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HIGHLIGHTS

Basic

REVIEW

ILC-poiesis: Ensuring tissue ILC differentiation at the right place and time

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Innate lymphoid cells (ILCs) represent a family of innate effector cells including NK cells, lymphoid tissue inducer (LTi) cells, and distinct ILC1, ILC2, and ILC3 subsets that produce IFN-γ, IL-5/IL-13, and IL-17A/IL-22, respectively. ILCs accumulate at mucosal sites and can promote the first-line defense against infection. ILCs are also implicated in tissue repair and can either pre-empt, or alternatively, exacerbate inflammation. Studies in mice have identified ILC precursors in fetal liver and adult BM that have diverse lineage potential. As such, these sites have been considered as the 'factories' to generate mature ILC. Here, we summarize knowledge concerning murine and human ILC development and discuss the recent identification of circulating multipotent and unipotent ILC precursors. We propose an alternative model of "ILC-poiesis", whereby blood ILC precursors migrate into tissues to complete their differentiation into mature ILC subsets under the influence of local environmental factors. Within this framework, ILC-poiesis guarantees appropriate ILC generation at the right place and the right time. We further discusss the potential applications of circulating ILC precursors for cell therapy of human disease.

Keywords: cell therapy · development · hematopoiesis · innate lymphoid cells · precursor

Murine and human innate lymphoid cell development

Innate lymphoid cells (ILCs) were initially categorized into three major groups based on their transcription factor profiles and functional outputs (reviewed in [1, 2]). Mouse and human ILC nomenclature was recently updated [3] and five distinct groups are now recognized, including cytotoxic NK cells, ILC1, ILC2, ILC3, and lymphoid tissue inducer (LTi) cells. Type 1 cytokine production (IFN-γ and TNF-α) in NK cells and ILC1 is regulated by T-BET. Expression of the transcription factor EOMES distinguishes NK cells (EOMES$^+$) from ILC1 (EOMES$^-$). ILC2 express GATA-3 and RORα and secrete the type 2 cytokines IL-5 and IL-13. ILC3 utilize RORγt to drive production of the T_H17-associated cytokines IL-17A and/or IL-22; LTi cells use this same transcription factor to promote secondary lymphoid tissue formation during the fetal period via LTβ [1, 3]. In general, ILC groups show strong similarities with functional T cell subsets (NK cells with CD8$^+$ CTL; ILC1 with CD4$^+$ Th1 cells; ILC2 with CD4$^+$ Th2 cells; and ILC3 with CD4$^+$ Th17 and CD4$^+$ Th22 cells) leading to the notion that ILCs represent 'innate' versions of differentiated CD4$^+$ and CD8$^+$ T cells of the adaptive immune system.

In humans, ILC subsets can be identified by excluding lineage (Lin) positive cells (including CD3$^+$ T cells, CD19$^+$ B cells, CD94$^+$ NK cells, and CD14$^+$ monocyte/myeloid cells) and gating on cells expressing IL-7 receptor (CD127). This Lin$^-$CD127$^+$ population includes several distinct ILC subsets (depending on the tissue origin) that can be further identified using additional markers such as CD161, CRTh2, CD117, and NKp44 (reviewed in [2]). This general gating strategy is widely used to identify human ILC1 (CD161$^+$CD117$^-$CRTh2$^-$), ILC2 (CRTH2$^+$), and ILC3 (CRTh2$^-$CD117$^+$NKp44$^{+/-}$) in various healthy and disease tissues. Nevertheless, further analysis of transcription factor expression (TBX21, EOMES, GATA3, RORC2) combined with cytokine production (IFN-γ, IL-13, IL-17A, IL-22) are usually required in

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order to unambiguously ascertain human ILC identity. While the above Lin⁻CD127⁺ gating excludes most mature human NK cells ($CD56^{dim}$ that co-express CD16 and $CD56^{Br}$ that are CD16⁻), some $CD56^{+}$ cells are present that include ILC3 and immature $CD56^{Br}$ NK cells [3].

ILC differentiation in the mouse is regulated by more than a dozen transcription factors, including *Id2*, *Nfil3*, *Zbtb16*, *Tcf7*, *Gata3*, *Ets1*, and *Tox* that drive differentiation of mature ILC subsets from common lymphoid precursors (CLP) (reviewed in [4]). Mice bearing fluorescent reporters for some of these factors allowed the identification of several distinct ILC precursors (ILCP) from fetal liver and adult BM, including α—lymphoid precursors, early ILC progenitors, and common 'helper' ILCPs [5–9]. A stepwise model of ILC differentiation in the mouse has emerged that begins with commitment of lymphoid-restricted precursors to the ILC fate, thereby generating multi-potent (able to generate two or more ILC subsets) and uni-potent (able to generate one ILC subset) ILCPs (Fig. 1). These then further differentiate into mature ILC subsets that inhabit different tissues and circulate in the blood. Still, many issues remain unanswered, particularly the precursor–product relationships between different multipotent ILCPs and the transcriptional regulators that dictate the choice of mature ILC subset fate [4].

