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The Development of Adult Innate Lymphoid Cells

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Abstract

Innate lymphoid cells (ILC) are a specialized family of effector lymphocytes that transcriptionally and functionally mirror effector subsets of T cells, but differ from T cells in that they lack clonally-distributed adaptive antigen receptors. Our understanding of this family of lymphocytes is still in its infancy. In this review, we summarize current understanding and discuss recent insights into the cellular and molecular events that occur during early ILC development in adult mice. We discuss how these events overlap and diverge with the early development of adaptive T cells, and how they may influence the molecular and functional properties of mature ILC.

Introduction

Despite the lack of adaptive antigen receptors, innate lymphoid cells (ILC) transcriptionally and functionally parallel T cells [1-4]. Analogous to T cells, adult ILC can be broadly divided into four lineages: conventional NK cells express T-bet and Eomes and exert cytotoxic function; group-1 innate lymphoid cells (ILC1) express T-bet and secrete IFN- γ ; group-2 innate lymphoid cells (ILC2) express GATA-3 and produce type-2 cytokines such as IL-5 and IL-13; group-3 innate lymphoid cells (ILC3) express ROR γ t and make IL-17 and IL-22 (**Figure 1**) [5]. Lymphoid tissue-inducer cells (LTi) are also innate lymphocytes, whose development is likely elicited by unique microenvironmental signals in fetal life; these cells are not further discussed here. Recent studies have revealed interesting similarities and differences between the development and function of innate lymphoid cells and T cells. In this Review, we discuss the recent advances that illuminate our perspective on the formation of an innate lymphoid cell.

Property of bone marrow innate lymphoid cell-committed progenitors

Unlike T cells that mature in the thymus, early stages of ILC specification and commitment occur in the bone marrow (BM) [2,4]. ILC derive from BM lymphoid progenitors [6-8], and

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several ILC-committed progenitors have been recently identified in the BM. A developmental history of expression of the transcription factor PLZF was observed in several cytokine-producing helper ILC subsets, but not in most conventional NK cells [9,10]. BM PLZF-expressing progenitors efficiently matured into several cytokine-producing helper ILC subsets, but not into conventional DX5⁺ NK cells or CD4⁺ ILC3 [10]. A similar subset of BM progenitor cells, termed common helper innate lymphoid cell progenitors (CHILP), consists of both PLZF⁺ and PLZF⁻ progenitors. CHILP give rise to all helper ILC subsets, but not to conventional NK cells [11]. BM CD122⁺NK1.1⁻ NK progenitors (NKP) may develop into mature NK cells [12], although their capability to generate other ILC subsets has not been assessed. α -lymphoid progenitors [8] are a heterogeneous subset in adult mice that contain CHILP, ILC2 cell precursors (ILC2p) [13,14], ILC3 cell precursors, and some progenitors with residual T cell potential [15]. Among them, CXCR6⁺ α -LP cells may give rise to both conventional NK cells as well as helper ILC [8,15,16]. However, the rarity of CXCR6⁺ α -LP suggests that other physiological early ILC progenitors likely exist [15]. Upregulation of TCF-1 expression identifies a subset of early innate lymphoid cell progenitors (EILP) in the BM [17]. EILP lack efficient T/B cell potentials, but develop into conventional NK cells and various helper ILC subsets *in vivo*, indicating that they might be the earliest identified ILC progenitors [17]. Similar to CLP, EILP also possess dendritic cell (DC) potential. Whether EILP are obligate precursors to all ILC subsets is unknown, but the identification of these ILC-committed progenitors provides a base to decipher the gene and molecular regulatory events that specify the ILC fate.

Transcription factors that direct both thymic early T cell development and BM early ILC development

Interestingly, multiple studies indicate that ILC express many transcription factors characteristic of the adaptive T cell lineage. ILC express the core T-lineage transcriptional regulators, such as TCF-1, GATA-3 and Bcl11b. They also express many T cell receptor (TCR) associated signaling molecules such as LAT, LCK, ITK, ICOS and PD-1 [18-23], and similar levels of cytokine and chemokine receptors as their adaptive counterparts. Genome-wide transcriptional analysis indicates that ILC are more similar to T cells than other leukocytes [24]. The transcriptional and functional similarities between mature populations of ILC and T cells raise the question of whether similar early developmental events at distinct anatomical sites underlie the functional and molecular similarities in mature T cells and ILC (reviewed in [2]).

