NK cells, which are likely ILC1s [36]. Therefore, the thymic pathway of ILC development is unlikely limited to ILC2s.

Conclusion

In conclusion, ILC development is a multifaceted process involving diverse pathways beyond the bone marrow. IL-18R α + progenitors found in both bone marrow and peripheral tissues like the lung exhibit plasticity, differentiating into various ILC subsets under the influence of local cues. The fetal intestine contributes unique progenitors to this landscape, while the thymus might emerge as an alternative site for ILC2 development. This understanding highlights the adaptability of ILCs to specific microenvironments and offers potential therapeutic targets for modulating tissue-specific immune responses.

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