Over the past several years, a corresponding map of human ILC development has been under construction (Fig. 1, Table 1). Earlier studies identified multipotent lymphoid-restricted progenitors in human BM with $CD34^{+}CD10^{+}CD62L^{+}$ phenotype that have the potential to give rise to B cells, T cells, NK cells, as well as monocytic and dendritic cells but are devoid of myeloid and erythroid potential [10]. Further analysis using additional markers and single cell assays identified $CD34^{+}CD38^{-}CD45RA^{+}CD90^{-}$ cells as putative human CLP equivalents that can generate B cells, T cells, and NK cells [11]. Further analysis using additional markers and single cell assays identified $CD34^{+}CD38^{-}CD45RA^{+}CD90^{-}$ cells as putative human CLP equivalents that can generate B cells, T cells, and NK cells . More recently, Canque and colleagues proposed a bipartite architecture of human lymphocyte development that segregates $CD34^{+}CD45RA^{+}$ CLP into $CD127^{-}$ early T cell precursors and $CD127^{+}$ early B cell precursors [12]. Interestingly, NK cell, ILC1, and ILC3 potential was observed in both compartments, suggesting multiple paths for human innate lymphocyte development.

Consistent with this bipartite model, committed NK precursors (NKP; Table 1) have been identified in fetal liver, fetal BM, cord blood, and adult tonsil as $CD34^{+}$ cells expressing CD45RA, CD10, and CD7 but not CD127 [13]. At the transcriptional level, human NKP express TBX21, EOMES, ID2, and GATA3 transcripts suggesting a rather advanced stage of differentiation, although protein expression for these transcription factors was not validated. Human NKP were able to generate fully differentiated cytotoxic NK cells after in vitro co-culture on OP9-DLL1 stroma in the presence of IL-2, IL-3, IL-7, IL-15, FLT3L, and SCF. In vivo transfer of cord blood NKP to irradiated immunodeficient NOD/SCID/$Il2rg^{null}$ mice generated mature NK cells 11 weeks after transplantation. Both in vitro and in vivo experiments showed that NKP lacked potential for T cell, B cell, non-NK cell ILC subsets, or myeloid lineages [13]. This study provided the first evidence for a committed lymphoid precursor restricted to the NK cell lineage in humans.

Romagnani and colleagues identified committed ILC3 precursors (ILC3P; Table 1) that were present in tonsil and intestinal lamina propria but not detected in peripheral blood, thymus, or BM [14]. These human ILC3P expressed CD34 as well as CD45RA, CD117, α4β7, and RORγt but not CD7 or CD127. Interestingly, human ILC3P shared transcriptional signatures with mature ILC3 including strong RORC, ID2, KIT, and NCR1 expressions and a subset of cells was able to produce IL-17A and IL-17F in response to pharmacological stimulation. In vitro bulk culture of human ILC3P with OP9 stroma cells in the presence of IL-7, IL-15, and FLT3L generated primarily $ROR\gamma t^{+}$ ILC3 but also $EOMES^{+}$ NK cells. Further, single cell cloning experiments showed that 20% of clones maintained both NK and ILC3 potential indicating that $CD34^{+}CD117^{+}$ ILC3P harbored bipotent ILC3/NKP. The ability of human ILC3P to generate T cells, B cells, myeloid, and other ILC subsets was not fully explored [14]. This study was the first to identify human ILC3Ps and further suggested that lymphoid tissues (tonsil) as well as nonlymphoid tissues (intestinal lamina propria) could be potential sites for ILC3 development.

An important study from Freud and colleagues identified human common ILC progenitors (CILCP; Table 1) with the $CD34^{+}CD45RA^{+}CD117^{+}IL\text{-}1R1^{+}integrin\beta7^{+}$ phenotype in secondary lymphoid tissues (tonsil and spleen) but not present in cord blood, peripheral blood, thymus, or BM [15]. Following in vitro culture on Notch ligand expressing stromal cells (OP9-DLL1) with IL-7 and FLT3, CILCP could generate cytotoxic NK cells as well as helper ILC subsets but not T cells or DC. Curiously, in vivo transfer of CILCP to immunodeficient hosts demonstrated robust NK potential but no potential for other ILC subsets. This may reflect a deficiency in certain homeostatic and/or stimulatory cytokines (IL-2, IL-7) in this context. The cell surface phenotype (CD34, CD117, CD45RA) and transcription factor profiles (RORγt) of tonsillar CILCP showed remarkable similarities to previously identified ILC3P but interestingly differed with respect to IL-1R1 expression. While the role for IL-1R ligands in stimulating CILCP was not explored in this report, this finding suggests that inflammatory signals may be 'sensed' by ILCPs (see more below). The authors further detected low level of *RORC2* transcripts in human NK cells and mature helper ILCs (with confirmation of low levels of RORγt protein) suggesting this transcription factor is not uniquely expressed by ILC3. They propose a model for human ILC development in which ILC develop through a RORγt-dependent pathway [15].