Transcriptional regulators implicated in thymic early T cell development also play important roles in the development of innate lymphoid cells. T cell factor-1 (TCF-1), a sequence-specific HMG protein, is highly expressed in thymus where it drives the earliest stages of T cell lineage specification and differentiation [25-29]. TCF-1, however, is also required for the generation of all known adult ILC subsets as well as their bone marrow precursors [17,30,31]. Ectopic expression of TCF-1 in BM multi-potent LSK (Lin⁻Sca-1⁺Kit⁺) elicits the expression of many genes shared by T lineage cells and ILC, such as the transcription factors GATA-3, ETS-1 and Bcl11b [27,30]. GATA-3 is another transcriptional driver of early T cell development. Recent work indicated that GATA-3 is also highly expressed in

BM PLZF⁺CHILP [10,11]. GATA-3 deficiency abolished the development of IL7R α ⁺ helper-ILC, fetal LTi, and BM CHILP [13,32-35]. GATA-3 is dispensable for the generation of conventional splenic NK cells, but GATA-3^{-/-} NK cells are inefficient in producing IFN- γ , indicating GATA-3 expression during ontogeny is also important to confer functional integrity of conventional NK cells [36]. ETS-1 is required for thymic TCR β -selection, but is also expressed in NK cells, and is required for NK cell development [37]. ETS-1^{-/-} mice have markedly reduced number of BM NK cells as well as NK cell progenitors termed refined-purity NKP (rNKP) [38], indicating that ETS-1 acts early in the BM [39]. ETS-1 is also highly expressed in ILC2 [40], although its role in non-NK ILC subsets remains to be deciphered. Bcl11b, another direct target gene of TCF-1 [27], was identified as a T cell-lineage commitment factor that can repress innate cell fates in T lineage-committed cells [41-43]. However, recent work shows that Bcl11b is also expressed in ILC2 and maintains ILC2 identity by preventing them from becoming IL-17-producing ILC3-like cells, indicating a broader role of Bcl11b in maintaining identity of both ILC and T cell lineages [44-47]. Ectopic expression of TCF-1 in BM LSK also induces the expression of many other genes shared by ILC and T lineages, such as the TCR-associated signaling molecules LAT, LCK, ITK and ICOS [27,30,48-50]. These molecules mediate TCR-signaling, and have also been recently implicated in the activation of innate lymphoid cells [18-20,51,52]. Hence, early BM ILC development may share key gene regulatory circuits with early thymic T cell development, which might underlie the striking function and gene expression similarity between innate lymphoid cells and adaptive T cells.

Possible mechanisms that separate innate lymphoid cell fate from the T cell fate

Recent results also provide insights into some differences between early ILC development and early T cell development. Early T cell development requires constraint of myeloid potentials by the transcriptional repressor Hes-1 [53], whereas Hes-1 is dispensable for ILC development [30], indicating that other mechanisms may repress myeloid fates during ILC fate specification. In contrast, the bZIP transcription factor Nfil3 is required for all adult ILC development, but is dispensable for T cell development [15,54-56]. Nfil3 is expressed as early as BM common lymphoid progenitors (CLP), and its expression is further up-regulated in BM EILP [15,54-56]. Nfil3 promotes the development of all known ILC subsets, including NK cells, adult helper-ILC, and fetal LTi, as well as BM CHILP, CXCR6⁺ α -LP, and NKP [15,54-56]. The expression of Nfil3, however, is undetectable in thymic T lineage progenitors [17], indicating that the maintenance of Nfil3 expression might be important in separating ILC fate from T cell fate. Nfil3 can direct expression of Id2, the inhibitor of E proteins [57]. Id2 is highly expressed in, and is required for the generation of all known mature ILC [58,59]. A high level of Id2 expression blocks T cell development at multiple stages, indicating that Id2 might play a role in the separation between ILC fate and T cell fate [60]. However, evidence indicates that Id2 might be dispensable for the earliest stage of ILC fate specification, because Id2^{-/-} mice possess several ILC-committed precursors, including EILP, CXCR6⁺ α -LP, NKP and rNKP [11,17,46,61]. Nevertheless, Id2^{-/-} EILP have significantly increased expression of Id1 and Id3, suggesting the possibility that other Id proteins up-regulate their expression to compensate for the loss of Id2 [17]. Nfil3 can also directly bind the promoter region and control the expression of TOX, a sequence-non-specific high mobility group (HMG) protein [15]. TOX is highly expressed in BM EILP and