The identification of human NKP, ILC3P, and CILCP provided a series of novel landmarks on the map of human ILC development (Fig. 1, Table 1). Compared with the current mouse ILC model, the parallels are apparent and suggest a conserved, stepwise process that sequentially restricts lymphoid precursors toward distinct ILC and NK lineages. An outstanding question remains how these different multi-potent ILC and NKPs are related in terms of precursor–product relationships. In the mouse, $PLZF^{+}$ ILCP appear

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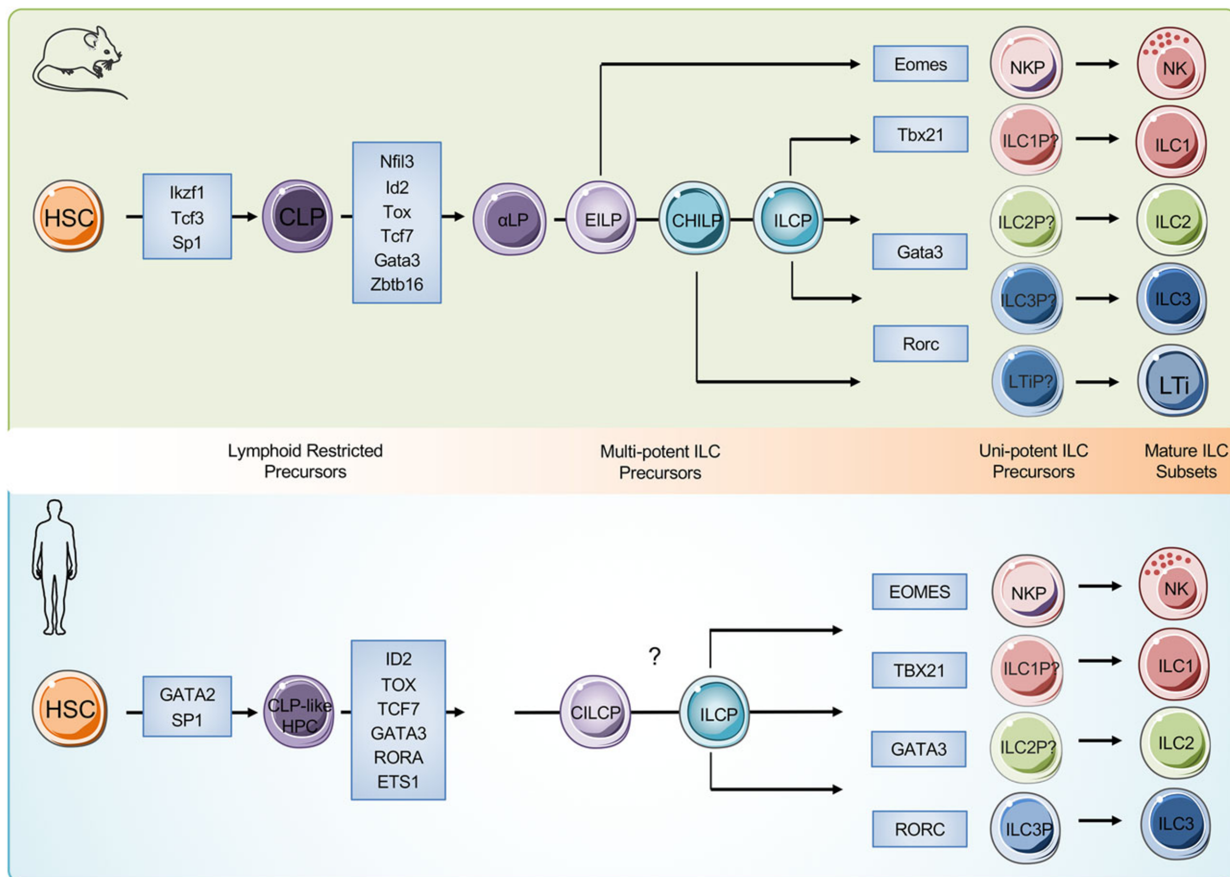

Figure 1. Parallels in mouse and human ILC development. Mouse and human ILC differentiation process proceeds in a stepwise fashion from hematopoietic stem cells (HSC) to lymphoid lineage restricted precursors, including common lymphoid progenitors (CLP) in mouse and CLP-like hematopoietic progenitor cells (HPC) in human. CLP further commit to the ILC/NK lineage via multi-potent ILCPs including α-lymphoid precursors (α-LP), early innate lymphoid precursors (EILP), common helper innate lymphoid precursors (CHILP) and ILCPs in mice and via CILCP, bipotent ILC3P/NKP, and multipotent ILCPs in humans. Subsequent differentiation generates unipotent ILCP that further generate mature tissue-resident ILC subsets. Key transcription factors for ILC development in human and mouse models are indicated.

downstream of common 'helper' ILCPs [7, 8]. Similarly, EILP are presumed to be the most upstream precursor as this population has both NK and ILC potential [6]. The transcriptional regulation that generates these different precursors from CLP and dictates their final ILC or NK cell fate is likewise unclear. Interestingly, each of the different human ILCPs so far described (NKP, ILC3P, and CILCP) expresses CD34^{+} (that is also expressed by HSC and early multilineage progenitors) and were identified in secondary lymphoid tissues but were rare or absent from the circulation. As such, it remains unclear how these CD34^{+} ILCP are developmentally related to mature ILC subsets that reside primarily in mucosal tissues.

Where do ILCs develop?

The fetal liver and BM are generally considered as the 'factories' where the various ILC subsets found in embryonic and adult tissues, respectively, are generated. This concept is in part supported by studies in mice where multipotent ILCPs and immature ILC2 have been discovered [16]. Another study in mice has identified immature ILCs in fetal and adult intestine [17] suggesting that sites other than fetal liver and BM may be permissive for ILC differentiation. Earlier studies in mice detected NKP outside the BM (in spleen, blood, and lymph nodes) raising the possibility that lineage committed lymphoid precursors could exit the BM and complete their differentiation in the periphery [4]. An analogous model for ILC differentiation would predict the existence of circulating ILCPs that could then give rise to different ILC subsets in tissues.