CHILP [17,62], and is required for the generation of all known ILC subsets, including conventional NK cells, functional lymphoid tissue inducer cells (LTi), and the known adult helper ILC subsets [62,63]. Interestingly, TOX is also highly expressed in thymocytes but is dispensable for early T lineage fate specification [64]. The zinc finger protein PLZF is another transcriptional regulator that is highly expressed in ILC progenitors but not in T lineage progenitors [10]. PLZF promotes the development of some adult helper ILC subsets, such as peripheral ILC2 and liver ILC1, but is dispensable for the generation of ILC3, fetal LTi and conventional NK cells. A transient expression of PLZF might also be important to restrain L-selectin expression in adult helper-ILC, possibly contributing to the establishment of a non-circulating, tissue-resident ILC pool at diverse sites [10]. The expression of these ILC-specific regulators during early ILC development could endow ILC with certain unique properties in later life, such as their relative abundance at mucosal barrier sites. How these ILC-unique regulators may collaborate with shared ILC/T lineage factors to specify the ILC fate awaits further investigation.

The expression of recombination-activating gene (RAG) during innate lymphoid cell development

Recombination-activating genes, Rag-1 and Rag-2, enable the diversity of adaptive lymphocytes by mediating productive V(D)J recombination. During lymphocyte development, expression of Rag genes initiates in the earliest lymphoid-biased progenitors in the BM [65,66]. ILC still develop in Rag^{-/-} mice that lack T and B lymphocytes. However, fate mapping shows that a history of RAG expression can be detected in many innate lymphoid cells, including around 50% of lung ILC2 and 25% of splenic NK cells, indicating that a large percentage of ILC derive from RAG-expressing lymphoid progenitors [7,67]. Interestingly, NK cells from RAG deficient mice display cell-intrinsic hyper-responsiveness with diminished survival and persistence during MCMV infection, indicating that RAG activity during ontogeny might promote cellular fitness of innate lymphocytes [67]. CHIP-seq analysis revealed that RAG2 co-localizes with H3Kme4 and binds at over 24,000 sites throughout the genome, but the biological importance of this extensive binding remains unknown [68]. Whether and how RAG activity influences lymphocyte biology beyond its role as a V(D)J recombinase requires further study. In addition, NK cell memory responses can still be elicited in Rag^{-/-} mice, indicating that other molecules might also contribute to some adaptive-lymphocyte-like attributes of ILC [69,70].

Possible extramedullary maturation of innate lymphoid cells

ILC are relatively abundant in non-lymphoid tissues, such as adipose tissues, liver and various mucosal barriers. The identity of BM ILC progenitors that migrate to non-lymphoid tissues, however, remains unknown. BM ILC progenitors express $\alpha 4\beta 7$, including EILP, CHILP and ILC2p, suggesting their ability to home to mucosal barrier sites [10,11,13,17]. Several extramedullary ILC precursor subsets have also been identified in mice and humans, indicating that some steps of ILC maturation might occur locally, in peripheral organs. CD34⁺Ror γ t⁺ ILC3 progenitors have been found in human tonsils and intestinal lamina propria, but not in the BM and peripheral blood, indicating that some human ILC might mature locally at mucosal barrier sites [71]. In mouse intestinal lamina propria, a more immature ILC precursor subset, identified as Lin⁻Sca-1^{lo}Kit^{int}IL-7R α ⁺Ror γ t⁻, possesses the

capability to differentiate into NKp46⁺Roryt⁻ ILC1-like cells and Roryt⁺ILC3-like cells *in vitro* [8,16]. Early hematopoietic progenitors are known to express Pattern Recognition Receptors (PRR) such as Toll-like receptors (TLR), and they display distinct proliferation and differentiation patterns in responses to different pathogen-derived stimuli [72-74]. It is thus conceivable that tissue-resident ILC progenitors might be able to orchestrate local immune and inflammatory responses via differentiation in the highly specialized micro-environments of non-lymphoid organs.

Recent work indicates possible influences of mucosal microenvironments on the generation of resident innate lymphoid cells. Interestingly, the distribution of individual ILC subsets differs at distinct mucosal barrier sites. In adult mice, ILC3 are the predominant ILC in the small intestine laminal propria, whereas ILC2 are the major lung-resident ILC [75]. The greater abundance of ILC3 in the small intestines might be partly due to the enrichment of certain dietary compounds such as Vitamin A and aryl hydrocarbon receptor (AHR) ligands. Vitamin A deficiency results in diminished ILC3 and expansion of ILC2 in the small intestines, indicating an adaptation to micronutrient deficiency that confers augmented defense against intestinal helminth [75]. Retinoic acid, a Vitamin A metabolite, also controls fetal LTi development [76]. Vitamin A metabolites may directly modulate the proliferation of mature ILC2 and ILC3 via the nuclear receptor Retinoic acid receptor- α (RAR α), but their effects on possible extramedullary mucosal-resident ILC precursors are yet to be assessed [75]. Another nuclear factor, AHR, is required for the efficient generation of intestinal Roryt⁺ ILC3 in mice [77-79]. How AHR promotes ILC3 generation, however, remains to be clarified. Mouse intestinal lamina propria and human mucosal-associated lymphoid tissues also contain a unique subset of NCR⁺ILC3, the abundance of which might be influenced by intestinal commensal bacteria [80-84]. Interestingly, although ILC3 are barely detectable in the lungs of adult mice at homeostasis, IL-17-producing ILC3 accumulate in the lungs in a mouse model of obesity [85]. It will be interesting to examine whether these ILC3 (or ILC3-like cells) are recruited from other sites, are converted from other lung-resident ILC, or develop from yet unknown mucosal-resident precursors with multi-ILC lineage potential. Whether and how the activity of tissue-resident ILC precursors contributes to the diversity of ILC responses at distinct organs, and under different pathological stresses, warrants further investigation.

Another extramedullary organ, the thymus, is the major site of T cell maturation. Some mature ILC subsets, such as NK/ILC1-like cells and CD4⁺ LTi-like cells, have been detected in mouse thymus [86,87]. More work is needed to understand whether these cells develop in the thymus, or whether they migrate there from other anatomic sites.

Concluding Remarks

The discovery of the innate lymphoid cell family has greatly expanded our knowledge of lymphocytes, and we have a lot to learn about this unique family of lymphocytes. What controllers determine the fate of ILC? How do ILC essentially differ from other innate immune cells? What endows them with certain adaptive lymphocyte-like attributes? And what confers their defining differences with adaptive lymphocytes? Understanding the

fundamental biology of ILC will eventually uncover new mechanisms of immunopathology and inform novel strategies to combat many diseases.

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Highlights

- Early BM innate lymphoid cell development shares gene regulatory circuits with early thymic T cell development.
- Several transcriptional controllers separate innate from adaptive lymphocyte fates.
- A lymphoid origin may endow innate lymphoid cells with certain attributes of adaptive immune cells.
- Extramedullary maturation may account for the diversity of innate lymphoid cells at different anatomic locations and under distinct pathologic states.

Figure 1.

The developmental pathway of innate lymphoid cells. Molecules implicated in early ILC development are shown at developmental stages where they are expressed. Molecules that are expressed by both innate lymphoid cells (ILC) precursors and T cell precursors are in black font. ILC-unique transcriptional regulators are in red font. TOX is in orange font, because it is expressed by both ILC and T lineage precursors, but is required for ILC fate specification but not for early T lineage specification.