In a recent report [18], we found evidence for a novel circulating ILC subset that had the characteristics of human ILCPs. Lineage^{-}CD127^{+}CD117^{+} cells have been long considered as ILC3 since tonsillar ILC3 have precisely this phenotype [19]. In contrast, we found that peripheral blood CD7^{+}CD127^{+}CD117^{+} cells were not able to produce IL-17A or IL-22 following stimulation and expressed low levels (if any) of RORγt protein. In contrast, cultured peripheral blood CD7^{+}CD127^{+}CD117^{+} cells gave rise to both mature cytotoxic NK and helper ILC subsets (expressing the appropriate transcription factors and cytokine outputs) but

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Table 1. Phenotypic markers of the different subsets of human lymphoid precursors

		HSC	CLP-like cells	CILCP	ILCP	NKP	ILC3P
Distribution		BM, blood	BM	Tonsil	Fetal liver, blood, tonsil, lung	BM, cord blood	Tonsil, intestinal lamina propria
Surface molecules	CD34	+	+	+	–	+	+
	CD38	–	–	Nd	nd	+	nd
	CD45RA	–	+	+	+	+	+
	CD90	+	Int	nd	nd	nd	nd
	CD10	–	–	nd	nd	+	+/–
	CD7	–	+	nd	+	+	+/–
	CD127	–	–	+/–	+	–	–
	CD117	–	nd	+	+	+/–	+
	CD161	–	nd	+	nd	–	+
	IL-1R1	–	nd	+	+	nd	+/–
	Integrinβ7	–	nd	+	nd	nd	+
Transcription factors (protein)	EOMES	nd	nd	nd	–	nd	–
	T-BET	nd	nd	nd	–	nd	–
	GATA-3	nd	nd	nd	Int	nd	nd
	RORγt	nd	nd	+	Int	nd	+

The expression of cell-surface molecules and transcription factors in the above tables are presented as +, positive; –, negative; +/–, heterogeneous; int, intermediate; nd, not determined.

could not generate other hematopoietic lineages. As such, we refer to these cells as human ILCP. Extensive single cell cloning studies showed that human ILCPs are a heterogeneous population of multipotent and unipotent precursors. Moreover, human peripheral blood ILCP could generate tissue-resident ILC subsets in vivo in 'humanized' mice after transfer to immunodeficient hosts. In addition to adult blood, human ILCP with similar characteristics could be identified in fetal liver, cord blood, and various postnatal lymphoid (tonsil) and nonlymphoid tissues (lung). Whole transcriptomic and epigenetic analyses showed human ILCPs express TF known to control ILC differentiation in the mouse (ID2, TOX, TCF7, and RUNX3) but lack transcripts for mature ILC transcription factors (EOMES, TBET, and RORC) and cytokines (IFN-γ, IL-5, IL-13, IL-17A, and IL-22) consistent with their precursor activity. Taken together (Fig. 2), these results provided strong evidence for the existence of systemically distributed human ILCPs [18].

How do CD117$^+$ ILCP relate to the already described CD34$^+$ NKP and ILCP subsets? We found that blood ILCP express low levels of CD34, so they are distinct from other ILCP found in secondary lymphoid tissues. CD34 is proposed to have roles

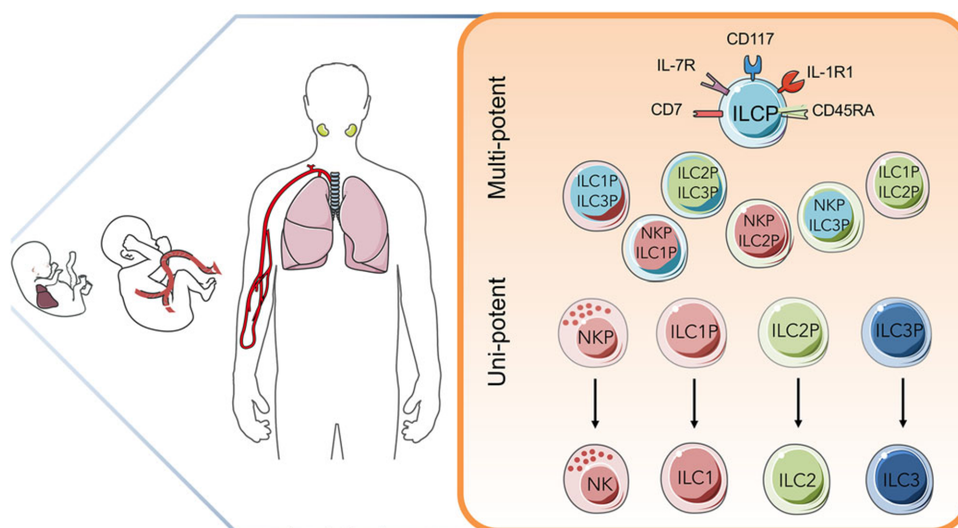

Figure 2. Human multi-potent and uni-potent ILCPs. Human ILCP have the Lin$^-$CD7$^+$CD127$^+$CD117$^+$IL-1R1$^+$CD45RA$^+$ phenotype and are present in the fetal liver, cord, and adult blood as well as adult tissues such as lung (shown) and tonsil (not shown). By in vitro cloning, whole transcriptomic and epigenetic analyses, we found that human circulating ILCPs are a heterogeneous population consists of multipotent and unipotent ILCP that can give rise to cytotoxic NK cells, helper ILC1, ILC2, and ILC3.

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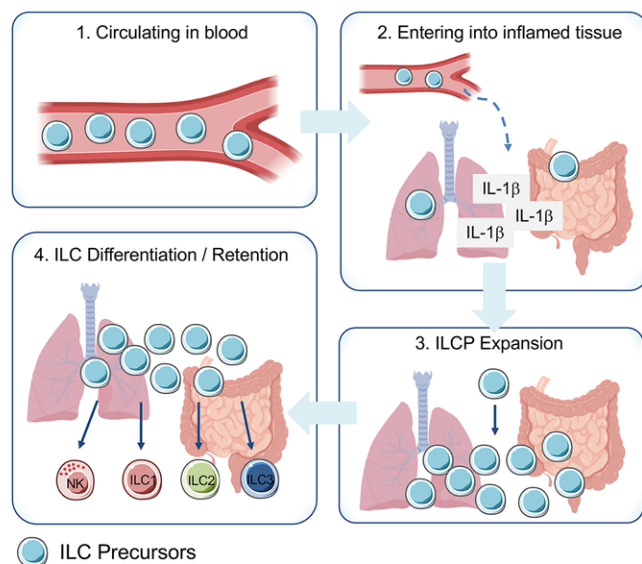

Figure 3. 'ILC-poiesis' model for tissue ILC generation. Under healthy conditions, ILCP circulating in the blood (panel 1). Local disturbances (for example, infection, inflammation, stress) trigger IL-1β production that may allow ILCP to enter tissues from the blood (panel 2). IL-1β can then drive ILCP expansion (panel 3) while the local environmental signals will further guide the appropriate ILC differentiation and tissue retention (panel 4).

in homing and adhesion and its expression may reflect a need for precursor interactions in specific tissue microenvironments. Loss of CD34 may accompany entry of ILCP into the circulation (reviewed in [4]); in this model, blood ILCP would represent immediate downstream progeny of $CD34^+$ ILCP and NKP.

A new model for ILC development: "ILC-poiesis"

During our characterization of human ILCPs, we discovered that IL-1β was a potent growth factor for these cells. In combination with IL-2 and IL-7, IL-1β allowed for robust ILCP proliferation and differentiation in vitro with subsequent generation of mature NK and ILC subsets. Human ILCP constitutively expressed the IL-1R, CD25, and CD127, thereby providing responsiveness to this cytokine mix. Several cell types generate IL-1β during inflammation [20], while IL-7 is normally produced by stromal cells in lymphoid and nonlymphoid tissues during steady state and following inflammation [21]. The importance of IL-1β for human ILCP differentiation suggested a four-step model (Fig. 3) whereby circulating ILCP (step 1) respond to local increases in tissue IL-1β levels secondary to inflammation (step 2). This would promote ILCP proliferation (step 3) with subsequent ILCP differentiation into mature ILC subsets dependent on local tissue signals (step 4). We termed this new model "ILC-poiesis" [18, 22]. Unlike models of ILC development that rely on BM differentiation to generate distinct NK cell and ILC subsets, our model of ILC-poiesis shifts the site of ILC generation to the tissues where inflammatory signals originate and where ILC functions are acutely required. In this way, ILC-poiesis provides 'on demand' ILC generation when and where it is needed.

Our single cell cloning assays using human ILCP generated a variety of distinct cell outputs, including cultures that contained one, two, or three ILC lineages with or without NK cells [18]. One interpretation of this result is that a homogeneous population of multipotent human ILCPs are present that stochastically give rise to different ILC and NK cell fates in a random fashion. This may be the case as our stromal system is devoid of polarizing cytokines (IL-12, IL-23, and IL-33) that may play a role in reinforcing particular ILC and NK cell fates. An alternative explanation is that blood ILCPs comprise a mixture of uni-potent committed NKP, ILC1 precursor, ILC2 precursor, and ILC3P as well as multipotent ILCP (Fig. 2). Perturbation of our single cell culture assays as well as single cell RNAseq analysis may help to distinguish between these two possibilities.

The multipotent ILCP that we observed in blood as well as tissues may represent the innate equivalent of naïve T cells that differentiate into Th and CTL of the adaptive immune system. ILCP share some commonalities with naïve T cells ('resting' state including reduced glycolysis and mitochondrial activity, lack of effector cytokine production, extensive proliferation once activated), although the mechanisms that enforce the quiescent phenotype associated with naïve T cells and ILCP remain to be elucidated.

Another unanswered question is how such phenotypic and functional diversity of ILC are generated from 'naïve' ILCP? It is likely that the ILC diversification is a process that is strongly influenced by extrinsic signals provided in the tissue environment. The nature of the signals required for ILCP differentiation to mature subsets via 'ILC-poiesis' in vivo remain poorly defined. Using a single cell in vitro culture system, we identified a cytokine mixture (IL-2, IL-7, IL-1β, and IL-23) and feeder cells (OP9) that can optimize the growth (burst size after 14–18 days) and differentiation (functional diversity) of ILCP. Much remains to be learned using this assay of ILCP differentiation in vitro: what are the signals delivered by stromal cells? What other cytokines influence this process? What type of ILCP intermediate are generated during culture? Notch signaling is a key determinant of cell-lineage commitment in lymphopoiesis (reviewed in [23]). T cell differentiation in the thymus from lymphoid precursors requires persistent Notch signaling through DLL4. Moreover, T_H differentiation is influenced by specific Notch ligands with DLL1 favoring the development of IFN-γ secreting Th1, whereas engagement of Jagged ligands preferentially induced development of Th2 and Treg [24, 25]. We found that Notch signaling via DLL4 could alter ILC cell fate in our in vitro system [18], although the effects of different Notch signaling (JAG1, JAG2, DLL1) on ILC lineage commitment remains to be tested.

Signals for tissue-specific ILC-poiesis

The ILC-poiesis model provides a starting point to understand tissue-specific ILC 'repertoires'. It is well appreciated that fetal and adult tissues in both mice and humans harbor different ILC subsets

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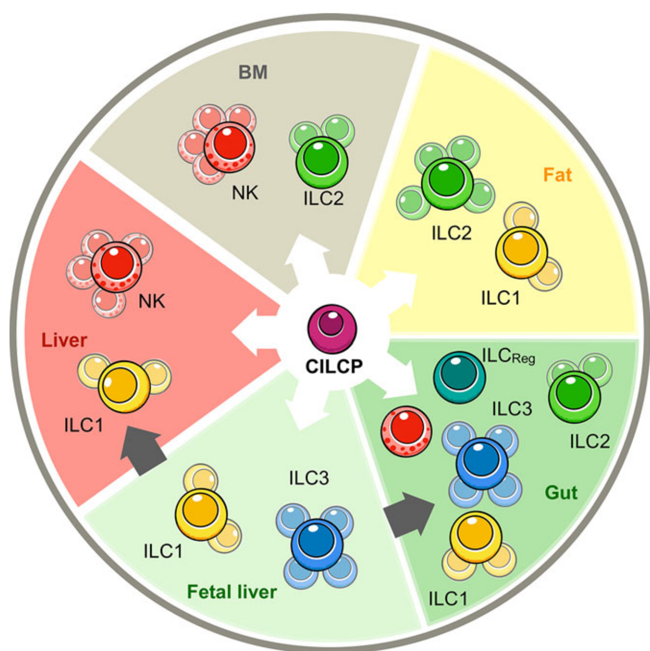

Figure 4. Tissue-specific ILC 'repertoires' via common ILC/NK cell precursors (CILCP). CILCP produced during fetal and adult life are a substrate for tissue ILC and NK cell development. Tissue-specific signals dictate the relative abundance of different ILC subsets and NK cells within tissues that may also contribute systemically to the circulation (for example, BM). Fetal 'waves' from liver may also contribute to distant sites (liver, gut) with potentially long-lived cells.

(reviewed in [1, 2]), but that the composition varies depending on age and state of activation (naïve versus infected or inflamed). For example, adipose tissue is rich in ILC1 and ILC2, whereas liver and BM have the highest proportions of NK cells (Fig. 4). Some tissues are nearly devoid of ILCs shortly after birth (lung, spleen) suggesting that environmental antigens drive ILC accumulation even under steady-state, non-infectious conditions. In other tissues, ILC seeding occurs in the fetal period, which may reflect dedicated pathways (LTi cells from liver to gut to create secondary lymphoid tissues) that occur during early development. As a myriad of tissue signals can provoke inflammation and IL-1β release, the expansion and subsequent differentiation of ILCs and NK cells may help to create ILC/NK cell 'repertoires' within tissues that are locally and temporally controlled via specific environmental signals.

It is interesting to consider human ILCP differentiation in tissues with the analogous process of adaptive T cell differentiation in lymph nodes. T cells enter secondary lymphoid tissues via HEV following chemokine gradients. Once inside LNs, T cells require a series of well-characterized signals (signal 1 = TCR triggering; signal 2 = co-stimulation; signal 3 = polarizing cytokines; reviewed in [26]). Do similar signals guide ILC differentiation? It is possible that the IL-1β signal that triggers human ILCP proliferation represents an equivalent of the TCR-mediated signal that is critical for T cell clonal expansion. Downstream effectors of IL-1β include NFκB and JNK pathways that are also triggered by cytokines including IL-18 and IL-33. Whether IL-1β signals are essential for the process of ILCP differentiation in vivo in humans or mice is not known.

Additional questions surround ILCP migration. How do ILCP enter inflamed tissues? Do chemokine receptors drive this process? Dendritic cells are critical orchestrators of T_H and CTL differentiation. Do these cells also guide ILCP differentiation? It will be interesting to compare the ability of different DC subsets and activation pathways on ILC differentiation from ILCP.

Tissue resident ILCs

A report from the Rudensky lab using parabiotic mice demonstrated that the vast majority of ILC subsets reside in tissues (lymphoid and nonlymphoid) for extended periods of time up (130 days) under physiological conditions [27]. Similarly, a study using HLA-mismatch liver transplant samples reported a unique subset of NK cells (marked by CXCR6 and CCR5 expression) that appear as long-lived tissue-resident cells even after a 13 year follow-up [28]. These results indicate that once ILCs have exerted their functions in tissues, they may remain tissue-resident and do not take up residence at distant sites. However, as these studies did not investigate the mechanisms that led to generation of these different tissue ILCs, they do not exclude the possibility that small numbers of ILC subsets (or their precursors) may enter the circulation and are available to enter inflammatory tissue sites in the context of the ILC-poiesis model. Along these lines, a recent study from Huang et al demonstrated that ILC2 residing in the intestinal lamina propria can migrate to distant tissues driven by sphingosine 1-phosphate-mediated chemotaxis under inflammatory conditions [29]. Further studies are needed to decipher whether ILC tissue residency is a feature of selective ILC subsets or a property dependent on specific environmental signals in some but not all organs.

ILCP as targets for cellular therapy?

Several studies have identified ILC subset structure is perturbed under various disease conditions. For example, NCR^+ ILC3 are decreased and ILC1 is increased in Crohn's disease patients [30]. ILC2 are frequently elevated in the inflamed nasal polyp of patients with chronic rhinosinusitis [31]. In colorectal cancer and in certain types of lung tumors, distinct ILC1 subsets are increased compared to the healthy controls [32], whereas ILC3 subsets are altered in inflammatory skin diseases [33]. Finally, ILC2 are decreased in adipose tissue of obese individuals, suggesting a general role for these cells in system metabolism [34]. Despite these observations, it remains unclear whether the alterations of ILC frequency in these different disease states are the cause or consequence of these conditions. Uncovering novel roles for ILCs in the prevention or exacerbation of disease progression will be an important step in potential targeting of ILCs in the clinic. The discovery of circulating ILCP coupled with a robust *in vitro* system for ILC differentiation opens the path to future ILC therapy through adoptive transfer of

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defined (and potentially genetically modified) ILCs. This approach could provide the means to redirect ILC effector functions in precise ways that could find applications in inflammatory conditions as well as for treating infectious diseases and cancer (reviewed in [35]).

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Conflict of Interest: The authors have submitted a patent describing the characterization and uses of human ILCPs. J.P.D. is a stakeholder in AXENIS (founder, member of the executive board).

References

1 **Artis, D. and Spits, H.**, The biology of innate lymphoid cells. *Nature* 2015. **517**: 293–301.

2 **Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., Koyasu, S. et al.**, Innate lymphoid cells–a proposal for uniform nomenclature. *Nat. Rev. Immunol.* 2013. **13**: 145–149.

3 **Vivier, E., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., Koyasu, S. et al.**, Innate lymphoid cells: ten years on. *Cell* 2018. **174**: 1054–1066.

4 **Serafini, N., Vosshenrich, C. A. and Di Santo, J. P.**, Transcriptional regulation of innate lymphoid cell fate. *Nat. Rev. Immunol.* 2015. **15**: 415–428.

5 **Xu, W., Domingues, R. G., Fonseca-Pereira, D., Ferreira, M., Ribeiro, H., Lopez-Lastra, S., Motomura, Y. et al.**, NFIL3 orchestrates the emergence of common helper innate lymphoid cell precursors. *Cell Rep.* 2015. **10**: 2043–2054.

6 **Yang, Q., Li, F., Harly, C., Xing, S., Ye, L., Xia, X., Wang, H. et al.**, TCF-1 upregulation identifies early innate lymphoid progenitors in the bone marrow. *Nat. Immunol.* 2015. **16**: 1044–1050.

7 **Klose, C. S., Flach, M., Möhle, L., Rogell, L., Hoyler, T., Ebert, K., Fabiunke, C. et al.**, Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell* 2014. **157**: 340–356.

8 **Constantinides, M. G., McDonald, B. D., Verhoef, P. A. and Bendelac, A.**, A committed precursor to innate lymphoid cells. *Nature* 2014. **508**: 397–401.

9 **Seehus, C. R., Aliahmad, P., de la Torre, B., Iliev, I. D., Spurka, L., Funari, V. A. and Kaye, J.**, The development of innate lymphoid cells requires TOX-dependent generation of a common innate lymphoid cell progenitor. *Nat. Immunol.* 2015. **16**: 599–608.

10 **Kohn, L. A., Hao, Q. L., Sasidharan, R., Parekh, C., Ge, S., Zhu, Y., Mikkola, H. K. et al.**, Lymphoid priming in human bone marrow begins before expression of CD10 with upregulation of L-selectin. *Nat. Immunol.* 2012. **13**: 963–971.

11 **Karamitros, D., Stoilova, B., Aboukhalil, Z., Hamey, F., Reinisch, A., Samitsch, M., Quek, L. et al.**, Single-cell analysis reveals the continuum of human lympho-myeloid progenitor cells. *Nat. Immunol.* 2018. **19**: 85–97.

12 **Alhaj Hussen, K., Vu Manh, T. P., Guimiot, F., Nelson, E., Chabaane, E., Delord, M., Barbier, M. et al.**, Molecular and functional characterization of lymphoid progenitor subsets reveals a bipartite architecture of human lymphopoiesis. *Immunity* 2017. **47**: 680–696.

13 **Renoux, V. M., Zriwil, A., Peitzsch, C., Michaëlsson, J., Friberg, D., Soneji, S. and Sitnicka, E.**, Identification of a human natural killer cell lineage-restricted progenitor in fetal and adult tissues. *Immunity* 2015. **43**: 394–407.

14 **Montaldo, E., Teixeira-Alves, L. G., Glatzer, T., Durek, P., Stervbo, U., Hamann, W., Babic, M. et al.**, Human RORγt(+)CD34(+) cells are lineage-specified progenitors of group 3 RORγt(+) innate lymphoid cells. *Immunity* 2014. **41**: 988–1000.

15 **Scoville, S. D., Mundy-Bosse, B. L., Zhang, M. H., Chen, L., Zhang, X., Keller, K. A., Hughes, T. et al.**, A progenitor cell expressing transcription factor RORγt generates all human innate lymphoid cell subsets. *Immunity* 2016. **44**: 1140–1150.

16 **Halim, T. Y., MacLaren, A., Romanish, M. T., Gold, M. J., McNagny, K. M. and Takei, F.**, Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. *Immunity* 2012. **37**: 463–474.

17 **Bando, J. K., Liang, H. E. and Locksley, R. M.**, Identification and distribution of developing innate lymphoid cells in the fetal mouse intestine. *Nat. Immunol.* 2015. **16**: 153–160.

18 **Lim, A. I., Li, Y., Lopez-Lastra, S., Stadhouders, R., Paul, F., Casrouge, A., Serafini, N. et al.**, Systemic human ILC precursors provide a substrate for tissue ILC differentiation. *Cell* 2017. **168**: 1086–1100.

19 **Cupedo, T., Crellin, N. K., Papazian, N., Rombouts, E. J., Weijer, K., Grogan, J. L., Fibbe, W. E. et al.**, Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells. *Nat. Immunol.* 2009. **10**: 66–74.

20 **Garlanda, C., Dinarello, C. A. and Mantovani, A.**, The interleukin-1 family: back to the future. *Immunity* 2013. **39**: 1003–1018.

21 **Mackall, C. L., Fry, T. J. and Gress, R. E.**, Harnessing the biology of IL-7 for therapeutic application. *Nat. Rev. Immunol.* 2011. **11**: 330–342.

22 **Di Santo, J. P., Lim, A. I. and Yssel, H.**, 'ILC-poiesis': generating tissue ILCs from naïve precursors. *Oncotarget* 2017. **8**: 81729–81730.

23 **Radtke, F., MacDonald, H. R. and Tacchini-Cottier, F.**, Regulation of innate and adaptive immunity by Notch. *Nat. Rev. Immunol.* 2013. **13**: 427–437.

24 **Amsen, D., Blander, J. M., Lee, G. R., Tanigaki, K., Honjo, T. and Flavell, R. A.**, Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* 2004. **117**: 515–526.

25 **Amsen, D., Antov, A. and Flavell, R. A.**, The different faces of Notch in T-helper-cell differentiation. *Nat. Rev. Immunol.* 2009. **9**: 116–124.

26 **Smith-Garvin, J. E., Koretzky, G. A. and Jordan, M. S.**, T cell activation. *Annu. Rev. Immunol.* 2009. **27**: 591–619.

27 **Gasteiger, G., Fan, X., Dikiy, S., Lee, S. Y. and Rudensky, A. Y.**, Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science* 2015. **350**: 981–985.

28 **Cuff, A. O., Robertson, F. P., Stegmann, K. A., Pallett, L. J., Maini, M. K., Davidson, B. R. and Male, V.**, Eomeshi NK Cells in Human Liver Are Long-Lived and Do Not Recirculate but Can Be Replenished from the Circulation. *J. Immunol.* 2016. **197**: 4283–4291.

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29 **Huang, Y., Mao, K., Chen, X., Sun, M. A., Kawabe, T., Li, W., Usher, N. et al.**, S1P-dependent interorgan trafficking of group 2 innate lymphoid cells supports host defense. *Science* 2018. **359**: 114–119.

30 **Bernink, J. H., Peters, C. P., Munneke, M., te Velde, A. A., Meijer, S. L., Weijer, K., Hreggvidsdottir, H. S. et al.**, Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat. Immunol.* 2013. **14**: 221–229.

31 **Mjösberg, J. M., Trifari, S., Crellin, N. K., Peters, C. P., van Drunen, C. M., Piet, B., Fokkens, W. J. et al.**, Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat. Immunol.* 2011. **12**: 1055–1062.

32 **Simoni, Y., Fehlings, M., Kløverpris, H. N., McGovern, N., Koo, S. L., Loh, C. Y., Lim, S. et al.**, Human Innate Lymphoid Cell Subsets Possess Tissue-Type Based Heterogeneity in Phenotype and Frequency. *Immunity* 2017. **46**: 148–161.

33 **Teunissen, M. B. M., Munneke, J. M., Bernink, J. H., Spuls, P. I., Res, P. C. M., Te Velde, A., Cheuk, S. et al.**, Composition of innate lymphoid cell subsets in the human skin: enrichment of NCR(+) ILC3 in lesional skin and blood of psoriasis patients. *J. Invest. Dermatol.* 2014. **134**: 2351–2360.

34 **Brestoff, J. R., Kim, B. S., Saenz, S. A., Stine, R. R., Monticelli, L. A., Sonnenberg, G. F., Thome, J. J. et al.**, Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* 2015. **519**: 242–246.

35 **Sonnenberg, G. F. and Artis, D.**, Innate lymphoid cells in the initiation, regulation and resolution of inflammation. *Nat. Med.* 2015. **21**: 698–708.

Abbreviations: **CLP**: common lymphoid precursor · **CILCP**: common ILC progenitor · **ILC**: Innate lymphoid cell · **ILC3P**: ILC3 precursor · **Lin**: lineage · **LTi**: lymphoid tissue inducer · **NKP**: NK precursor

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